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***Cryptococcus gattii* in Wildlife of Vancouver Island, British Columbia, Canada**

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ABSTRACT: Although *Cryptococcus gattii* has emerged as an important pathogen of humans and domestic animals on Vancouver Island, Canada since 1999; its distribution in regional wildlife species is largely unknown. Opportunistic sampling methods were employed to obtain nasal swabs for fungal culture from wild mammal species residing within the coastal Douglas fir biogeoclimatic zone on the southeast coast of the island. Samples were collected from 91 animals representing 14 species. *Cryptococcus gattii* was isolated from the nasal swabs of two eastern gray squirrels (*Sciurus carolinensis*) trapped in Duncan, British Columbia. The relative proportion of nasal colonization in wild mammal species is consistent with findings in domestic animals, suggesting that animals may be good indicators of environmental organisms.

Key words: Canada, *Cryptococcus gattii*, eastern gray squirrel, *Sciurus carolinensis*, wildlife.

Since 1999 *Cryptococcus gattii*, a species now distinct from *Cryptococcus neoformans* (Kwon-Chung et al., 2002), has emerged as an important pathogen of humans and animals in southwestern British Columbia (Stephen et al., 2002; Hoang et al., 2004; Kidd et al., 2004; Lester et al., 2004). Previously only *C. neoformans* had been isolated from animals or humans in Canada and *C. gattii* was thought to be restricted to the tropics and subtropics (Kwon-Chung et al., 1984; Sorrell, 2001). Clinical illness associated with *Cryptococcus* has been identified in humans and numerous domestic animal species in British Columbia including cats, dogs, ferrets, llamas, domestic birds, and a horse. Cases are clustered on the east coast of Vancouver Island largely within the coastal Douglas fir (CDF) biogeoclimatic zone (Fig. 1) (Duncan et al., 2005).

Cryptococcus gattii has been routinely isolated from soil, air, and vegetation within the CDF zone since 2001 (Bartlett, 2003).

Since 2000, 11 Dall's porpoises (*Phocoenoides dalli*) found washed up on the southern coast of mainland British Columbia or Vancouver Island have been diagnosed with cryptococcosis on post-mortem examination; all isolates have been *C. gattii* (Stephen et al., 2002; Raverty, unpubl.). Given the airborne nature of the organism it may be assumed that wildlife species and feral animals are also exposed, but infection has been largely unnoticed. Asymptomatic carriage of *C. gattii* has been recognized in companion-animal species of British Columbia, presumably as a result of contact with airborne infectious material (Bartlett, 2003; Duncan et al., 2005). Environmental exposure and asymptomatic colonization of the respiratory tract has been proposed to be much more common than clinical disease (Malik et al., 1997; Connolly et al., 1999); however, variables influencing the initiation of infection remain unclear. The prevalence of the organism in other British Columbia wildlife is unknown. The objective of this study was to identify terrestrial mammalian wildlife species that have been exposed to or infected with *C. gattii* on Vancouver Island, British Columbia.

Between February and August 2004, samples were collected for fungal culture. Sources of live and dead animals included wildlife rehabilitation facilities, veterinarians, biologists, and registered trappers. Any mammalian species live-trapped or killed and known to reside in the CDF zone on Vancouver Island was eligible for

