Am. J. Trop. Med. Hyg., 91(3), 2014, pp. 584–588
doi:10.4269/ajtmh.14-0102

Seroepidemiological Study of Outdoor Recreationists' Exposure to Spotted Fever Group *Rickettsia* in Western Australia

Mohammad Y. Abdad,* Angus Cook, John Dyer, John Stenos, and Stanley G. Fenwick

Australian Rickettsial Reference Laboratory, Geelong, Victoria, Australia; School of Population Health, University of Western Australia, Perth, Western Australia, Australia; Fremantle Hospital, Fremantle, Western Australia, Australia; School of Veterinary and Biomedical Science, Murdoch University, Murdoch, Western Australia, Australia

Abstract. Bushland activity has previously been linked to rickettsial exposure in eastern and central regions of Australia, whereas little is known about the risks in Western Australia. The isolation of *Rickettsia gravesii* sp. nov. from *Amblyomma triguttatum* ticks and anecdotal reports of low-grade illness among bush recreationists raised the possibility of rickettsial transmission in the State. This study investigated rickettsial seroprevalence and potential risk of exposure to the spotted fever group rickettsiae in rogainers. Our results showed that rogainers active in the bush had a significantly higher risk of seropositivity (immunofluorescence total antibody titer ≥ 128) for the spotted fever group *Rickettsia* (odds ratio [OR] = 14.02, 95% confidence interval [CI] = 1.38–142.07) compared with a reference population, the overall seroprevalence in the rogainer group being 23.1%.

INTRODUCTION

Members of the genus *Rickettsia* are endemic worldwide and are the etiologic agents for typhus and a range of spotted fevers.[1,2] Although there are variously named spotted fevers worldwide with varying disease severity, they share many common symptoms such as fever, myalgia, headache, malaise, maculopapular rash, and, in a proportion of cases, cutaneous eschar. There are several well-documented *Rickettsia* spp.[2] in Australia of clinical importance: *Rickettsia australis* (Queensland tick typhus),[3] *Rickettsia honei* (Flinders Island spotted fever),[4] *Rickettsia typhi* (murine typhus),[5] and *Rickettsia felis* (cat flea typhus).[6]

Despite its presence on the eastern parts of Australia, spotted fever group rickettsioses have rarely been reported from Western Australia (WA), a state that consists of roughly a third of the continent, or an area of over 2.5 million km^2. However, recent evidence shows that rickettsial organisms are relatively common throughout the state, and most importantly in the more densely populated south-west region.[7–11] The discovery of two new *Rickettsia*, *Rickettsia gravesii* sp. nov.,[12] and *Candidatus* "Rickettsia antechini," brings the total number of known *Rickettsia* in the state to four (alongside *R. felis* and *R. typhi*). *Rickettsia gravesii* is suspected to be the most widely distributed *Rickettsia* in WA bush with a high prevalence (> 70%) in tick populations infesting the local fauna.[8,13]

The confirmed presence of *Rickettsia* in both urban and bush areas of WA raises the question of whether, and the degree to which, rickettsial exposure is occurring in human populations. The mere presence of an endemic *Rickettsia* in an environment does not necessarily mean that it will be transmitted to humans, and hence a high degree of seropositivity cannot be assumed.

This study was designed to investigate the rate of rickettsial exposure in groups who are in the bush recreationally. These at-risk populations were selected because of the high prevalence of *R. gravesii* in tick populations in the recreational areas, together with reports of frequent tick bites. This particular study focused on members of the Western Australian Rogaining Association (WARA). Rogaining is an outdoor recreational sport that was first introduced in 1947 by students of Melbourne University, Australia. The sport resembles orienteering, with the main difference being a more significant cross-country element. Rogaining events last between 6 and 24 hours with participants traveling through the bush around a designated course. It is not uncommon for groups to stay out in the bush for the entire duration of the event, with minimal protection from arthropods. Rogaining events were organized within a 200 km range of the outskirts of Perth, the major WA population center.

MATERIALS AND METHODS

Recruitment criteria. The study was conducted in south-west WA in the 2006–2007 period. Two groups were recruited for this study: 1) the at-risk population: active rogainers and 2) the comparator population: staff and students of Murdoch University in metropolitan Perth. The eligibility criteria for rogainers were as follows: a current resident of WA, previous attendance of at least one rogaine event, a member of WARA, and > 18 years of age. Participant recruitment was performed at rogaining competitions in WA, which occurs once every 2 months. Members of the comparator population were required to be residents of WA who had not lived outside of the state for more than 6 months cumulatively in the past 5 years and were > 18 years of age.

