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Histoplasmosis, Blastomycosis & Cryptococcosis in Canada

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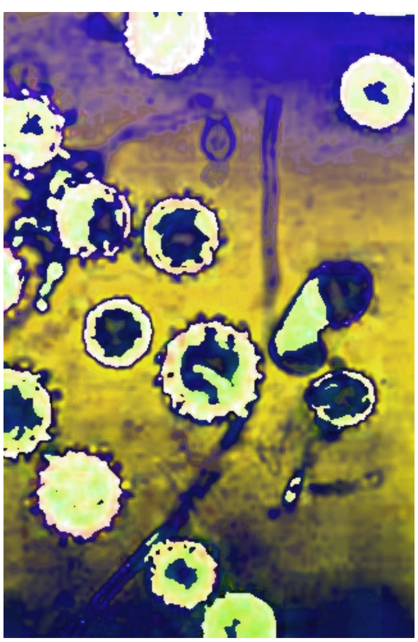

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EXECUTIVE SUMMARY

Canada includes defined regions that are natural habitat areas for three fungal species that can cause serious disease in otherwise-healthy, immunologically normal people. These fungi are *Blastomyces dermatitidis* (referred to in medical slang as 'blasto'), causal agent of the disease blastomycosis, *Histoplasma capsulatum* ('histo'), causal agent of histoplasmosis, and *Cryptococcus gattii* ('tropical crypto'), causal agent of tropical-type cryptococcosis.

Two of these fungi, *Blastomyces dermatitidis* and *Histoplasma capsulatum* are closely related but have different habitats. *Blastomyces dermatitidis* occurs in soil or plant debris mainly near the banks of streams and lakes; geographically, it is most common in parts of northern Ontario, Quebec and New Brunswick, as well as in a band of mostly forested area stretching across central Manitoba and into Saskatchewan. *Histoplasma capsulatum* mainly occurs in accumulations of bat, pigeon or starling guano in a zone including most of southern Ontario, the lower Ottawa River valley, the lower St. Lawrence River valley (excepting the north shore from an undefined point east of Montreal) and northwestern New Brunswick. It has also been recorded once from the Edmonton area. Both *B. dermatitidis* and *H. capsulatum* infect people (and animals) primarily via inhalation and may cause pneumonia-like diseases or potentially fatal widespread infection throughout the body (disseminated infection).

Cryptococcus gattii is a pathogenic yeast fungus that is not closely related to the other species considered here. It is mostly associated with Australia, tropical South America and the Mediterranean, and was unknown from Canadian sources until 2001, when a major outbreak occurred stemming from heavy environmental growth in forested parkland areas of eastern Vancouver Island. The persons affected were mainly park visitors who experienced a pneumonia-like disease or a disease affecting the brain, cryptococcal meningitis. There were a number of fatalities.

All three fungal pathogens can be successfully treated if diagnosed correctly, except in uncommon cases of massive infection. Prevention of infection, however, is a topic that has received relatively little attention. Partly this is because the habitat of the most common pathogen, *B. dermatitidis*, is very poorly understood and precise environmental sources of inoculum have very rarely been located. Similarly, the apparent invasion of a Canadian region by *C. gattii* resulted in an unprecedented pattern of environmental distribution for this species. It is usually associated with eucalypts and other tropical and subtropical trees, none of which occur in the affected area of British Columbia. The primary task of the present review was to generate workable information that home occupants and owners could use to reduce the probability of infection by these fungi in areas where they are known to occur.

In the case of *B. dermatitidis*, the buildup of high-nitrogen organic wastes such as canine excreta should be prevented in vulnerable damp, riparian (streamside or lakeside) areas, and exposure to dust from such areas should be prevented. In these riparian sites, outbuildings and crawlspaces that may have become contaminated with rodent or other small animal excreta should be considered potentially hazardous and cleaning or structural work should be done in an appropriate manner. Property buyers are recommended to inquire about history of blastomycosis or diseases suggestive of blastomycosis (including infections of dogs, a sentinel species for this disease) when purchasing new property in affected regions.

In *H. capsulatum* areas, interior building spaces that have heavy accumulations of bat or bird guano should be treated as highly hazardous. Buildings should be maintained to prevent colonization by guano-depositing animals. Guano accumulation in chicken facilities and around domesticated pigeon facilities should be prevented in endemic areas, and guano removal should be performed in a way that avoids dust formation.

In *C. gattii* areas, the generation of dust in activities involving the soil or wood should be minimized. Cultivated soils may be suitably limed or mulched to reduce the likelihood of *C. gattii* proliferation.

RÉSUMÉ

Le Canada compte des régions définies qui servent d'habitats naturels à trois espèces fongiques risquant d'entraîner de graves maladies chez les gens en santé qui présentent un système immunitaire normal. Il s'agit, en l'occurrence, de la *Blastomyces dermatitidis* (connue dans le jargon médical sous le nom de « blasto »), agent causant la blastomycose, *Histoplasma capsulatum* («histo »), agent causant l'histoplasmose, et *Cryptococcus gattii* (« crypto tropical »), agent causant la cryptococcose tropicale.

Deux de ces espèces fongiques, *Blastomyces dermatitidis* et *Histoplasma capsulatum,* ont des liens étroits, mais vivent dans des habitats différents. La *Blastomyces dermatitidis* se manifeste dans le sol ou dans les résidus de plantes se trouvant surtout à proximité de la rive de cours d'eau et de lacs; sur le plan géographique, on la retrouve le plus souvent dans le nord de l'Ontario, au Québec et au Nouveau-Brunswick, ainsi que dans une bande de zones surtout boisées s'étendant du centre du Manitoba jusqu'en Saskatchewan. La *Histoplasma capsulatum* se trouve principalement dans les accumulations de guano de chauve-souris, de pigeons et d'étourneaux dans une zone regroupant la majeure partie du sud de l'Ontario, les basses terres de la vallée de la rivière des Outaouais, la vallée du Bas St-Laurent (à l'exception de la rive nord depuis un point non défini à l'est de Montréal) et le nord-ouest du Nouveau-Brunswick. Elle a également été décelée à une occasion dans la région d'Edmonton. La *B. dermatitidis* et la *H. capsulatum* infectent les gens (et les animaux) par inhalation surtout et risquent d'entraîner des maladies semblables à la pneumonie ou d'infecter mortellement tout le corps (infection dispersée).

La *Cryptococcus gattii* est une espèce fongique pathogène (levure) qui n'a pas de lien étroit avec les autres espèces envisagées ici. Elle est le plus souvent associée à l'Australie, à l'Amérique du Sud tropicale et à la Méditerranée; elle était d'ailleurs inconnue au Canada jusqu'en 2001, alors qu'une importante épidémie a surgi, à la suite d'une forte croissance dans les parcs-forêts de l'est de l'île de Vancouver. Les personnes infectées étaient en majorité des visiteurs qui avaient contracté une maladie s'apparentant à la pneumonie ou la méningite cryptococoque, maladie affectant le cerveau. Des décès lui avaient été imputables.

