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Prevalence of tick-borne pathogens in the South Okanagan, British Columbia: Active surveillance in ticks (*Dermacentor andersoni*) and deer mice (*Peromyscus maniculatus*)

Although results from testing ticks and mice in the South Okanagan suggest the risk is low for contracting Lyme disease and other zoonoses in the area, the public should be informed of protective personal practices that reduce tick bites.

ABSTRACT:

***Background*: Tick-borne pathogens are rare in the South Okanagan region of British Columbia, but there is still public concern about the diseases they can cause.**

***Methods*: Ticks (*Dermacentor andersoni*) and deer mice (*Peromyscus maniculatus*) were collected and tested to determine the prevalence of tick-borne pathogens and hantavirus.**

***Results*: No ticks contained evidence of *Borrelia burgdorferi senso stricto*, *Anaplasma phagocytophilum*, *Bartonella henselae*, or *Rickettsia rickettsii*. *R. peacockii* (nonpathogenic) was present in 32.5% of ticks tested and *R. rhipicephali* (unknown pathogenicity) was present in 12.5%. Deer mice tested had no antibodies to *B. burgdorferi s.s.* or *B. henselae*; one mouse was seropositive for *A. phagocytophilum*; 12.3% were positive for *R. rickettsii*, but this might represent cross-reactivity to *R. rhipicephali*; 4.1% had significant antibody titres to Sin Nombre virus, a species of hantavirus.**

***Conclusions*: Given the low prevalence of tick-borne pathogens, health professionals should focus on improving public understanding of the true risk of tick-borne zoonoses in the area studied, and raise awareness of the personal protective practices (e.g., tucking pants into socks; regular self checks after being in tick habitat) that can further reduce the already low risk of infection.**

Background

Concerns about zoonoses

Several species of ticks—arthropod ectoparasites such as *Ixodes pacificus*—transmit pathogens that cause the most frequently contracted zoonoses in North America.[1] Human cases of these diseases (e.g., Lyme disease, anaplasmosis, human babesiosis) are rare in the South Okanagan region of British Columbia.[2] Indeed, Lyme disease, caused by the pathogen *Borrelia burgdorferi sensu stricto*, has a human incidence rate of less than 0.1/100 000 in all of BC.[3] However, there is increasing public concern and controversy about the risk of contracting diseases from wildlife or domestic animals, particularly Lyme disease.[4]

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The low risk of contracting tick-borne zoonoses in the South Okanagan region is supported by passive surveys for tick-borne pathogens. Between 1993 and 2006 health practitioners and private citizens sent ticks collected from humans for analysis at the British Columbia Centre for Disease Control (BCCDC), resulting in the identification of 5801 *I. pacificus* and 1151 *I. angustus* samples. Of the ticks collected, approximately 80 were found to harbor *B. burgdorferi s.s.*[2] However, while passive surveys can provide an idea of the distribution of tick-borne pathogens (i.e., presence or absence), they do not constitute a representative sample of ticks in a given region. Passive surveys are indirect methods that do not reveal the prevalence of pathogens within tick populations—that is, the likelihood of being exposed to a pathogen after being bitten by a tick.

Active surveys needed

Active surveys can help clarify the prevalence of tick-borne pathogens by directly examining ticks and their main disease host for the presence of pathogens. In BC, tick-borne pathogens are maintained in the environment by the interaction between ticks and the main disease host, deer mice (*Peromyscus maniculatus*).[5] Examining ticks allows us to estimate the current prevalence of pathogens, while examining the antibodies in the serum of deer mice can allow us to confirm the prevalence found in ticks, as well as identify past exposures to pathogens. As well, different species of ticks transmit different pathogens (e.g., *Dermacentor andersoni* transmit *Rickettsia rickettsii*; *I. pacificus* transmit *B. burgdorferi s.s.* and *Anaplasma phagocytophilum*).[6] Thus, active surveillance not only helps determine the prevalence of the pathogens, but also the tick species present and the pathogens they are likely to transmit.