Questionnaire design. Questionnaires were designed for the control and target group (rogainers) to collect information on bush activity and tick exposure. All participants completed their questionnaire at the time of recruitment. Information was also requested on age, gender, occupational responsibilities, recreational activities, frequency of such activities, and exposure to bush and ticks, duration of activities, clinical symptoms presenting after extended periods of bush activity, and past medical information.

Blood collection. Blood was collected from study participants at the time of recruitment by a qualified phlebotomist. Approximately 18 mL of blood were collected from each participant in two 10 mL serum tubes. Tubes were centrifuged at 1200 × *g* for 5 minutes and serum collected for immunofluorescence assay (IFA).

*Address correspondence to Mohammad Y. Abdad, Australian Rickettsial Reference Laboratory, Geelong Hospital, Geelong, Victoria 3220, Australia. E-mail: yazid.abdad@hotmail.com

IFA. The IFA was performed on the collected sera as originally described.[11,14] Briefly, rickettsial whole cell antigen preparations (Australian SFG isolates, i.e., *R. australis* strain JC, *R. honei* strain Kaplan, and *R. gravesii* strain BWI-1) were acetone fixed to 40-well slides. Sera were diluted 1/128 with 2% casein buffer and incubated with antigen slides in a humid environment at 35°C for 30 minutes. Slides were washed 3 times with 10% phosphate buffered saline solution and air-dried. Goat fluorescein isothiocyanate labeled antibodies (KPL, Gaithersburg, MD) specific for human antibodies (IgM + IgA + IgG) were applied and incubated in a humid environment for 30 minutes at 35°C. Slides were washed and coverslips mounted using fluorescent mounting fluid (Dako, Glostrup, Denmark). Antibody titers were read using an illuminator-equipped microscope (Leica, Solms, Germany). Samples with titers of more than 1/128 were considered positive as presently used by the Australian Rickettsial Reference Laboratory, Geelong.

Statistical analysis. Statistical analyses were performed on the relationship between the predictive variables and major outcome variable (seropositivity for SFG *Rickettsia* at titers equal to or higher than 1/128). Descriptive statistics were generated for activity-related descriptors and the age and gender of the study population. To determine the risk of infection in the WA bush, final risk estimates were modeled using logistic regression. The outcomes of the survey conducted on participants were converted to binary data and odd ratios (ORs) were calculated for the rogainer group versus the control (baseline) group. Other risk factors were also assessed, such as bush activities in other states and overseas and the presence of major health issues. Both crude and adjusted risk estimates were calculated by statistical modeling using Stata/MP 10 (College Station, TX).

Ethics. Human ethics approval for this study was obtained from the Fremantle Hospital, WA (05/609) and Murdoch University, WA (2006/023), human ethics committees. Informed consent was obtained from all human adult participants.

RESULTS

Descriptive epidemiology. During the duration of the study (2006–2009), there were ~1,500 members registered with WARA. Of these, a total of 61 individuals participated in both components of the study; completion of the questionnaire and serological testing (an estimated participation rate of 4.1%). The descriptive results are provided in Table 1. The gender distribution between the control and rogainer groups was comparable. The median age for the rogainers was 49 years as opposed to 24 years for the control group.

All study participants from the rogainers' group reported being in the bush at least once a month for recreational activities (Table 1). As expected, the control group had minimal bush exposure for both recreational and occupational purposes.

Serology (IFA) results. A total of 47 sera were collected from the baseline control group and 61 from rogainers at the first collection. Based on the 1/128 benchmark, seroprevalence among rogainers was 23.0% (14 of 61) and 2.1% for the baseline control group (1 of 47).

OR analysis. Logistic regression and calculation of ORs showed that there were significant associations ($P < 0.05$) between SFG seropositivity and age, frequency of bush activities and frequency of tick bites. However, no significant associations were observed between variables such as gender or with self-reported symptoms relating to tick bites with positive serology subjects. Data are summarized and presented in Table 2. The ORs are presented unadjusted and adjusted for age and gender.

