Exposure to infectious agents in dogs in remote coastal British Columbia: Possible sentinels of diseases in wildlife and humans

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Abstract

Ranked among the top threats to conservation worldwide, infectious disease is of particular concern for wild canids because domestic dogs (Canis familiaris) may serve as sources and reservoirs of infection. On British Columbia’s largely undeveloped but rapidly changing central and north coasts, little is known about diseases in wolves (Canis lupus) or other wildlife. However, several threats exist for transfer of diseases among unvaccinated dogs and wolves. To gain baseline data on infectious agents in this area, including those with zoonotic potential, we collected blood and stool samples from 107 dogs in 5 remote communities in May and September 2007. Serology revealed that the dogs had been exposed to canine parvovirus, canine distemper virus, Bordetella bronchiseptica, canine respiratory coronavirus, and Leptospira interrogans. No dogs showed evidence of exposure to Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis, or Cryptococcus gattii. Of 75 stool samples, 31 contained at least 1 parasitic infection, including Taeniid tapeworms, the nematodes Toxocara canis and Toxascaris leonina, and the protozoans Isospora sp., Giardia sp., Cryptosporidium sp., and Sarcocystis sp. This work provides a sound baseline for future monitoring of infectious agents that could affect dogs, sympatric wild canids, other wildlife, and humans.

Résumé

Classées mondialement parmi les trois premières menaces à la conservation, les maladies infectieuses sont une préoccupation particulière pour les canidés sauvages étant donné que les chiens domestiques (Canis familiaris) peuvent servir comme source et réservoir d’infection. Sur les côtes centrales et boréales de la Colombie-Britannique, largement peu développées mais rapidement changeantes, relativement peu de choses sont connues des maladies chez les loups (Canis lupus) ou autres animaux de la faune sauvage. Toutefois, plusieurs menaces existent pour le transfert de maladies parmi les chiens non-vaccinés et les loups. Afin d’acquérir des données de base sur les agents infectieux dans cette région, incluant ceux ayant un potentiel zootonique, nous avons amassé des échantillons de sang et de fèces de 107 chiens dans cette région, incluant ceux ayant un potentiel zoonotique, nous avons amassé des échantillons de sang et de fèces de 107 chiens dans 5 communautés éloignées au cours des mois de mai et septembre 2007. Les analyses sérologiques ont révélé que la population canine avait été exposée au parvovirus canin, au virus du distemper, à Bordetella bronchiseptica, au coronavirus respiratoire canin et à Leptospira interrogran. Aucun chien n’a montré d’évidence d’exposition à Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi, Dirofilaria immitis ou Cryptococcus gattii. Parmi les 75 échantillons de fèces, 31 contenaient au moins 1 infection parasitaire, incluant des ténias, les nématodes Toxocara canis et Toxascaris leonina et les protozoaires Isospora sp., Giardia sp., Cryptosporidium sp. et Sarcocystis sp. Cette étude fournit des données de base pour la surveillance future des agents infectieux qui pourraient affecter des canidés sauvages sympatriques, d’autres animaux de la faune et les humains.

(Traduit par Docteur Serge Messier)
**Introduction**

Emerging infectious disease is considered among the top threats to conservation worldwide (1). Although rarely the sole reason for declines and extinction of species, disease makes populations more susceptible to factors such as climate change and habitat degradation (2). The threats of disease to wildlife, combined with increasing anthropogenic drivers of changes in disease distribution (3), highlight the need for generating baseline data and for continued surveillance of disease dynamics, especially those considered to be emerging.

Monitoring disease may be particularly important in canids, which have a higher risk of undergoing disease-related population declines or extinction compared with most other mammals (4). Domestic dogs (*Canis familiaris*) are likely the most important reason for disproportionately high disease risks in wild canids and have been implicated in disease outbreaks in canids and other wildlife around the world (5). Transfer of diseases from wildlife to dogs also occurs (6) and some diseases may be transmitted from dogs to humans. Indeed, dogs can be sources of many diseases in humans, most notably rabies (5), but also macroparasitic diseases such as hydatidosis and toxocariasis (7).

Whereas dogs are potential sources of disease, they are useful sentinels of pathogens to which wildlife and humans may be exposed (8,9). Dogs are logistically and ethically easier to sample than wildlife or humans. Moreover, sampling can be coupled with vaccination campaigns that effectively reduce disease-related suffering in dogs, and risk of disease spill over to humans and wildlife (5). Recently, dogs have been used as sentinels of disease in species of conservation concern such as maned wolves (*Chrysocyon brachyurus*) (10) as well as other wildlife and humans (8).