To address public concern and clarify the risk of contracting tick-borne zoonoses, active surveillance was conducted in an area within the South Okanagan to determine the prevalence of tick-borne pathogens. From 2007 to 2009, ticks and deer mice serum samples were collected and analyzed for the following pathogens (ticks only) or antibodies to the pathogens (deer mice serum): *B. burgdorferi s.s.*; *R. rickettsii*; *A. phagocytophilum*; and *Bartonella henselae*. As we were concerned with the human exposure to pathogens during daily wilderness activities, our focus was on hard ticks (e.g., *Dermacentor* spp., *Ixodes* spp.), which are diurnal, and not soft ticks (e.g., *Ornithodoros* spp.), which are nocturnal. Deer mice serum samples were opportunistically tested for antibodies against Sin Nombre virus (SNV), a species of hantavirus that is transmitted by deer mice; human exposure to SNV can lead to respiratory failure.[7,8]

Methods

Collection of ticks and deer mice

Ticks were collected once a week from 1 April to 30 April 2008 and 1 April to 30 June 2009 at 11 sites in the study area (Figure). Ticks were collected by "flagging"[9] for 1 hour (from 6 to 10

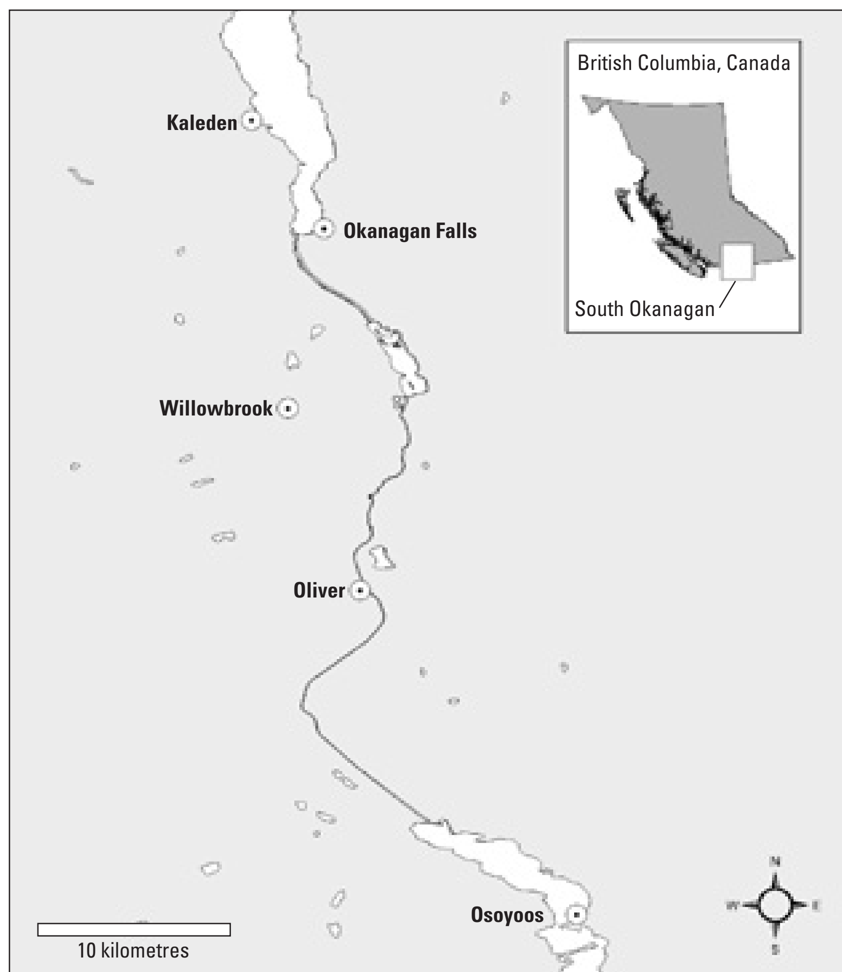

Figure. Study area (lat. 49 28.41 N, long. 119 35.43 W) within the South Okanagan.