TABLE 1
Summarized questionnaire data

Categories	Control baseline ($N = 47$)		Rogainers ($N = 61$)	
	n	%	n	%
Gender				
Male	20	42.6	30	49.2
Female	27	57.4	31	50.8
Age groups				
< 25 years of age	25	53.2	0	0
25 to 50 years of age	21	44.7	16	26.2
> 50 years of age	1	2.1	45	73.8
Recreational time in bush in WA				
Once a month or more	18	38.3	61	100
Rarely (< 2 times/year)	29	61.7	0	0
Occupational time in bush in WA				
Once a month or more	2	4.3	4	6.6
Rarely (< 2 times/year)	45	95.7	57	93.4
Time in bush in other Australian states				
More than once a year	4	8.5	8	13.1
Once per year or less	43	91.5	53	86.9
Time in bush overseas				
More than once a year	2	4.3	1	1.6
Once per year or less	45	95.7	60	98.4
Tick bite frequency				
Most or every time in bush (25% or more)	0	0	26	42.6
< 25% to never	47	100	35	57.4
Symptoms after tick bite				
No symptoms	NA		54	88.5
Systemic symptoms present			7	11.5
Symptoms with no bite history				
No symptoms	43	91.5	57	93.4
Systemic symptoms present	4	8.5	4	6.6
Medical conditions				
None	NA		42	68.9
Major (e.g., heart, asthma, diabetes)			19	31.1

DISCUSSION

Our findings suggest a high rate of SFG rickettsial transmission and infection for those active in the WA bush. There is a large and significant difference in seroprevalence between the rogainers and the baseline population, with members of the rogainer group ~14 times more likely to be seropositive for SFG *Rickettsia* (OR = 14.02, 95% confidence interval [CI] = 1.38–142.07). Although we anticipated that people involved in outdoor activities in WA would be exposed to ticks and rickettsiae, the elevated seroprevalence and risk of infection are higher than observed elsewhere in high risk "healthy" (asymptomatic) populations.[15,16] In other studies conducted in Australia, comparably high prevalence has only been observed in patients with overt symptoms that may have been attributable to past rickettsial infection.[17] Studies elsewhere have showed high seroprevalences to rickettsial exposure including; *R. africae* in Guadeloupe (French West Indies),[18] *Rickettsia japonica* in Korea[19] and *Rickettsia helvetica* in Denmark.[20] The low seroprevalence observed in the current

TABLE 2
Risk estimates for seropositivity to SFG *Rickettsia*

	(Sero +ve/ sero –ve)	Unadjusted odds ratio	95% CI (*P* value)	Adjusted odds ratio*	95% CI (*P* value)
Demographic variables					
Age in years					
	15/93	**1.04**	**1.00–1.08 (*P* = 0.035)**	**1.04**	**1.00–1.08 (*P* = 0.047)**
Age strata					
< 25 y (baseline)	1/24	1.00	–	1.00	–
25–50 y	8/49	3.92	0.46–33.15 (*P* = 0.210)	3.66	0.43–31.35 (*P* = 0.236)
> 50 y	6/20	7.20	0.80–64.89 (*P* = 0.078)	6.91	0.74–64.78 (*P* = 0.091)
Gender					
Female (baseline)	6/52	1.00	–	1.00	–
Male	9/41	1.90	0.63–5.78 (*P* = 0.257)	1.67	0.53–5.22 (*P* = 0.381)
Recreational classification					
Control group (baseline)	1/46	1.00	–	1.00	–
Rogainers (recreational)	14/47	**13.70**	**1.73–108.49 (*P* = 0.013)**	**14.02**	**1.38–142.07 (*P* = 0.025)**
Frequency of recreational activities in W.A. Bushland					
< 1× per month (baseline)	1/28	1.00	–	1.00	–
≥ 1× per month	14/65	6.03	0.76–48.11 (*P* = 0.090)	4.01	0.44–37.04 (*P* = 0.220)
Reported work in W.A. Bushland					
No	15/87	1.00	–	1.00	–
Yes	0/6	0.73†	0–5.46 (*P* = 0.797)	0.63†	0–4.79 (*P* = 0.694)
Experienced frequent tick bites in W.A. Bushland					
No	7/72	1.00	–	1.00	–
Yes	7/19	**3.79**	**1.18–12.13 (*P* = 0.025)**	2.37	0.73–9.30 (*P* = 0.138)
Reports of systemic symptoms specifically after tick bites∧					
No	9/37	1.00	–	1.00	–
Yes	3/4	3.08	0.58–16.29 (*P* = 0.185)	4.78	0.74–30.76 (*P* = 0.099)
Reports of any systemic symptoms (not necessarily after tick bites)					
No	15/85	1.00	–	1.00	–
Yes	0/8	0.53†	0–3.72 (*P* = 0.579)	0.50†	0–3.62 (*P* = 0.549)