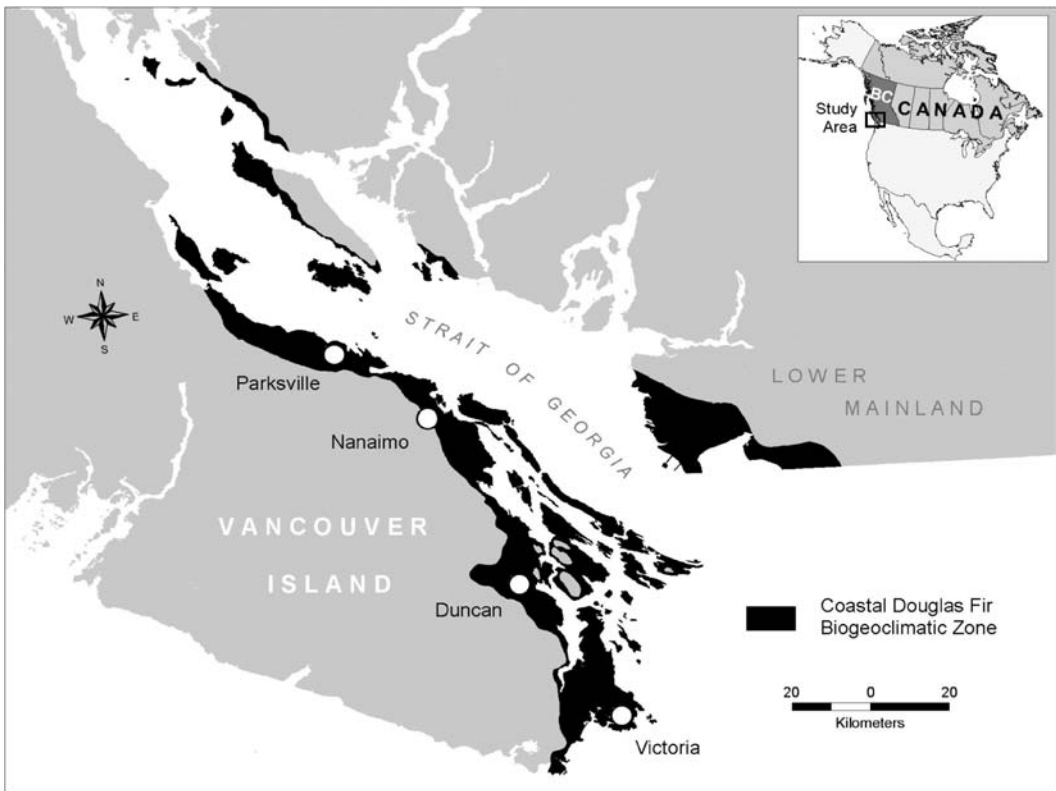


FIGURE 1. Map of coastal Douglas fir biogeoclimatic zone on Vancouver Island, British Columbia.

inclusion in the study. Material collected included a deep swab of the nasal mucosa using a Starplex StarSwab II (Starplex Scientific, Etobicoke, Ontario, Canada) and lung tissue where possible.

Culture swabs were plated onto Bird Seed Agar and incubated at 30 C for 48 hr. Plates were checked for growth daily for 10 days before being regarded as negative. Colonies conforming to cryptococcal morphology were identified and serotyped using agglutinating antibodies (Crypto-check, Iatron Laboratories, Tokyo, Japan). Lung tissue was splayed on a sterile surface and dissected to allow access to the interior surface using a scalpel blade sterilized by alcohol dip and flaming. Internal and external surfaces were swabbed using a cotton-tipped applicator (Puritan, Fisher Scientific Ltd., Ottawa, Ontario, Canada). The applicator was rolled across a differential agar (Bird

Seed Agar) and a rich nutrient agar (Saboraud Dextrose Agar, BBL, Becton Dickinson Diagnostics, Sparks, Maryland, USA). Agar plates were incubated and checked for growth as above.

Nasal swabs were collected from 91 individuals representing 14 species, including 19 living harbor seals (*Phoca vitulina*), and 72 postmortem samples. Postmortem material was collected from cottontail rabbits (*Sylvilagus* spp., $n=20$), black-tailed deer (*Odocoileus hemionus*, $n=15$), eastern gray squirrels (*Sciurus carolinensis*, $n=16$), North American river otters (*Lutra canadensis*, $n=6$), harbor seals (*Phoca vitulina*, $n=3$), red squirrels (*Tamiasciurus hudsonicus*, $n=2$), deer mice (*Peromyscus maniculatus*, $n=2$), raccoons (*Procyon lotor*, $n=2$), and a single black bear (*Ursus americanus*), one domestic rabbit (*Oryctolagus cuniculus*), one marten (*Martes americana*), one black rat

(*Rattus rattus*), one Vancouver marmot (*Marmota vancouverensis*), and a gray wolf (*Canis lupus*). Lung tissue was available for culture from 68 animals representing 13 species. *Cryptococcus gattii* was isolated from the nasal swabs of two eastern gray squirrels trapped at the same location in Duncan, a city in British Columbia. Lung tissue was available from one of these squirrels and fungal culture of the tissue yielded no *Cryptococcus* spp.