Here, we examine dogs as possible sentinels of disease in wolves (*Canis lupus*), other wildlife, and humans in coastal British Columbia (BC). Communities there are located on islands or remote mainland areas, are surrounded by dense temperate rainforest, and are accessible only by ferry or small plane. Most dogs are kept as pets but are often allowed to run free. Many are exposed to wildlife and their infectious agents in a number of ways, including: pursuing interloping bears (*Ursus* spp.) away from villages, fighting with wolves at the periphery of villages, encountering feces or urine, and scavenging in the same open garbage dumps as these species (Bryan et al., personal observation, 2007). Notably, no regular veterinary services are available in any of the communities.

Wolves are the only wild canid in the area and inhabit mainland areas and islands. A recent study revealed that this coastal population is genetically and ecologically divergent from continental populations, and should be classified as an “evolutionarily significant unit” (ESU) that deserves special conservation status (11). Inference from this work suggests that these coastal wolves might also be isolated from pathogens or their variants common in other wolf populations. In addition, impending climate change (12) combined with rapid increases in economic activities (13) in coastal BC might lead to introduction of new pathogens or altered dynamics of existing pathogens. However, little or no baseline information exists on endemic or emerging diseases occurring in wildlife in the area against which future conditions can be compared. Likewise, there is no published information about zoonotic diseases that might be present.

**Materials and methods**

**Sample collection**

We offered 1- to 3-day dog health clinics in 5 remote communities in coastal BC in spring and fall 2007 attempting to include as many
Table II. Serology for select pathogens of potential importance to dogs, wolves, other wildlife or humans. Samples were collected from dogs in 5 remote communities on the central and north coasts of BC, Canada, in May and September, 2007

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Testa</th>
<th>Location of test</th>
<th>Dogs tested</th>
<th>Interpretationb,c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine distemper (CDV)</td>
<td>VN</td>
<td>Prairie Diagnostic Services, Saskatoon, Saskatchewan</td>
<td>56</td>
<td>&lt; 1:18 low, 1:18–1:1400 medium, &gt; 1:1400 high</td>
</tr>
<tr>
<td>Canine parvovirus (CPV)</td>
<td>VN</td>
<td>Prairie Diagnostic Services</td>
<td>102</td>
<td>&lt; 1:20 low, 1:20–1:1800 medium, &gt; 1:1800 high</td>
</tr>
<tr>
<td>Canine respiratory coronavirus (CRCoV)</td>
<td>ELISA</td>
<td>Diagnostic Virology Laboratory, WCVM, Saskatoon, Saskatchewan</td>
<td>102</td>
<td>&lt; 20 low, 20–80 medium, &gt; 80 high</td>
</tr>
<tr>
<td>Bordetella bronchiseptica</td>
<td>ELISA</td>
<td>Diagnostic Virology Laboratory, WCVM</td>
<td>102</td>
<td>&lt; 20 low, 20–80 medium, &gt; 80 high</td>
</tr>
<tr>
<td>Leptospira spp. (7 serovars)</td>
<td>MAT</td>
<td>Animal Health Laboratory, Guelph, Ontario</td>
<td>100 (44 pooled, 22 single)</td>
<td>&lt; 80 negative, 80–160 suspicious, &gt; 160 positive</td>
</tr>
<tr>
<td>Cryptococcus gattii</td>
<td>Antigen</td>
<td>IDEXX Laboratories, Langley, Westbrook, Maine</td>
<td>98 (44 pooled, 10 single)</td>
<td>≥ 1:2 positive</td>
</tr>
<tr>
<td>Vector-borne diseases</td>
<td>4Dx</td>
<td>Four pathogen test kit</td>
<td>88</td>
<td>scored as positive/negative</td>
</tr>
<tr>
<td>(Borrelia burgdorferi, Ehrlichia canis,)</td>
<td>Snap</td>
<td>IDEXX Laboratories, Westbrook, Maine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplasma phagocytophilum, Dirofilaria immitis</td>
<td>Test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a VN — virus neutralization, ELISA — enzyme-linked immunosorbent assay, MAT — microscopic agglutination test.
b For CPV, CDV, and C. gattii, titers are reported as the highest dilution of test sera that reacted with a reference antigen or antibody. For L. interrogans, reciprocal titers are reported. For B. bronchiseptica and CRCoV, results are reported as ELISA units.
c Titers to CPV, CDV, CRCoV, and B. bronchiseptica were classified as providing high, medium, or low evidence of exposure (natural or vaccine).