Significant findings in bold;
*Adjusted for age and gender.
†Median unbiased estimator (MUE) using exact logistic regression for subjects who reported only tick bites.
CI = confidence interval.

control group was similar to that observed in other studies where prevalences of < 5% have been reported in a general Australian population.[11]

We also observed increasing odds of seropositivity with increasing age, with a 4% increase in risk of SFG *Rickettsia* per year (OR = 1.04 per year, 95% CI = 1.00–1.08). An association between age and seropositivity is observed in many human infectious diseases, as a result of cumulative seroconversion over time. It is notable that a significant proportion of participants from the rogainer group were > 50 years of age, and therefore age was an independent factor in predicting the likelihood of exposure to ticks.

Unadjusted ORs suggested an association between frequent tick bites and SFG seroprevalence (OR = 3.79, 95% CI = 1.18–12.13), but this was no longer significant once the estimates were adjusted for gender and age. The lack of a significant association between self-reported tick bites and seropositivity in this study may not be an accurate reflection of the true exposure profile as the majority of SFG rickettsioses worldwide are likely transmitted by an unrecognized tick exposure event.

The information gathered in this study shows the potential risk that SFG *Rickettsia* may pose to humans active in the WA bush. The high rate of infection/exposure observed among high-risk populations reinforces the hypothesis that there are endemic SFG rickettsiae in WA, and that the most likely etiologic agent may be *R. gravesii*. Rather than try to differentiate antibody isotypes, or determine the species of rickettsiae that is causing infection, focus was made on determining if there is rickettsial exposure. An anti-human antibody mix was used to measure total antibody level. This allowed us to determine the risk of exposure in the target group and will hopefully lead to more in-depth studies on rickettsial infection in WA.

The tick *Amblyomma triguttatum* had been identified previously as a potential reservoir and vector for *R. gravesii*,[13] and the affinity of this tick for large vertebrate mammals in the bush is well documented by Roberts (1970).[21] The other rickettsiae known endemic to WA, *R. typhi* and *R. felis*, presents serologically as TG rickettsiae and are transmitted by fleas.[6,22] It is more likely that the seroprevalence observed in bush-active groups in south-west WA is a result of exposure to an SFG *Rickettsia*. Furthermore, except for those described,

no other SFG *Rickettsia* has been found in ectoparasites that commonly bite humans in WA.

Clinically confirmed disease from SFG *Rickettsia* exposure is extremely rare in WA. There have been anecdotal reports of "*Rickettsia*-like" disease by physicians: although the majority of these have not been supported by laboratory findings, one of the authors recently saw a clinically compatible case in a heavily tick-exposed individual who had been bush walking in the south west of the state, with definitive seroconversion (> 4-fold rise in antibody titer) to SFG *Rickettsia* and positive polymerase chain reaction but insufficient product for DNA sequencing on biopsied skin tissue taken from an eschar (Dyer J, submitted for publication). This pattern of underdetection has been observed to occur with other rickettsial species elsewhere.[23,24] It is therefore possible that most human SFG rickettsial infection in WA is mild or asymptomatic. A previous WA study investigating *A. triguttatum* tick infestations of humans failed to identify a clear link between rickettsial-like illness and tick bites,[25] although delayed hypersensitivity reactions to tick bites (24–48 hours) and secondary bacterial infections were reported. Considering the symptoms of rickettsiosis usually present 7–14 days after infection, any presenting symptoms may not be attributed by patients to preceding tick bites. Many symptoms in association with tick bites are likely caused by an immediate allergic reaction, which is not uncommonly recorded in Australia.[26,27]

There are a number of potential limitations to the current study. All participants in the study were volunteers and thus may have particular characteristics that could have influenced the outcome of the result as it is known that volunteers in community-based studies have demographic attributes that may not be representative of the more general population.[28] Although characteristics of volunteers vary between studies, it has often been observed that healthier individuals in the community are more inclined to participate.[29–31] Alternatively, given the focus of the study on infectious disease, those individuals who were more concerned about their health or who had experienced symptoms that they attributed to tick exposure may have been inclined to participate.