a.m.) in suitable tick habitat at each site, and all collected ticks were identified by species and life stage.[10]

Deer mice were collected every 2 weeks using 36 Longworth live traps[11] placed in suitable habitat for deer mice and ticks (i.e., areas with mixed vegetational structure)[12] at 10 sites in the study area from 1 May to 30 May and 1 August to 31 August 2007 and 1 April to 30 April 2008. Blood samples were collected from captured deer mice and all animals were examined for attached ticks. Animal handling and manipulations were approved under the University of British Columbia animal care protocol (approval number A08-0711).

There is increasing public concern and controversy about the risk of contracting diseases from wildlife or domestic animals, particularly Lyme disease.

Testing for pathogens in host-seeking ticks

In total, 330 adult *D. andersoni* ticks collected from the different sampling sites in 2008 and 2009 were tested for infection with *B. burgdorferi s.s.*, *A. phagocytophilum*, *B. henselae*, and spotted fever rickettsial species. Testing of *B. burgdorferi s.s.* was conducted at the BCCDC on 110 ticks collected in 2008, while the remaining 220 ticks collected in 2008 and 2009 were tested at the National Microbiology Laboratory (NML) in Winnipeg. Ticks were tested individually at BCCDC, while at the NML they were tested in pools of five ticks. DNA was extracted from ticks using QIAGEN commercial extraction kits.[13]

At the BCCDC, tick samples were screened for *B burgdorferi s.s.* DNA as previously described.[14] Briefly, polymerase chain reaction (PCR) was used to target amplicons of borrelial DNA and amplify a portion of the variable spacer region between two conserved structures, the 3' end of the 5S rRNA (rrf) and the 5' end of the 23S rRNA. The PCR was performed using a GE illustra PuReTaq Ready-To-Go PCR Beads on Stratagene Robocycler.

At the NML, DNA from ticks was screened for *A. phagocytophilum*, *B. henselae*, and spotted fever rickettsial species using real-time PCR. The primers and probes were directed toward the *msp2* gene,[15] *321s* and *H495as* segments,[16] and *gltA* gene, respectively.[17] All reactions were performed using Taqman Fast Universal PCR Master Mix (Applied Biosystems) with a final concentration of 0.3 mM of each primer and 0.1 mM of the probe. Determination of the rickettsial species in positive ticks was performed by amplification of DNA in a semi-nested PCR.[18] All amplicons were purified using the QIAquick PCR Purification kit (QIAGEN) and sequenced by the NML Genomics Core Facility in Winnipeg. The sequences were analyzed using DNASTAR Lasergene 7 software. Homologous sequences were detected using the National Center for Biotechnology Information search engine.

Serological assays on deer mouse sera

Serological assays were performed at the NML to detect antibodies against *B. burgdorferi s.s.*, *A. phagocytophilum*, *R. rickettsii*, and SNV in deer mouse serum samples.

Antibodies to *B. burgdorferi s.s.* were detected by enzyme-linked immunosorbent assay (ELISA) and Western blot assay as previously described.[19]

Serum samples were screened for antibodies to *A. phagocytophilum* in an immunofluorescence assay (IFA) similar to that described by Nieto and Foley in 2008.[20] Modifications included using FTA Hemagglutination Buffer as sample diluent, Evan's Blue for conjugate dilution, and commercially prepared slides (Fuller Laboratories, CA). Reactive samples were confirmed by an IgG Western blot assay similar to that described by Walder and colleagues in 2006,[21] but using a conjugate specific to *Peromyscus* antibodies.

Serum samples were screened for antibodies to *R. rickettsii* using an IFA protocol similar to that described in a National Center for Infectious Diseases-CDC publication,[22] though modified for use with rodent sera. Briefly, serum samples diluted 1:64 were applied to slides pre-coated with *R. rickettsii* and performed as per the *A. phagocytophilum* IFA above. Reactive samples were further titrated and samples with titres equal to or greater than 1:64 were considered positive for this study.