Serology

Sera were analyzed by standard enzyme-linked immunosorbent assay (ELISA), virus neutralization, hemagglutination inhibition, or snap tests for evidence of exposure to 10 pathogens: canine distemper virus (CDV), canine parvovirus (CPV-2), canine respiratory coronavirus (CRCoV), Bordetella bronchiseptica, Cryptococcus gattii, Borrelia burgdorferi, Ehrlichia canis, Anaplasma phagocytophilum, Dirofilaria immitis, and Leptospira interrogans serovars autumnalis, grippotyphosa, pomona, icterohaemorrhagiae, hardjo, bratislava, and canicola (Table II). To economize sample volume and costs for C. gattii and L. interrogans testing, sera were pooled from 2 dogs to create one sample. Separate tests were conducted on both individual samples for pooled samples that were found to be positive. Although pooling samples slightly increases the chances of false negatives (Type II error), it is unlikely that sera with high titers (indicative of recent exposure) were missed. Serology was mainly conducted by commercial laboratories (Table II), but ELISAs for Bordetella bronchiseptica and canine respiratory coronavirus (CRCoV) were carried out at the Western College of Veterinary Medicine (WCVM) virology laboratory as described in (14) and (15), respectively. The CRCoV ELISA procedure differed from that described by Priestnall et al (15) in that antibody concentrations are expressed as “Units” which are calculated as the percentage of the optical density of the wells with test sera, compared with the optical density in wells containing known positive controls (dog serum from a CRCoV respiratory outbreak in Calgary, Alberta).

Analysis of fecal samples

A sugar flotation procedure was used to detect parasite eggs, oocysts, and larvae in dog feces (16). In brief, 4 g of feces was...
mixed with 40 mL of water and strained through cheesecloth. As a wash step, a 4 mL aliquot was centrifuged with 8 mL of water for 10 min at 1500 rpm. The pellet was re-dissolved in Sheather’s sugar solution (specific gravity 1.26) and centrifuged again. Parasite eggs and oocysts were collected on a coverslip and transferred to a microscope slide for identification and counting. A commercial immunofluorescent assay (Cyst-a-glo; Waterborne, New Orleans, Louisiana, USA) was used to determine the presence or absence of Cryptosporidium oocysts and Giardia cysts. Statistical software (SPSS version 16.0; SPSS, Chicago, Illinois, USA) was used for all statistical tests with α = 0.05.

Results

Serology

The proportion of unvaccinated dogs with medium or high titers to CPV-2 ranged from 0 to 93% across communities and was 59% overall (Figure 2a). Unvaccinated dogs showed evidence of recent exposure to CPV-2 in Bella Bella (6 of 19) and Oweekeno (13 of 15), but not in the other communities. Among 53 dogs that had been previously vaccinated, 85% had high titers to parvovirus (Figure 2b).

At least one unvaccinated dog with a medium or high titer to CDV occurred in all communities except Ocean Falls (Figure 2c). However, the proportion of unvaccinated dogs with elevated titers was moderate (6 of 37), and only one dog showed evidence of recent exposure to CDV. Among 19 dogs vaccinated at least once in their lives, 47% had high titer to CDV (Figure 2d).

Across communities, there was a wide range of titers to B. bronchiseptica in dogs, with 65% of 102 dogs having elevated titers (Figure 2e). Although owners were not asked specifically about previous B. bronchiseptica vaccination, dogs reported to have had previous veterinary care had higher titers than those without previous veterinary care (Mann-Whitney U = 669, n1 = 46, n2 = 53, P < 0.01). Overall, 21% of 102 dogs had elevated titers to CRCoV (Figure 2f). Notably, dogs with elevated titers occurred only in Bella Bella where the dog population was ≥ 45 and Ocean Falls with a dog population of 6.

At least one dog had a positive or suspicious titer to Leptospira serovar autumnalis, grippotyphosa, or pomona in each community (Table III). Although we have no data on vaccination status of dogs to L. interrogans, 3 dogs with positive or suspicious titers had never received veterinary care, and routine vaccination of dogs against L. interrogans would be very rare in these communities. No dogs showed evidence of exposure to L. interrogans serovars icterohaemorrhagiae, hardjo, bratislava, or canicola or to C. gattii, B. burgdorferi, D. immitis, A. phagocytophilum, or E. canis.