The questionnaire required participants to recall information as far back as 5 years from the time of the questionnaire application. The reliability of the information provided may therefore be questionable representing either over-reporting or under-reporting of information deemed useful (or unrelated) to the study. For example, mild symptoms after tick bites may not have been accurately recalled or may not have been linked to an exposure event.

Our findings have significant public health implications, and have already resulted in an increased awareness of arthropod-borne infections by the affected populations. By the end of this project, precautionary measures including frequent checking for ticks during prolonged periods in the bush and use of insect repellents were being routinely implemented by rogaining participants on recommendation of the WARA.

Received February 13, 2014. Accepted for publication May 21, 2014.

Published online June 16, 2014.

Financial support: Funding for this study was provided by the Fremantle Hospital Research Foundation. Authors declare no conflicts of interest.

Authors' addresses: Mohammad Y. Abdad, and John Stenos, Australian Rickettsial Reference Laboratory, Australian Rickettsial Reference Laboratory, Douglas Hocking Research Institute, The Geelong Hospital, Geelong, Victoria, Australia, E-mails: yazid.abdad@hotmail.com and johns@barwonhealth.org.au. Angus Cook, University of Western Australia, School of Population Health, Perth, Western Australia, Australia, E-mail: angus.cook@uwa.edu.au. John Dyer, Fremantle Hospital, Infectious Diseases Service, Fremantle, Western Australia, Australia, E-mail: john.dyer@health.wa.gov.au. Stanley G. Fenwick, Murdoch University, School of Veterinary and Biomedical Sciences, Murdoch, Western Australia, Australia, E-mail: Stanley_Fenwick@dai.com.

REFERENCES

1. Walker DH, Ismail N, 2008. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nat Rev Microbiol 6:* 375–386.
2. Parola P, Paddock C, Socolovschi C, Labruna M, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE, Raoult D, 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev 26:* 657–702.
3. McBride WJ, Hanson JP, Miller R, Wenck D, 2007. Severe spotted fever group rickettsiosis, Australia. *Emerg Infect Dis 13:* 1742–1744.
4. Unsworth NB, Stenos J, Graves SR, Faa AG, Cox GE, Dyer JR, Boutlis CS, Lane AM, Shaw MD, Robson J, Nissen MD, 2007. Flinders Island spotted fever rickettsioses caused by "marmionii" strain of *Rickettsia honei*, Eastern Australia. *Emerg Infect Dis 13:* 566–573.
5. Simon NG, Cremer PD, Graves SR, 2011. Murine typhus returns to New South Wales: a case of isolated meningoencephalitis with raised intracranial pressure. *Med J Aust 194:* 652–654.
6. Williams M, Izzard L, Graves S, Stenos J, Kelly J, 2010. First cases of probable *Rickettsia felis* (cat flea typhus) in Australia. *Med J Aust 194:* 41–43.
7. Owen H, Clark P, Stenos J, Robertson I, Fenwick S, 2006. Potentially pathogenic spotted fever group rickettsiae present in Western Australia. *Aust J Rural Health 14:* 284–285.
8. Owen H, Unsworth NB, Stenos J, Robertson I, Clark P, Fenwick S, 2006. Detection and identification of a novel spotted fever group *Rickettsia* in Western Australia. *Ann N Y Acad Sci 1078:* 197–199.
9. Schloderer D, Owen H, Clark P, Stenos J, Fenwick SG, 2006. *Rickettsia felis* in fleas, Western Australia. *Emerg Infect Dis 12:* 841–843.
10. O'Connor LF, Kelly HA, Lubich JM, Lindsey RJ, McComish MJ, 1996. A cluster of murine typhus cases in Western Australia. *Med J Aust 165:* 24–26.
11. Graves S, Wang L, Nack Z, Jones S, 1999. *Rickettsia* serosurvey in Kimberley, Western Australia. *Am J Trop Med Hyg 60:* 786–789.
12. Sentausa E, Abdad MY, Robert C, Stenos J, Raoult D, Fournier PE, 2013. Genome sequence of *Rickettsia gravesii*, isolated from Western Australian ticks. *Genome Announc 1:* e00975–e001013.
13. Li AY, Adams PJ, Abdad MY, Fenwick SG, 2010. High prevalence of *Rickettsia gravesii* sp. nov. in *Amblyomma triguttatum* collected from feral pigs. *Vet Microbiol 146:* 59–62.
14. Graves SR, Dwyer BW, McColl D, McDade JE, 1991. Flinders Island spotted fever: a newly recognized endemic focus of tick typhus in Bass Strait. Part 2. Serological investigations. *Med J Aust 154:* 99–104.
15. da Costa PS, Brigatte ME, Greco DB, 2005. Antibodies to *Rickettsia rickettsii, Rickettsia typhi, Coxiella burnetii, Bartonella henselae, Bartonella quintana*, and *Ehrlichia chaffeensis* among healthy population in Minas Gerais, Brazil. *Mem I Os Cr 100:* 853–859.
16. Pinter A, Horta MC, Pacheco RC, Moraes-Filho J, Labruna MB, 2008. Serosurvey of *Rickettsia* spp. in dogs and humans from an endemic area for Brazillian spotted fever in the State of Sao Paulo, Brazil. *Cad Saude Publica 24:* 247–252.
17. Unsworth N, Graves S, Nguyen C, Kemp G, Graham J, Stenos J, 2008. Markers of exposure to spotted fever rickettsiae in patients with chronic illness, including fatigue, in two Australian populations. *QJM 101:* 269–274.
18. Parola P, Vestris G, Martinez D, Brochier B, Roux V, Raoult D, 1999. Tick-borne rickettsiosis in Guadeloupe, the French West Indies: isolation of *Rickettsia africae* from *Amblyomma variegatum* ticks and serosurvey in humans, cattle, and goats. *Am J Trop Med Hyg 60:* 888–893.