Tous les agents pathogènes fongiques peuvent être traités avec succès si le bon diagnostic est posé, sauf dans les cas exceptionnels d'infection massive. La prévention de l'infection, par contre, est un sujet qui a retenu peu l'attention, sans doute, en partie à cause que l'habitat de l'agent le plus commun, en l'occurrence *B. dermatitidis*, est très mal compris et que des sources environnementales précises d'inoculum ont très rarement été situées. De même, l'invasion manifeste d'une région canadienne par la *C. gattii* a donné lieu à une modèle sans précédent de distribution de cette espèce. Elle est d'habitude associée aux eucalyptes ainsi qu'à d'autres arbres tropicaux ou subtropicaux, dont aucuns n'existent dans la région touchée de la Colombie-Britannique.

Quant à la *B. dermatitidis*, on doit prévenir l'accumulation de déchets organiques à forte teneur en azote, telles les déjections canines, dans les zones humides riveraines

vulnérables (bords de lacs ou de cours d'eau), de même que l'exposition à la poussière de cette provenance. Dans ces zones riveraines, les annexes et les vides sanitaires qui ont pu être contaminés par les excréments de rongeurs ou d'autres petits animaux doivent être considérés comme à risques; il faut donc procéder à leur nettoyage ou envisager des travaux structuraux de façon appropriée. Les acheteurs fonciers sont invités à se renseigner sur la blastomycose ou les maladies évoquant la blastomycose (y compris les infections causées par les chiens, espèce sentinelle de cette maladie) au moment d'acheter une propriété dans les régions touchées.

Dans les régions où se manifeste la *H. capsulatum*, l'intérieur des bâtiments qui comportent de fortes accumulations de guano de chauves-souris ou d'oiseaux doit être considéré comme hautement dangereux. L'entretien des bâtiments doit être exécuté de façon à prévenir l'établissement de colonies par le guano d'animaux. On doit prévenir l'accumulation de guano dans les installations avicoles et autour d'installations pour pigeons domestiqués dans les zones endémiques et enlever le guano de manière à ne pas produire de poussière.

Dans les zones touchées par la *C. gattii*, la formation de poussière lors d'activités mettant en cause le sol ou le bois doit être réduite au minimum. Ainsi, les sols cultivés pourront être couverts de chaux ou de paillis pour réduire les possibilités de prolifération de l'espèce *C. gattii.*

Background

Many bacteria and viruses have gained the ability to cause serious systemic diseases such as respiratory infection and brain infection in immunologically normal humans, but very few fungi manifest parallel abilities. Of the fewer than ten fungal species that pose this type of danger, four are established as resident in at least some parts of Canada: *Blastomyces dermatitidis, Histoplasma capsulatum, Cryptococcus neoformans* and *Cryptococcus gattii.* Corresponding disease names are blastomycosis, histoplasmosis, cosmopolitan cryptococcosis, and tropical cryptococcosis. These fungal infections differ from most bacterial and viral infections in that they are not acquired from infected humans or animals, but rather, directly from the environment. The causative fungi all have a primary habitat that includes materials such as soil, guano and trees. People in good health engaging in work and recreation are particularly likely to be infected. In all cases, animals are also likely to be affected. Cases are mainly reported in small domesticated animals such as dogs and cats, but also occur in farm animals and wild animals.

The present review outlines the principal clinical dangers posed by three of these organisms, *Blastomyces dermatitidis, Histoplasma capsulatum,* and *Cryptococcus gattii.* It gives notes on their ecology, epidemiology, and environmental detection. Prevention of exposure is stressed, especially in reference to residential housing and other residentially associated sites.

1. Blastomycosis

Overview

Blastomycosis is a potentially very serious disease that typically begins with a characteristically subtle pneumonia-like infection that may progress, after circa 1 – 6 months, to a disseminated phase that causes lesions to form in capillary beds throughout the body, most notably the skin, internal organs, central nervous system and bone marrow. The causal organism is a fungus living in soil and wet, decaying wood, often in an area close to a waterway such as a lake, river or stream. Indoor growth may also occur, for example, in accumulated debris in damp sheds or shacks. The fungus is endemic to parts of eastern North America, particularly boreal northern Ontario, southeastern Manitoba, Quebec south of the St. Lawrence River, parts of the U.S. Appalachian mountains and interconnected eastern mountain chains, the west bank of Lake Michigan, the state of Wisconsin, and the entire Mississippi Valley including the valleys of some major tributaries such as the Ohio River. In addition, it occurs rarely in Africa both north and south of the Sahara desert, as well as in the Arabian Peninsula and the Indian subcontinent. Though it has never been directly observed growing in nature, it is thought to grow there as a cottony white mould, similar to the growth seen in artificial culture at 25 C. In an infected human or animal, however, it converts in growth form and becomes a large-celled budding yeast. Blastomycosis is generally readily treatable with systemic antifungal drugs once it is correctly diagnosed; however, delayed diagnosis is very common except in highly endemic areas.

Causal agent

The causal agent of blastomycosis is an ascomycetous fungus, *Blastomyces dermatitidis.* In accord with the conventions of fungal nomenclature, this same organism also has a second correct name for its sexual state, *Ajellomyces dermatitidis.* Along with two other important human-pathogenic fungi, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis,* this species belongs to a newly recognized fungal family, the *Ajellomycetaceae* (Untereiner *et al*. 2004). The three principal pathogens in this family are all grouped physiologically as “dimorphic fungi”: fungi that switch from a mould-like (filamentous) growth form in the natural habitat to a yeast-like growth form in the warm-blooded animal host. *Blastomyces dermatitidis* itself is a sexual organism, occurring in nature as both a + mating type and a – mating type. This is epidemiologically important for two reasons: firstly, it implies that the organism will be genetically variable, potentially leading to variations in disease severity, treatment response and habitat preference; secondly, it implies that a suitable, stable habitat must exist for the complex process of sexual reproduction to take place. This habitat is as yet unknown. In its asexual form, the fungus grows as a typical colonial microfungus, comparable to *Penicillium* or *Rhizopus* mould forms commonly seen on mouldy bread. It forms a network of thread-like mycelium that penetrates the substratum on which it grows, and then after 3 – 5 days of growth begins to reproduce asexually with small (2 – 10 µm) conidia (asexual spores). These conidia are probably the main infectious particles produced by the fungus. They form on individual short stalks and readily become airborne when the colony is disturbed; their size places them well within the respirable range for particulates (Lippman 2001), meaning that they can deposit deeply in