Fecal analysis

At minimum, 7 parasitic genera were identified in 75 dog feces, including several with zoonotic potential (Table IV). Overall, 30% of feces were positive for one or more parasites. Of these, 91% contained evidence of single infections. Giardia cysts were detected most frequently, notably in samples from Hartley Bay (Table IV). Counts of parasite larval stages were generally low (< 60 eggs or oocysts/g feces), although 3 samples contained > 1000 eggs or oocysts/g feces. Pups < 6 mo old (3 of 8) had a higher proportion of parasitic infections than dogs > 6 mo old (17 of 67), and this difference was significant (Fisher’s test, P = 0.043). Overall, there were no relationships between parasitic infection and diet (Fisher’s test, P = 1.0), housing (X2 = 0.11, df = 1, P = 0.74), previous deworming (X2 = 0.44, df = 1, P = 0.51), or sex (X2 = 0.37, df = 1, P = 0.54).

Discussion

These results indicate that several infectious agents of significance to human, wildlife, and domestic animal health occur in dogs in...
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Table III. Number (%) of dogs with positive (≥ 320) and suspicious (80 to 160) titers to Leptospira serovars. Dogs with titers to ≥ 1 serovar were considered exposed to the serovar with the highest titer or, if titers were equal, dogs were considered exposed to multiple serovars. Sera were collected from dogs in 5 communities on the central and north coasts of British Columbia, Canada, in May and September 2007

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Community (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bella Bella</td>
</tr>
<tr>
<td></td>
<td>n = 43</td>
</tr>
<tr>
<td>Autumnalis</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Suspicious</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Suspicious</td>
</tr>
<tr>
<td>Pomona</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Suspicious</td>
</tr>
</tbody>
</table>

Table IV. Intestinal parasites detected in fecal samples from 75 dogs. Samples were collected from five remote communities on the central and north coasts of British Columbia, in May and September, 2007

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Number positive (%)</th>
<th>Community (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bella Bella</td>
<td>Hartley Bay</td>
</tr>
<tr>
<td>Cryptosporidiuma</td>
<td>1 (3)</td>
<td>—</td>
</tr>
<tr>
<td>Eimeriidaeb</td>
<td>—</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Giardiaa</td>
<td>4 (12)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Sarcocysts</td>
<td>2 (6)</td>
<td>—</td>
</tr>
<tr>
<td>Taeniidaeab</td>
<td>4 (12)</td>
<td>—</td>
</tr>
<tr>
<td>Toxascaris leonina</td>
<td>1 (3)</td>
<td>—</td>
</tr>
<tr>
<td>Toxocara canisa</td>
<td>—</td>
<td>1 (9)</td>
</tr>
</tbody>
</table>

a Parasites with zoonotic potential.
b Eggs/oocysts could only be identified to family.

remote communities of coastal BC. Evidence that dogs had been exposed to CPV-2 and, to a lesser extent CDV, is consistent with clinical cases of these infections reported in pups from Bella Bella (G. Moerkerken, personal communication, Big Heart Rescue Society, 2007), and highlights the continued need for vaccination to prevent morbidity and mortality of dogs and possibly also transmission to sympatric wildlife. Indeed, dogs have been implicated in transmission of CPV and CDV to wolves or other canids, in which the viruses can cause mortality or population declines (17–19).

Although we did not directly test the association, dog population density in each village might influence disease dynamics as there was strong evidence of recent exposure to CPV-2 in Bella Bella and Oweekeno, communities with the highest dog populations. This might be an indication that dog populations in the other communities are not large enough to maintain the infection. Alternatively, CPV-2 might be sporadic in all communities following introduction from an imported dog, a wildlife reservoir, a human with recent exposure to an infected dog, or an immunocompromised or otherwise healthy dog that is shedding modified live vaccine virus. In any case, periodic outbreaks in some or all communities could occur in dogs because titers induced by natural exposure (which would normally provide temporary protection against clinical disease) can wane between epizootics (18). This provides a good argument that regular vaccination of dogs in these communities is important.

Dogs with high titers to B. bronchiseptica occurred in all communities, so it is likely that B. bronchiseptica is endemic in dogs in coastal BC. In contrast, only dogs in Bella Bella and Ocean Falls showed titers consistent with exposure to CRCoV, a virus considered to be emerging in the canine infectious respiratory disease complex (20). This finding suggests that CRCoV and possibly other infectious agents may spread rapidly even to remote communities. Notably, Bella Bella is the largest community in the study area with the most commercial and tourist boat traffic, putting it at highest risk to be exposed to novel pathogens. Alone, B. bronchiseptica and CRCoV can cause mild clinical signs but in combination with other pathogens they can cause mild to severe disease (20). In addition to infecting dogs, both pathogens could affect wildlife (21,22).