19. Jang WJ, Kim JH, Choi YJ, Jung KD, Kim YG, Lee SH, Choi MS, Kim IS, Walker DH, Park KH, 2004. First serologic evidence of human spotted fever group rickettsiosis in Korea. *J Clin Microbiol 42:* 2310–2313.
20. Nielsen H, Fournier PE, Pedersen IS, Krarup H, Ejlertsen T, Raoult D, 2004. Serological and molecular evidence of *Rickettsia helvetica* in Denmark. *Scand J Infect Dis 36:* 559–563.
21. Roberts FHS, 1970. *Australian Ticks*. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organization.
22. Graves S, Stenos J, 2009. Rickettsioses in Australia. *Ann N Y Acad Sci 1166:* 151–155.
23. Higgins JA, Radulovic S, Schriefer ME, Azad AF, 1996. *Rickettsia felis*: a new species of pathogenic *Rickettsia* isolated from cat fleas. *J Clin Microbiol 34:* 671–674.
24. Paddock CD, Sumner JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SL, Tamminga CL, Ohl CA, 2004. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clin Infect Dis 38:* 805–811.
25. Pearce RL, Grove DI, 1987. Tick infestation in soldies who were bivouacked in the Perth region. *Med J Aust 146:* 238–240.
26. Brown AF, Hamilton DL, 1998. Tick bite anaphylaxis in Australia. *J Accid Emerg Med 15:* 111–113.
27. Gauci M, Loh RK, Stone BF, Thong YH, 1989. Allergic reactions to the Australian paralysis tick, *Ixodes holocyclus*: diagnostic evaluation by skin test and radioimmunoassay. *Clin Exp Allergy 19:* 279–283.
28. Ganguli M, Lytle ME, Reynolds MD, Dodge HH, 1998. Random versus volunteer selection for a community-based study. *J Gerontol 53:* M39–M46.
29. Lindsted KD, Fraser GE, Steinkohl M, Beeson WL, 1996. Health volunteer effect in a cohort study; temporal resolution in the Adventist Health. *J Clin Epidemiol 49:* 783–790.
30. Froom P, Melamed S, Kristel-Boneh E, Benbassat J, Ribak J, 1999. Healthy volunteer effect in industrial workers. *J Clin Epidemiol 52:* 731–735.
31. Austin MA, Criqui MH, Barrett-Connor E, Holdbrook MJ, 1981. The effect of response bias on the odds ratio. *Am J Epidemiol 114:* 137–143.