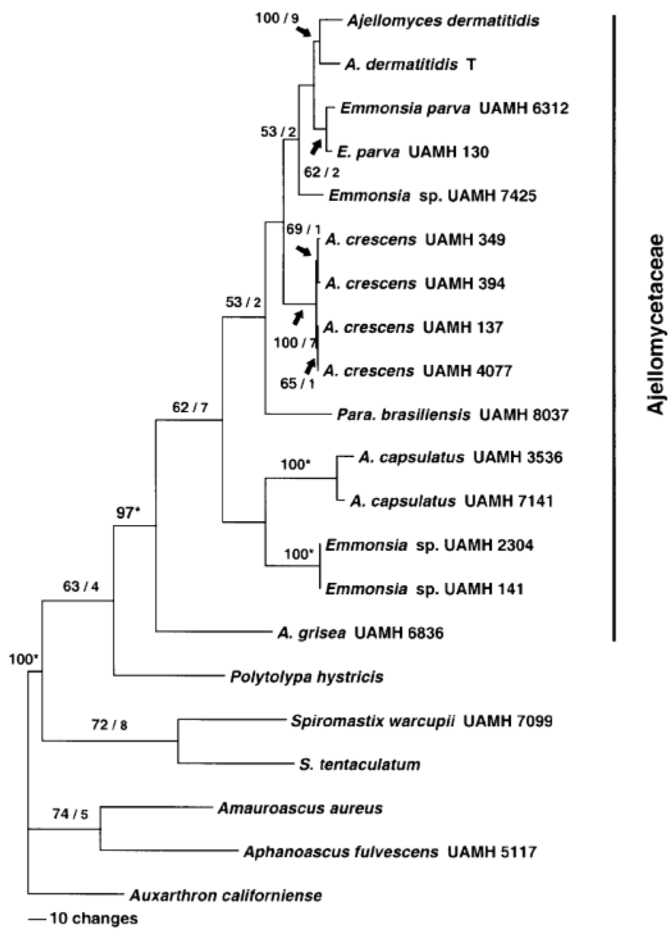

Figure 1. Phylogenetic relationships within the Ajellomycetaceae inferred from the combined dataset (ITS and partial LSU rDNA sequences). This is the single MPT (L 5 803) generated from an heuristic analysis of 1149 bp for 21 taxa (CI 5 0.654, RI 5 0.632). Bootstrap values greater than 50% calculated from 1000 replicates are given either above branches or to left of the diagonal lines adjacent to branches. Bremer support is shown either below the branches or the diagonal lines adjacent to branches. An asterisk indicates clades retained in trees 10 steps longer than the MPT. A ''T'' designates strains derived from the type specimen. Outgroup taxon is Auxarthron californiense. From Untereiner et al (2004)

the lungs when inhaled. Sexual reproduction by the fungus requires the meeting of colonies of + and – mating type, probably a relatively rare event, and results in the production of small ascomata (sexual fruiting bodies) 200 – 350 µm, looking, to the naked eye, similar to a woollen fuzz ball, and in microscopic view consisting of a layer of spiralling, springy guard hairs surrounding a fertile core in which groups of 8 ascospores (sexual spores) are produced in small round reproductive sacs (asci). The ascospores, at

1.5 – 2 µm, are among the smallest reproductive particles produced by fungi, and are within the respirable size range (Lippman 2001).

The budding yeast cells seen in infected tissues and bodily fluids are generally relatively large (ca. 8 – 15 µm) and characteristically bud through a broad base or neck, making them highly recognizable to the pathologist. A small ("nanic") form is rarely seen with cells under 6 µm.

Geographic distribution and geographic variants

One of the unexplained regularities of nature is that there are several fungi of different phylogenetic ancestry that show a similar pattern of existence: dimorphism (conversion from a filamentous form in the environment to a yeast form in warm-blooded host tissues), virulent pathogenesis (ability to cause a significant infection in an animal host that is otherwise in good health), pulmonary infectivity (infection mainly via the lungs) and sharply delimited endemism (occurrence in only a limited geographic range.). *Blastomyces dermatitidis* is one of these fungi; the others are *H. capsulatum, P. brasiliensis, Coccidioides immitis, C. posadasii* and *Penicillium marneffei*.

The geographic range of *B. dermatitidis* is largely focused around the waterways of the St. Lawrence and Mississippi River systems of North America. There is a widely distributed and much republished, partially erroneous map (e.g., see Kwon-Chung & Bennett, 1992) that shows the U.S. portion of this range accurately, inclusive of occurrence in Minnesota, Wisconsin, Ohio, Kentucky, Arkansas, Tennessee, North and

South Carolina, the Virginias, Mississippi, Louisiana, and a few regions of states adjacent to those named.

The Canadian range of *B. dermatitidis* is the inaccurate portion of the cited map: the map shows an abundance of blastomycosis in broad areas north and south of the St. Lawrence River in Quebec, as well as high endemicity along the north shore of Lake Erie and the low endemicity in southeastern corner of Manitoba. In fact, though the Quebec distribution shown in the map is reasonably accurate, the rest of Canada is strongly misrepresented. *Blastomyces dermatitidis* is absent or nearly so from the Lake Erie area, but occurs sporadically on the north shore of Lake Ontario, including metropolitan Toronto (Lester *et al.* 2000), and, most notably, has areas of high endemicity throughout northern Ontario (Kane *et al.* 1983). Remarkably high incidence is noted for some parts of the Kenora area and climatologically similar areas of northwestern Ontario (Dwight *et al*. 2000). To the west, the range of endemic blastomycosis extends across southern Manitoba and into adjacent Saskatchewan (Vallabh *et al.* 1988). A few cases have been reported from north central Alberta, e.g., the Edmonton area, though in these cases an atypical genetic group of the fungus may be involved (Sekhon 1982).

In the rest of the world, *B. dermatitidis* occurs at low levels in various parts of Africa, from Algeria to South Africa, as well as in and near the Arabian Peninsula. The African isolates are divided into two biologically different antigen groups: isolates from north of the Sahara are similar to North American isolates in having A and K antigens, while southern African isolates lack the A antigen (Frean *et al.* 1988). Isolates from the middle east possess both antigens. The sub-Saharan African isolates differ in the laboratory from other isolates by being exceedingly difficult to convert to the yeast phase, and they also show some enzymatic distinctions (Summerbell *et al.* 1990).