Lastly, serum samples were tested for evidence of infection with SNV using the protocol as previously des-

cribed,[7] but with Black Creek Canal virus used as the coating antigen in the initial screening ELISA.

Results

Tick and small mammal collection

A total of 5557 ticks were collected. Although other species are known to be present in the area (e.g., *I. angustus*),[23] *D. andersoni* was the only species collected and more than 99% of these ticks were adult males and females. No larvae and only 37 *D. andersoni* nymphs were collected, of which 17 were collected from the small mammals. A total of 276 deer mice were captured, and serum samples were collected from 219 animals. The deer mice collected were mainly adults (more than 80%), while the rest were juveniles. Other types of rodents were collected: 22 chipmunks, *Tamias townsendii*; 17 Great Basin pocket mice, *Perognathus parvus*; and 5 Montane voles, *Microtus montanus*. These rodents were examined for ticks and released without further processing as per animal care guidelines.

Pathogens detected in ticks

DNA of *B. burgdorferi s.s*, *A. phagocytophilum*, *B. henselae*, and *R. rickettsii* was not detected in any of the ticks tested by PCR. *R. peacockii* was present in all the pooled tick samples except for 2, and confirmed in 13 of 40 ticks tested; *R. rhipicephali* was present in 8 of the pooled samples, and confirmed in 5 of 40 ticks tested; an unknown rickettsial agent was detected in 3 of 40 ticks.

Antibodies in deer mouse sera

The deer mouse sera results were as follows: 0% (0/219) positive for *B. burgdorferi s.s.*, 0.46% (1/219) positive for *A. phagocytophilum*, and 12.3% (27/219) positive for *R. rickettsii*. The single positive for *A. phagocytophilum* suggests that the pathogen is present, but given the absence of *Ixodes* spp. collected, it is at a low prevalence in the environment. Similarly, the positive results for *R. rickettsii* confirm the low prevalence found among the ticks. However, because *R. rhipicephali* was present in the ticks at our study sites, but not *R. rickettsii*, the seropositivity may be due to cross-reactions in the IFA.[24] Finally, 4.1% of the deer mice had evidence of infection with SNV.

Conclusions

Low prevalence of pathogens confirmed

The prevalence of tick-borne pathogens was low in the study area, suggesting there is little risk that humans will be exposed to the pathogens and contract the diseases they can cause. The ticks tested showed no evidence of *B. burgdorferi s.s.*, *A. phagocytophilum*, *B. henselae*, or *R. rickettsii*. *R. peacockii* was present in nearly all tick pools tested, but this is not surprising as it is an endosymbiont of *D. andersoni*; notably, it is nonpathogenic. *R. rhipicephali* was found in 12.5% of ticks tested, but is of unknown pathogenicity. The results from the ticks were confirmed with the deer mouse sera tested: 0% *B. burgdorferi s.s.*, 0.4% *A. phagocytophilum*, and 12.3% possible positives for *R. rickettsii*. The evidence of positive *R. rickettsii* in the deer mouse sera is questionable, given that *R. rickettsii* was found at low titres (≥1:64) and this finding may be the result of a cross-reaction with *R. rhipicephali* or other rickettsial agents.[24] In addition, only *D. andersoni* ticks were collected, suggesting that pathogens transmitted by those ticks (i.e., *R. rickettsii*) are more common in the region and of more concern than those transmitted by *Ixodes* spp. (i.e., *B. burgdorferi s.s.*). Finally, 3.1% of the samples were positive for hantavirus, confirming the presence of the disease as previously found in human cases in BC.[8]