Our findings suggest that L. interrogans likely occurs throughout the study area, although at low levels. Serovars we detected are among the most common found in healthy North American dogs in recent years (23,24). Evidence that dogs had been exposed to serovar pomona is particularly significant for wildlife in coastal BC, as dogs have been identified as risk factors in sea lion (Zalophus californianus) mortality from this serovar (25). The seroprevalence of L. interrogans was ≤ 10%, which is lower than that reported recently in healthy dogs in Washington (17%, n = 158) (23) and Michigan (24.9%, n = 1241). A possible reason for this difference is that dogs were sampled mainly in the spring, whereas seroprevalence increases in the fall (23). Alternatively, it is possible that dogs in coastal BC are exposed to fewer risk factors for L. interrogans exposure, including contact with livestock and peri-domestic wildlife reservoirs (26), both of which are absent in
northern and central coastal BC (26). Another possibility is that low L. interrogans exposure in dogs reflects a lower prevalence in the area. Currently, leptospirosis is rare in BC, although evidence from dogs suggests it is becoming more prevalent in Canada (24).

Although we found no evidence that dogs had been infected with C. gattii, an emerging pathogen on the northwest coast of North America (27), this finding does not necessarily reflect an absence of C. gattii in the area. Dogs may be asymptomatic carriers of C. gattii and do not always show detectable antigen titers following exposure (28). Our sample size may have been too small to detect C. gattii, especially if it exists at low prevalence. Indeed, sampling of environmental sites and multiple collections of both nasal swabs and serum from domestic animals would likely be required to detect C. gattii (29). Nonetheless, other studies suggest that domestic and wild animals are good sentinels of C. gattii prevalence (29,30), indicating that based on our findings there is currently low risk of C. gattii infection in humans and animals on the central and north coasts of BC.

No dogs showed evidence of exposure to the tick-borne zoonoses A. phagocytophilum, E. canis, or B. burgdorferi or to infection with the mosquito-borne nematode D. immitis. These results are consistent with case reports and other surveys for these pathogens in BC dogs (31–34) and provide further support that prevalence is currently low.

Egg counts and the number of parasite taxa detected in dog feces may have been underestimated in this study for methodological reasons. Freezing of samples prior to analysis, which we considered necessary for safety and practical reasons, may affect detection (35). Sugar flotation methods may also compromise recovery of some parasite taxa; however, this technique is appropriate for detection of many common dog parasites including important zoonoses (35,36). Therefore, it is likely that our findings reflect the most common gastrointestinal parasite infections to which dogs and potentially humans and wildlife in coastal BC have been exposed.

All parasites detected in dog feces have also been reported in wolves, and in general, likely have little effect on populations or individuals unless they are co-infections with more pathogenic agents (37). However, several of the parasites are of importance to human health, most notably Toxocara canis and tapeworms in the family Taeniidae, which include Echinococcus spp. (7). Some strains of Cryptosporidium and Giardia can infect both humans and dogs; transmission occurring through contact with infected feces or a common water source.

The proportion of feces with parasitic infections was low compared with studies of dogs in other remote communities, in part because we found no evidence of hookworm infection. Others using similar methods have reported high frequencies (up to 92%) of hookworm infections in dog feces (8,36). Additional factors relating to the low prevalence of parasites in coastal dog feces are husbandry practices, nutritional status, and diet of coastal dogs. Unlike sled dogs in northern communities, coastal dogs are not usually tied up, nor are they kept together in moderate to large groups. Their feces, therefore, are deposited over a wider area, reducing the chances of transmission of parasites with direct lifecycles. Moreover, only 23% of dogs were reported to have been fed wild game, which likely explains the low prevalence (6%) of parasites transmitted to dogs through consumption of raw intermediate prey. Although prevalence of parasites in dog feces was generally low, regular de-worming of dogs, especially puppies, would further reduce the chance that these parasites could be transmitted to humans.

In conclusion, this study provides a solid baseline of micro- and macroparasitic agents of domestic dogs on the north and central coasts of BC and forms a framework for future monitoring of canine and zoonotic diseases in the area. Monitoring will be important because climate change and habitat alteration are predicted to alter the distribution and prevalence of many diseases and may favor selection of more pathogenic strains (12,24,38,39). Regular veterinary presence in remote communities in coastal BC and elsewhere will be essential in long-term disease monitoring and management.

Acknowledgments

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