Spectrum of disease

Blastomycosis manifests as a pulmonary primary infection in ca. 70% of cases (Kwon-Chung & Bennett 1992). The onset is relatively slow and symptomatology is suggestive of pneumonia, often leading to treatment with antibacterials. Occasionally, if a lesion is seen on X-ray in a cigarette smoker, the disease may be misdiagnosed as carcinoma, leading to swift excision of the pulmonary lobe involved (Summerbell, unpublished data). Upper lung lobes are involved somewhat more frequently than lower lobes (Kwon-Chung & Bennett 1992). If untreated, many cases progress over a period of months to years to become disseminated blastomycosis. In these cases, the large *Blastomyces* yeast cells translocate from the lungs and are trapped in capillary beds elsewhere in the body, where they cause lesions. The skin is the most common organ affected, being the site of lesions in circa 60% of cases (Kwon-Chung & Bennett 1992). The signature image of blastomycosis in textbooks is the indolent, verrucous or ulcerated dermal lesion seen in disseminated disease. Osteomyelitis is also common (12 – 60% of cases). Other recurring sites of dissemination are the genitourinary tract (kidney, prostate, epididymis; collectively ca. 25% of cases) and the brain (3 – 10% of cases). These figures are from Kwon-Chung and Bennett (1992); somewhat differing figures are given in other sources.

An uncommon but very dangerous type of primary blastomycosis manifests as acute respiratory distress syndrome (ARDS); for example, this was seen in 9 of 72 blastomycosis cases studied in northeast Tennessee (Vasquez *et al.* 1998). Such cases may follow massive exposure, e.g., during brush clearing operations (unpublished data). The fatality rate in the ARDS cases in the Tennessee study was 89%, while in non-ARDS cases of pulmonary blastomycosis, the fatality rate was 10%.

Blastomycosis also affects an indefinitely broad range of mammalian hosts, and dogs in particular are a highly vulnerable sentinel species (Kwon-Chung & Bennett. 1992). They generally suffer a disease that begins with acute respiratory symptoms and rapidly progresses to death. Cats are the animals next most frequently detected as infected (Blondin *et al.* 2007).

Ecology

Blastomyces dermatitidis is one of the most ecologically mysterious organisms causing human and animal disease. Prediction of disease risk and prevention of disease are both made extraordinarily difficult by our very poor understanding of where and how this organism normally grows in nature.

Despite decades of attempts at isolating organisms from epidemiological foci, *B. dermatitidis* has only been isolated from the environment 21 times (Burgess *et al.* 2006). Most of these isolations have been based on the arduous isolation techniques outlined by Ajello and Weeks (1983): suspension of soil or other environmental materials in aqueous medium with antibacterial antibiotics, and injection of mice with these materials, followed by sacrifice of the animals when they appear ill or at the end of six weeks. The internal organs of the mice are then checked microscopically for evidence of blastomycosis. Needless to say, the cost and complexity of performing such studies is imposing, especially as the ethical clearance procedures for work involving animals become ever more involved. More direct and economical mycological techniques for environmental isolation, such as dilution plating, have never yielded positive results for *Blastomyces* growth. Since *B. dermatitidis* will grow readily from clinical samples on common laboratory media, the lack of success in isolating it from environmental materials is generally ascribed to the inhibitory effects of co-occurring common moulds and antibiotic-resistant bacteria.

In just one experiment (Baumgardner & Paretsky 1999), a single positive *B. dermatitidis* culture was gained via use of a novel enrichment broth technique.

Recently, in an important breakthrough, a specific PCR technique was developed that was able to detect *B. dermatitidis* in three environmental samples from a dog kennel that had been experiencing problems with blastomycosis (Burgess *et al.* 2006).

What has been learned from direct isolation and recent PCR studies is that *B. dermatitidis* tends to be associated with soils and wood debris in areas "characterized by an acidic pH, high organic content (due to rotting or decayed wood or vegetation and animal or bird droppings), abundant moisture, and proximity to waterways" (Baumgardner *et al.* 1992). Recent PCR detections, for example, concerned a Kentucky dog kennel where 35 of 100 dogs had contracted blastomycosis (Burgess *et al.* 2006). Previous isolations have been from comparable sites such as soil and wood debris from an abandoned Wisconsin beaver dam (Klein *et al.* 1986), and woody materials from a Wisconsin woodpile (Baumgardner & Paretsky 1999). Isolation of *B. dermatitidis* was also accomplished from an earthen floor indoors on one occasion (Bakerspigel *et al.* 1986).

Further environmental associations not substantiated by direct isolation are reviewed under epidemiology, below.

There has been a long history of justifiable speculation that *B. dermatitidis* may associate in nature with one or more indigenous North American mammalian host species. To date, however, all the animal species that have been subjected to focused investigation have been exonerated of this specific connection. Unsubstantiated suspicion has particularly focused on the beaver (Klein *et al*., 1987; Bradsher 1987; Gaus *et al.* 1996), but the shrew (Baumgardner *et al*. 2005b), the bat (Chaturvedi *et al.* 1986) and the prairie dog (De Groote *et al.* 2000) have also been focal points of interest, with no conclusive interspecies association being demonstrated to date. Interestingly, the closely related pathogenic fungus *P. brasiliensis* in South America has a well substantiated, though not well understood, ecological link with the nine-banded armadillo, *Dasypus novemcinctus* (Silva Vergara & Martinez 1999). This member of the mammalian order Edentata has no close relatives in the geographic range of *B. dermatitidis*.

Epidemiology

The study of outbreaks as well as trends in individual cases of blastomycosis has clarified a number of important matters. Some of these relate to the ongoing effort to understand the source of infectious inoculum of this species, while others relate to which groups of people are especially likely to become infected.

In overview, human blastomycosis is primarily associated with forested areas and open watersheds (DiSalvo 1992; Kwon-Chung & Bennett, 1992; Baumgardner *et al.* 2005a; Baumgardner *et al.* 2006). It primarily affects otherwise healthy, vigorous people, mostly middle-aged (Klein *et al.* 1987), who acquire the disease while working or undertaking recreational activities in sites conventionally considered clean, healthy and in many cases beautiful (Rippon 1988, Kwon-Chung & Bennett 1992, Parmar 2005). Repeatedly associated activities include hunting, especially raccoon hunting (Armstrong *et al.* 1987), where accompanying dogs also tend to be affected, as well as working with wood or plant material in forested or riparian areas (Kwon-Chung & Bennett, 1992; Kesselman *et al.* 2005), involvement in forestry in highly endemic areas (Vaaler *et al.* 1990), excavation (Baumgardner & Brockman 1998) fishing (Klein *et al.* 1987; Baumgardner *et al.* 1992) and possibly gardening and trapping (Baumgardner & Brockman 1998, Dwight *et al.* 2000).