The absence of *B. burgdorferi s.s.* in our results, in both the ticks and the deer mice, confirms the rarity of *Ixodes* spp. in the region[25] and indicates that *D. andersoni* is not a competent vector for the pathogen, but is competent for rickettsial agents such as *R. rhipicephali*.[24] If *B. burgdorferi s.s.* was significantly present in the environment within *Ixodes* spp. ticks—which we did not collect (as further discussed below)—its presence would also have been indicated through the antibodies in the deer mouse sera tested. The prevalence of pathogens found is in accordance with reports of patient cases and passive surveys in the region.[24,26] Other BCCDC surveys of deer mice spurred by suspected human cases of Lyme disease have found that 3.66% (6/164) of deer mice caught tested positive for *B. burgdorferi s.s.* antibodies.[3] The presence of pathogens is due to the collection of deer mice in regions of BC that have populations of *Ixodes* spp. present rather than the *D. andersoni* collected in the South Okanagan. Indeed, recent predictive modeling of *I. pacificus* and *I. angustus* distributions based on habitat conditions in BC suggest they are mainly located along the coast of BC.[25]

Limitations of study

Clearly, this study had several limitations. First, a restricted area was examined—which is the trade-off of active surveys: though our sampling intensity allowed for an understanding of the prevalence of tick-borne pathogens in specific locations, other locations in the South Okanagan not sampled could harbor different pathogens or even *Ixodes* spp. Also, as previously mentioned, other areas of BC have had human cases of Lyme

disease and evidence of *Ixodes* spp., so the tick-borne pathogens in those areas are likely different. Our results can thus not be generalized to other parts of BC. Further, we were limited to examining only the main disease reservoir, deer mice, and did not examine other species we collected (e.g., pocket mice) because they were deemed at-risk species or we lacked the permits to collect them. Previous work has shown that other species can be competent disease reservoirs,[27] so other tick-borne pathogens may have been overlooked.

Importantly, our collection of only *D. andersoni* and no *Ixodes* spp. ticks was likely the result of our study design and our choice of sampling times and locations: although *Ixodes* spp. and nymph ticks are present in the region,[23] they are more active in cooler months and at earlier hours in the day. However, we selected our sampling times because they reflected the times and locations of human activity so that our results would be meaningful for evaluating the risk of human exposure to tick-borne pathogens. The South Okanagan is an arid and hot region, where the temperatures can be in excess of 30 °C. In these conditions, only the hardiest ticks will be active: *Ixodes* spp. are more sensitive to heat than *D. andersoni*;[28] similarly, being smaller, nymphs are more sensitive to heat and desiccation than adults. As well, *Ixodes* spp. and nymphs may prefer to quest for hosts on leaf litter rather than vegetation, thus making them unavailable for collection in this study.[28] Although we were not able to survey all the ticks in the region for pathogens (i.e., pathogens from *Ixodes* spp. and the nocturnal soft ticks), our results suggest that, during daily activities in the wilderness, humans are most likely to encounter *D. andersoni* ticks and their associated pathogens.

The prevalence of tick-borne pathogens was low in the study area, suggesting there is little risk that humans will be exposed to the pathogens and contract the diseases they can cause.

Recommendations

While prevalence of tick-borne pathogens is low and exposure to them is unlikely, the zoonoses they can cause remain a source of public concern.[4] Addressing tick-borne zoonoses in the South Okanagan may be primarily a communication challenge. Members of the public need to know both the true risk of contracting a disease and the personal protective practices that can effectively reduce the likelihood of contact with pathogens (e.g., tucking pants into socks; regular self-checks after being in tick habitat).[29] Risk communication is particularly important in the South Okanagan, given the region's expanding human population and economic growth. More people are recreating and working in the human-wildlife interface and many of these are new residents who are unfamiliar with local environmental hazards, such as ticks, and do not know how to protect themselves.[30]

In summary, the prevalence of tick-borne pathogens was found to be low in the South Okanagan, and there is little risk of contracting zoonoses when exposed to a tick in the region. Health professionals should thus focus on improving public understanding of tick-borne zoonoses, and on promoting personal protective practices. Future research should continue to actively examine other sites in the South Okanagan as well as other locations in BC that are expected to have populations of *Ixodes* spp.[25]

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Competing interests

None declared.

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