Recent investigation into subclinical infection in companion animals identified 4.3% of cats and 1.1% of dogs residing within the CDF zone of Vancouver Island as having *C. gattii* in their nasal cavities (Duncan et al., 2005). The organism was present on 1.5% of nasal swabs collected from horses within the same region, including one horse from property adjacent to the area inhabited by the positive gray squirrels (Duncan, unpublished). *Cryptococcus gattii* was isolated from 2% of wild mammals tested in this study; a finding similar to that of companion animals and horses.

The presence of *C. gattii* in nasal passages is likely the result of environmental exposure. Lung tissue was available from one of the positive squirrels but the organism was not isolated. Without isolation of the organism from a normally sterile site, histological examination of tissue from the nasal cavity, or serum upon which to run a cryptococcal antigen test, it is difficult to make inferences about the status of the organism within the respiratory tract. Both gray squirrels were trapped and humanely euthanized on private property because the species is considered an invasive alien in this area; both were presumed to be healthy. On gross postmortem examination no lesions suggestive of clinical cryptococcosis or other diseases were observed. Failure to identify pathology or systemic infection in an individual suggests nasal colonization resulting from environmental exposure and not clinical infection.

Both positive animals were from the

same geographic location. The city of Duncan is central in the region in which clinical cryptococcosis cases have been reported (Duncan et al., 2003). A cross-sectional study in dogs and cats identified Duncan to have a higher proportion of colonized or subclinically infected animals (Duncan et al., 2005) and investigation into nasal colonization in horses revealed a similar distribution (Duncan, unpubl.). Because the two squirrels were the only mammal species submitted from this region, it is difficult to draw conclusions regarding the relative role of location compared to species; however, concurrent environmental sampling revealed the second highest airborne concentrations of organism to be in the Duncan area (Bartlett, unpubl.). In Australia it has been proposed that heavily colonized or infected koalas may contaminate previously culture-negative vegetation (Krockenberger et al., 2002). Eastern gray squirrels were introduced to Vancouver Island in the Victoria area; their northward expansion may facilitate transmission of *C. gattii* to regions currently free of the organism.

Over 74% of animals sampled in this study were collected from wildlife rehabilitation facilities. Although younger species are often over represented, this sampling technique is an inexpensive way to collect samples from multiple species in a short period of time. It is, however, important to consider the effect of a young sample population on the results; younger animals will have had a shorter duration of exposure and, given the long incubation period of *Cryptococcus* spp., younger animals may not manifest signs of clinical disease at the time of sampling.

Failure to culture the organism from the nasal cavity of any living animals may be influenced by the inability to sample with the same intensity used postmortem; swabs collected from living animals were more superficial than those collected postmortem. *Cryptococcus gattii* has been repeatedly isolated from the nasal cavity of living wild and domestic animals; however, there

are conflicting results concerning the agreement between deep and superficial nasal swabs (Duncan et al., 2005; Connolly et al., 1999). Standardization of sampling techniques is important in cross-species studies; given the lack of data for agreement between the sampling techniques in wild mammals, it should be noted that samples collected from living animals differed from those samples collected post-mortem. The sensitivity of nasal culture in animals is unknown and it is possible that the organism is missed during a nasal swab, or that infection is present in a site other than the nasal passage. Antigenemia, but failure to isolate the organism from the nasal cavity, has been reported in asymptomatic animals (Duncan et al., 2005) and clinical cases (Duncan, unpubl.). The methodology used in this study may not be sufficient to identify exposure or infection in all animal samples.

The recent emergence of *C. gattii* in western Canada dictates the need to identify the population at risk; including wild animals. Wildlife, by virtue of living outdoors all the time and therefore being constantly exposed to airborne organisms, may be a better 'environmental indicator' of human risk than are companion animals. The collection of nasal swabs from wildlife species residing within endemic regions of British Columbia may be an inexpensive way to survey the environment and quantify environmental load. The impact of environmental *Cryptococcus* spp. on wildlife of British Columbia remains largely unknown; further investigation is warranted.

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