There is also a developing profile of urban and other domestic blastomycosis cases, beginning with an outbreak tentatively attributed to construction dust in Westmount, Illinois (Kitchen *et al.* 1977). The city of Rockford, Illinois, was also documented as a hyperendemic area based on incidence rates as high as 6.67 per 100,000 population for some areas of the city. Though proximity to open watersheds was linked to incidence in some areas (Baumgardner *et al.* 2006), suggesting that outdoor activity within the city may be connected to many cases, there is also an increasing body of evidence that even the interiors of buildings may be risk areas. An early case concerned a prisoner who was confined to prison during the whole of his likely blastomycotic incubation period (Renston *et al*. 1992). An interesting epidemiological survey done by Baumgardner *et al.* (1992) found that although many patients who contracted blastomycosis had engaged in fishing, hunting, gardening, outdoor work and excavation, the most strongly linked association in patients was living or visiting near waterways. Citing a similar finding in a Louisiana study (Lowry *et al.* 1989), the authors suggested that place of residence might be the most important single factor in blastomycosis epidemiology in north central Wisconsin. Follow-up epidemiological and case studies indicated that clusters of cases were often associated with particular domiciles, often spread out over a period of years, and that there were uncommon but regularly occurring cases in which pets kept mostly or entirely indoors, in particular cats, contracted blastomycosis (Baumgardner & Paretsky 2001, Blondin *et al.* 2007). The occurrence of blastomycosis, then, is an issue strongly linked to housing and domestic circumstances.

Seasonality and weather also appear to be linked to contraction of blastomycosis. Many studies have suggested an association between blastomycosis contraction and cool to moderately warm, moist periods of the spring and autumn (Rudmann *et al.* 1992; Kwon-Chung & Bennett, 1992; Dwight *et al.* 2000) or, in relatively warm winter areas, Arceneaux *et al*. 1998). However, the entire summer or a known summer exposure date is included in the association in some studies (Klein *et al.* 1987; Archer *et al.* 1987). Occasional studies fail to detect a seasonal link (e.g., Chapman *et al.* 1997). In terms of weather, both unusually dry weather (Proctor *et al.* 2002) and unusually moist weather (De Groote *et al.* 2000) have been cited. The seemingly contradictory data can most likely be reconciled by proposing that *B. dermatitidis* prospers in its natural habitats in times of moisture and moderate warmth, but that inoculum formed during these periods remains alive for some time and can be released into the air by subsequent dust formation under dry conditions. Indeed, dust per se or construction potentially linked to dust has been associated with several outbreaks (Kitchen *et al.* 1977; Baumgardner & Burdick 1991; Vasquez *et al.* 1998). The data, then, tend to link blastomycosis to all weather, climate and atmospheric conditions except freezing weather, periods of snow cover, and extended periods of hot, dry summer weather in which soil is not agitated.

Sex is another factor inconstantly linked to contraction of blastomycosis: though many studies show more men than women affected (Kwon-Chung & Bennett, 1992; Morris *et al.* 2006), some show no sex-related bias (Dwight *et al*. 2000; Baumgardner & Brockman 1998). As mentioned above, most cases are in middle aged adults, but all age groups are affected, and cases in children are not uncommon (Kwon-Chung & Bennett 1992; Dwight *et al*. 2000; Morris *et al.* 2006).

Ethnic group or race is frequently investigated in epidemiological studies of blastomycosis, but is potentially profoundly conflicted by differences in residence and in quality and accessibility of medical care, factors that have not been stringently controlled for to date. In the USA, a disproportionately high incidence and/or mortality rate is occasionally shown for blacks (Lemos *et al.* 2000; Cano *et al.* 2003; Dworkin *et al.* 2005; Manetti 1991); aboriginals in Canada are disproportionately linked to blastomycosis in some studies (Kepron *et al.* 1972; Dwight *et al.* 2000) but not others (Crampton *et al.* 2002). Incidence in aboriginal children may be unusually high (Dwight *et al.* 2000). The

Canadian data in some areas may be confounded or explained by the tendency to establish aboriginal communities in wooded, riparian, northern areas corresponding to the core habitat of *B. dermatitidis*, often with known *B. dermatitidis* habitats such as woodpiles and beaver constructions in the near vicinity.

There are a very small number of cases of human-to-human transmission of *B. dermatitidis* related to dermal contact (Bachir & Fitch 2006) or sexual transmission of disseminated blastomycosis of the genital tract (Kwon-Chung & Bennett 1992) among spouses.

Incidences in most endemic areas are circa 0.5 per 100,000 population, with occasional local areas attaining as high as 12 per 100,000 (Rippon 1988; Manetti 1991; Kwon-Chung & Bennett 1992; Cano *et al*. 2003). Most Canadian data fit this picture. Ontario overall, inclusive of endemic and non-endemic areas, is calculated as having 0.3 cases per 100,000; northern Ontario, mostly endemic, has 2.44 per 100,000 (Morris *et al*. 2006). Manitoba is calculated at 0.62 cases per 100,000 (Crampton *et al.* 2002). Remarkably higher incidences were shown for the Kenora, Ontario region: 117 per 100,000 overall, with aboriginal reserve communities experiencing 404.9 per 100,000 (Dwight *et al.* 2000). The most closely similar figures obtained in the USA are from the city of Eagle River, Vilas County, Wisconsin, which has an incidence rate of 101.3 per 100,000; the county as a whole has been shown in two successive studies to have an incidence of ca. 40 cases per 100,000 (Baumgardner *et al.* 1992; Baumgardner & Brockman 1998). An incidence of 277 per 100,000 was roughly calculated based on 9 cases seen in a

Wisconsin aboriginal reservation during a time in which extensive excavation was done for new housing construction (Baumgardner *et al.* 2002).

Synopsis and recommendations on prevention of infection

Synthesis of the above data suggests that, when all epidemiological confounders are taken into account, it will be found that all persons are more-or-less equally likely to be infected by *B. dermatitidis.* Exposure will most likely occur when people are either residing or visiting near a waterway in a highly endemic area, or when they are working or taking part in recreational activities in these areas. Work involving excavation or digging, dust dissemination, brush or tree clearance, wooden pole replacement, and extensive contact with riparian sites (e.g., trapping) is particularly likely to be involved. Potentially problematic recreational activities are stream- or lakeside fishing, hunting and camping. Highly endemic areas themselves will be often attractive stream- or lakeside sites with sandy soil, pine or spruce forest, evidence of animal activity or occupation (beaver dams, dog kennels, prairie dog mounds) and accumulations of woody debris (woodpiles, beaver dams, natural windfall). Though most exposures leading to disease will occur outdoors, infection can also be acquired indoors.

This combination of factors makes blastomycosis one of the most difficult of all regularly non-communicable diseases to avoid. An additional factor needs also to be considered, which is the relatively uncommon incidence of the disease even in most endemic areas: understandably, there may be reluctance to implement stringent, costly or otherwise inconvenient protective measures for a treatable disease with an incidence of 1 or 2 per

100,000. Finally, the fact that the actual ecological foci giving rise to the organism are very poorly understood mean that blastomycosis is very difficult to link to sites needing treatment or ecological conditions that need to be altered or avoided. Individual dwellings such as the McDougall Township, (Ontario cottage mentioned by Morris *et al.* 2006), may be hotspots connected with multiple cases of human and canine blastomycosis, yet extensive attempts to detect the source of infection and to presumptively disinfect the area may prove futile.Some recommendations for homeowners or occupants can be made even in this difficult situation:

a. In the purchase or rental of a:

- new home outside built-up urban areas,
- a new home near an undeveloped urban waterway, or
- a vacation home, cottage or vacation property

anywhere in south or central Manitoba, northern Ontario, the Lake Huron area, southern Quebec and northern New Brunswick, inquiry should be made about any known history of blastomycosis in humans, dogs, cats or livestock living or kept on the property during the time of the incumbent ownership. Consider including in any contract of sale or rental a clause stating that no human or domestic animal resident or kept on the property has been diagnosed with blastomycosis during the time of the vendor or landlord's ownership, and the vendor has no knowledge that previous vendors in the last 10 years have experienced problems potentially connected to in-situ blastomycosis.

