Seroepidemiological Study of Outdoor Recreationists’ Exposure to Spotted Fever Group

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

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. Although there are variously named spotted fevers and are the etiologic agents for typhus and a range of spotted fevers, R. typhi (typhus), R. conorii (Q fever), R. slovaca (European tick typhus), R. montана (Rocky Mountain spotted fever), and R. rickettsii (cat flea typhus) are widespread in humans throughout the world.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.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 partic-

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 at 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).
The IFA was performed on the collected sera as originally described. 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 previously 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 WARAJ. 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.

## 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. 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. Studies elsewhere have shown high seroprevalences to rickettsial exposure including *R. australis* in Guadeloupe (French West Indies), *Rickettsia japonica* in Korea and *Rickettsia helvetica* in Denmark. The low seroprevalence observed in the current
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*, and the affinity of this tick for large vertebrate mammals in the bush is well documented by Roberts (1970). The other rickettsiae known endemic to WA, *R. typhi* and *R. felis*, presents serologically as TG rickettsiae and are transmitted by fleas. 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 under-detection has been observed to occur with other rickettsial species elsewhere. 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, 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 pre-existing 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. 

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. Although characteristics of volunteers vary between studies, it has often been observed that healthier individuals in the community are more inclined to participate. 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 regaining participants on recommendation of the WARAC.

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