Blastomycosis known or reasonably believed to have been acquired off the property can be excepted, but should be disclosed.

b. In a known endemic area for blastomycosis, especially near sites associated with previous known cases of blastomycosis, residents and renters are recommended to refrain from keeping, within 400 m of a waterway (Baumgardner *et al*. 1995; Baumgardner *et al.* 2005a):

- a dog kennel, run or yard confining dogs to an enclosed area where soil becomes enriched with their wastes
- an outdoor woodpile in which a portion of the wood remains in situ for over a year and may be extensively frequented by animals
- a beaver lodge, raccoon nest or other large animal dwelling within or under a boathouse or other enclosed wooden structure
- a subterranean earthen chamber intended to be at least occasionally entered by a person but potentially extensively entered by animals, such as a poorly enclosed root cellar, timber fort, subfloor crawlspace, electrical vault or other pole housing, etc. (if construction of such spaces is unavoidable, they should only be entered by persons wearing a fit-tested, NIOSH-compliant N100 respirator)

c. In the case of a dwelling coming under strong suspicion as associated with a blastomycosis source, direct testing of this with conventional research methods is impractical and unreliable; therefore, as a precautionary measure, areas where high moisture combines with woody debris and a presumed moderate to high level of animal waste input (except wastes or enclosures associated with large herbivores such as cattle, horses, deer and moose, which have never been associated with blastomycotic outbreaks), should be considered for disinfection using the methods recommended by Ajello and Weeks (1983).

d. In a known endemic area for blastomycosis, especially near sites associated with previous, known cases of blastomycosis, gardening, digging, brush clearing, excavating, etc., should be done in a way that minimizes exposure to dust and organic particulates. Activities generating heavy dust or extensive soil exposure, such as mechanical brush clearing, should only be undertaken while wearing a fit-tested, NIOSH-compliant N100 respirator.

e. In a known endemic area for blastomycosis, especially near sites associated with previous known cases of blastomycosis, no outbuilding should be maintained (workshed, garage, boathouse, woodshed), within 400 m of a waterway, that combines enclosure, rodent or other small animal inhabitation or infestation, woody organic debris and soil.

f. To encourage disclosure of *Blastomyces* “hot spots” and thus to prevent further cases of infection, municipalities, regions and other polities collecting property taxes should consider instituting very substantial tax relief for properties associated with two or more cases of blastomycosis, potentially acquired on the property, over the preceding ten years. The properties should be assessed to determine their loss in value, and taxed at a substantially lower rate on the condition that the public health status of the site will be disclosed in future contracts of sale. These conditions can expire and the property return to standard assessed value after 10 years of normal occupation in which no potentially epidemiologically linked case of blastomycosis occurs in human residents or visitors, or domesticated animals kept on the property.

In addition, a general recommendation is strongly mandated in the case of blastomycosis:

As a priority matter in the health care of northern Canadians, in particular aboriginals in areas endemic for blastomycosis, the recently developed molecular biological techniques for environmental detection of *B. dermatitidis* should be promptly and appropriately applied in an energetic Canadian research effort aimed at detecting and understanding the environmental reservoirs of this disease agent.

2. Histoplasmosis (in Canadian perspective)

Overview

Substantial areas of Canada lie within the main endemic zone of blastomycosis, but with respect to the related disease histoplasmosis, Canadian regions tend to be marginal or to fall outside the endemic region completely. Scattered cases and moderate to low levels of subclinical exposure are only found in southern areas of Quebec, Ontario, and Manitoba, as well as a few parts of New Brunswick and Nova Scotia. Infection acquired within Canada is usually associated with exposure to highly contaminated sites characterized by accumulations of bat or bird guano. Histoplasmosis, caused by *Histoplasma capsulatum*, resembles blastomycosis in being a disease that is mostly acquired by inhalation of environmental particulates. It differs by causing an unnoticed, subclinical infection in the great majority (95%+) of persons who are exposed (Kwon-Chung & Bennett, 1992). Such cases are mostly detected by later serological tests or by the detection of small pulmonary calcifications on x-ray. Clinically significant pulmonary histoplasmosis in general is often related to inhalation of a large inoculum load, or to the presence of an underlying immunodeficiency in the patient. Like *Blastomyces dermatitidis*, *H. capsulatum* appears to grow in the natural habitat as a cottony white mould, and it converts to a budding yeast phase in infected human or animal tissue. When treated, it generally responds well to systemic antifungal drugs.

Causal agent

Histoplasma capsulatum is an ascomycetous fungus closely related to *Blastomyces dermatitidis.* It is potentially sexual, and its sexual state, *Ajellomyces capsulatus*, can readily be produced in culture, though it has not been directly observed in nature. As mentioned above, *H. capsulatum* groups with *B. dermatitidis* and the South American pathogen *P. brasiliensis* in the recently recognized fungal family *Ajellomycetaceae* (Untereiner *et al.* 2002; Untereiner *et al.* 2004). It is dimorphic and switches from a mould-like (filamentous) growth form in the natural habitat to a small budding yeast form in the warm-blooded animal host.

Histoplasma capsulatum has two mating types +and –, as with *B. dermatitidis*. The great majority of North American isolates belong to a single genetic type (Karimi *et al.* 2002; Kasuga *et al.* 2003) but a study of multiple genes suggests a recombining, sexual population (Kasuga *et al*. 2003). A recent analysis has suggested that the prevalent North American genetic type and a less common type should be considered separate phylogenetic species, distinct from *H. capsulatum* isolates obtained in Central and South America and other parts of the world. These entities are temporarily designated NAm1 (the rare type, which includes a famous experimental isolate designated “the Downs strain”) and NAm2 (the common type; Kasuga *et al*. 2003). There is as yet no well established clinical or geographic distinction among these two genetic groups. To our knowledge, no Canadian isolate has been analysed to determine which group it belongs to.

In its asexual form, the fungus grows as a colonial microfungus strongly similar in macromorphology to *B. dermatitidis*. A microscopic examination shows a marked distinction: *H. capsulatum* produces two types of conidia, globose macroconidia, 8 – 15 µm, with distinctive tuberculate or finger-like cell wall ornamentation, and ovoid microconidia, 2 – 4 µm, which appear smooth or finely roughened. It is not clear whether one or both of these conidial types is more important than the other as the principal main infectious particles. They form on individual short stalks and readily become airborne when the colony is disturbed. Ascomata of the sexual state are 80 – 250 µm, and are very similar in appearance and anatomy to those described above for *B. dermatitidis*. The ascospores are similarly minute, averaging 1.5 µm.

The budding yeast cells formed in infected tissues are small (ca. 2 – 4 µm) and are characteristically seen forming in clusters within phagocytic cells, including histiocytes and other macrophages, as well as monocytes. An African phylogenetic species, *H. capsulatum* var. *duboisii*, often but not always forms larger yeast cells to 15 µm. Imported cases of this variant are rarely seen in Canada (Nethercott *et al*., 1978).

Geographic distribution, with emphasis on Canada

The endemic zones of *Histoplasma capsulatum* can be roughly divided into core areas, where the fungus occurs widely in soil or on vegetation contaminated by bird droppings or equivalent organic inputs, and peripheral areas, where the fungus occurs relatively rarely in association with soil but is still found abundantly in heavy accumulations of bat or bird guano in enclosed spaces such as caves, buildings, and hollow trees. The principal core area for this species includes the valleys of the Mississippi, Ohio and Potomac rivers in the USA as well as a wide span of adjacent areas extending from Kansas, Illinois, Indiana and Ohio in the north to Mississippi, Louisiana and Texas in the south (Kwon-Chung & Bennett, 1992). In some areas, such as Kansas City, skin testing with the histoplasmin antigen preparation shows that 80 – 90% of the resident population have an antibody reaction to *H. capsulatum*, probably indicating prior subclinical infection (Kwon-Chung & Bennett, 1992). Northern U.S. states such as Minnesota, Michigan, New York and Vermont are peripheral areas for histoplasmosis, but have scattered counties where 5 – 19% of lifetime residents show exposure to *H. capsulatum*. One New York county, St. Lawrence county (across the St. Lawrence River from the Cornwall – Preston – Brockville area of Ontario) shows exposures over 20% (Kwon-Chung & Bennett, 1992).

The distribution of *H. capsulatum* in Canada is not as well documented as in the US. The St. Lawrence Valley is probably the best known endemic region based both on case reports and on a number of skin test reaction studies that were done between 1945 and 1970. The Montreal area is a particularly well documented endemic focus, not just in the agricultural regions surrounding the city (Guy *et al.* 1949b) but also within the city itself (Leznoff et al., 1964). The Mount Royal area in central Montreal, especially the north and east sides of Mt. Royal Park, showed exposure rates between 20 and 50% in schoolchildren (Leznoff *et al.* 1969) and locally lifetime-resident university students (MacEachern *et al.* 1971). A particularly high rate of 79.3% exposure was shown in St. Thomas, Ont., south of London Ont., after 7 local residents had died of histoplasmosis in 1957 (Haggar *et al.* 1957). Based on numerous small regional studies summarized by

Haggar et al. (1957), MacEachern *et al.* (1971), Rostocka and Hiltz (1966), Hoff & Fogle (1970) and Mochi & Edwards (1952), histoplasmin skin test reactors form ca. 10 – 50% of the population in much of southern Ontario and in Quebec's St. Lawrence Valley, ca. 5% in southern Manitoba and some northerly parts of Quebec (e.g., Abitibi), and ca. 1% in Nova Scotia. Exposure of aboriginal Canadians occurs remarkably far north in Quebec (Guy *et al.* 1949a; Schaefer 1966), but has not been reported in similar boreal biogeoclimatic zones in many other parts of Canada.

Recently and remarkably, a cluster of four indigenously acquired cases of histoplasmosis was shown to be associated with a golf course in suburban Edmonton, Alberta (Anderson *et al.* 2006). Examination suggested that local soil was the source.

Spectrum of disease

Histoplasmosis, as mentioned above, is usually a subclinical infection that does not come to the attention of the person involved. The organism tends to remain alive in the scattered pulmonary calcifications; therefore, some cases are detected by emergence of serious infection when a patient becomes immunocompromised, perhaps decades later. Frank cases are most often seen as acute pulmonary histoplasmosis, a disease that resembles acute pneumonia but is usually self-limited (Kwon-Chung & Bennett, 1992; Kauffman 2007). It is most often seen in children newly exposed to *H. capsulatum* or in heavily exposed individuals. Erythematous skin conditions arising from antigen reactions may complicate the disease, as may myalgias, arthralgias, and rarely, arthritic conditions. Emphysema sufferers may contract chronic cavitary pulmonary histoplasmosis as a disease complication; eventually the cavity formed may be occupied by an *Aspergillus* fungus ball (aspergilloma), potentially leading to massive hemoptysis (Kauffman 2007). Another uncommon form of histoplasmosis is a slowly progressing condition known as granulomatous mediastinitis, in which the lymph nodes in the mediastinal cavity between the lungs become inflamed and ultimately necrotic; the swollen nodes or draining fluid may ultimately affect the bronchi, the superior vena cava, the esophagus or the pericardium. A particularly dangerous condition is mediastinal fibrosis, in which a subset of patients with granulomatous mediastinitis develop an uncontrolled fibrotic reaction that may press on the lungs or the bronchi, or may cause right heart failure. There are a number of other rare pulmonary manifestations of histoplasmosis.

Histoplasmosis, like blastomycosis, may disseminate haematogenously to infect internal organs and tissues, but it does so in a very low proportion of cases, and half or more of these dissemination cases involve immunocompromise. Unlike blastomycosis, histoplasmosis is a recognized AIDS-defining illness in HIV patients; disseminated histoplasmosis affects approximately 5% of AIDS patients with CD4+ cell counts <150 cells/ µl in highly endemic areas (Hajjeh 1995). The incidence of this condition dropped significantly after introduction of current anti-HIV therapies (Kauffman 2007).

Other conditions very uncommonly associated with *H. capsulatum* include endocarditis and peritonitis (Kwon-Chung & Bennett, 1992; Rippon 1988).

Ecology and epidemiology

Histoplasma capsulatum appears to be strongly associated with the droppings of certain bird species as well as bats (Kwon-Chung & Bennett, 1992). A mixture of these droppings and certain soil types is particularly conducive to proliferation. In highly endemic areas there is a strong association with soil under and around chicken houses, and with areas where soil or vegetation has become heavily contaminated with faecal material deposited by flocking birds such as starlings and blackbirds. Bird roosting areas that are *Histoplasma*-free appear to be lower in nitrogen, phosphorus, organic matter and moisture than contaminated roosting areas (Kwon-Chung & Bennett 1992). Interestingly, the guano of gulls and other colonially nesting water-associated birds is rarely connected to histoplasmosis (Waldman *et al.* 1983). Bat dwellings, including caves, attics and hollow trees, are classic *H. capsulatum* habitats (Kwon-Chung & Bennett, 1992; Rippon 1988). Histoplasmosis outbreaks are typically associated with cleaning guano accumulations or clearing guano-covered vegetation, or with exploration of bat caves. In addition, however, outbreaks may be associated with wind-blown dust liberated by construction projects in endemic areas: a classic outbreak is one associated with intense construction activity, including subway construction, in Montreal in 1963 (Leznoff *et al*. 1964).

As with blastomycosis, a good understanding of the precise ecological affinities of *H. capsulatum* is greatly complicated by the difficulty of isolating the fungus directly from nature. Again, the mouse passage procedure originally devised by Emmons (1961) must be used. A direct PCR technique for detection of *H. capsulatum* in soil has been published (Reid & Schafer 1999) but to our knowledge, no studies applying it have appeared in the literature as yet.

As mentioned above, *H. capsulatum* appears particularly likely to cause clinical disease in young children, persons working in sites contaminated by conducive bird or bat droppings, persons exposed to construction dust raised from contaminated sites, immunocompromised patients, and emphysema sufferers.

Recommendations on prevention of infection

In areas where histoplasmosis is hyperendemic, it is widespread in the soil and ubiquitous in the air, and little or nothing can be done about it. Fortunately, very few areas of Canada fit this description. In the experience of the senior author of the present document, based on 15 years in Ontario's central public health laboratory and extensive discussions with medical mycologists from Quebec and Alberta, many or most of the indigenous histoplasmosis cases seen in Canada relate to attempts to renovate or clean buildings that are the sites of large accumulations of bird or bat guano. This may be because most clinical cases occurring in HIV-negative individuals are based on exposure to a much higher inoculum level than is needed for the minor illness that produces a histoplasmin skin test reaction. MacEachern *et al*. (1971) made an ecologically reasonable argument that the over 30% histoplasmin reaction level of some students living directly east of Mt. Royal Park in Montreal was due to extensive bird roosting on the mountain, but the situation is evidently not serious enough to have stimulated any public warnings or environmental cleaning efforts known to the present authors. Also, it is not clear what could be effectively done either in terms of warning or of cleanup. Certainly, study of some multi-case reports, like that done by Haggar *et al*. (1957) in reference to the St. Thomas, Ont. area, indicates that at least a few cases of clinical histoplasmosis occur in patients who have not been directly involved with classic sites of contamination. However, St. Thomas, at least at that time, was clearly an isolated pocket of hyperendemicity, with a local histoplasmin reactor prevalence of almost 80% of the population, including ca. half of children in the 1 – 9 yr age group. Control strategies for such local and regional hyperendemicity have yet to be devised – though the general advice to avoid allowing buildup of bird manure would perhaps be admitted as a common sense measure.Recommendations for Canadian homeowners and occupants, then, are:

1. ttics and other enclosed spaces *within* buildings, including sheds, barns and other outbuildings, should not be allowed to from becom nesting or roosting sites for pigeons, starlings, sparrows, or bats. In the case of bat colonies desired for conservation or insect-control purposes, cleanable bat boxes or treat nesting sites as hypothetically contaminated with *H. capsulatum*, anywhere within the provinces extending between Nova Scotia and Alberta. Attics or other spaces protected as valuable bat habitats should be well sealed off from any contiguous portions of buildings, and the deposition or spillover of droppings into wall cavities adjacent to lower floors should be prevented. Note that to date, *H. capsulatum* does not appear to have been linked to two valuable types of colonially dwelling insect-control birds, swallows and swifts. Excessive accumulation of dung of these species, however, should be prevented.

2. Also, to the greatest extent practicable, prevent excessive buildup of pigeon and starling dung on building surfaces, as well as ornamental objects, vegetation, etc., within 30 – 50 m of a house or other human dwelling. Though seldom linked to histoplasmosis, extensive depositions of gull or cormorant dung should also not be allowed to accumulate within 30 – 50 m of dwellings.

3. In endemic areas, keep chicken facilities in clean condition but avoid raising excessive dust while cleaning, e.g., by moderately wetting material to be cleaned. Occasionally turn or exchange the soil of outdoor chicken yards while it is in moist condition to prevent excessive guano buildup in the upper soil layers. Note the importance of not raising dust in endemic areas while turning soil: for example a recent outbreak causing 355 cases of acute histoplasmosis at an Indiana school was caused by dust raised by rototilling a courtyard known to be a bird roosting area (Chamany *et al*. 2004).

4. Attics and other building spaces from Nova Scotia through Alberta found to contain accumulations of bird of bat droppings should be treated as potentially contaminated with pathogenic fungi, including not just *H. capsulatum* but also *Cryptococcus neoformans*. (Outside this endemic zone, contamination with *C. neoformans* alone remains probable.) Though the material can in principle be disinfected prior to removal with the formalin drench procedures recommended by Ajello and Weeks (1983; specifically, 3.8 L [1 gallon] of 3% formalin per 0.093 m^2 [square foot]), the application of bulk formalin to buildings is generally not advised, since formalin itself is considered hazardous. Unfortunately, no alternative technique has as yet been scientifically demonstrated as effective. Steaming has been attempted to decontaminate guano in a Canadian historical

building (personal information) but the difficulty of assessing efficacy prevented any scientific report on the success of the procedure. Decontamination should follow stringent procedures developed for toxigenic indoor moulds (e.g. Canadian Construction Association, 2004). Currently there are no requirements for inactivation of these materials prior to disposal, however sterilization by autoclaving or incineration may be warranted.

5. Accumulations of bird droppings on building surfaces should be thoroughly wetted prior to removal, in order to prevent dust-associated histoplasmosis outbreaks. (See report by Stobierski *et al*. 1993 on an outbreak connected with sweeping bird droppings off a Michigan paper factory roof.)

6. HIV+ persons should as much as possible avoid contact with accumulations of bird or bat guano, as well as chicken raising facilities in general (Hajjeh 1995).

7. Hobby pigeon coops within endemic regions should be maintained in clean condition, and any soil heavily contaminated by guano (e.g., a nearby feeding area), should be regularly turned or tilled in a dust-free manner, and

8. **Priority research: development of an environmentally acceptable decontamination procedure for *H. capsulatum* in guano and soil accumulations is a high priority. This research could be carried out using artificially inoculated ("spiked") material if contaminated natural material is not available or cannot be reliably characterized. Linked to this research, recently developed molecular detection techniques for *H. capsulatum* in soil should be adapted for evaluation of**

other materials suspected to contain *H. capsulatum*, and reliable molecular techniques should be developed for specific detection of viable *H. capsulatum* in test or environmental materials.

3. Sylvan (or "tropical") cryptococcosis (in Canadian perspective)

Overview

The relatively well known fungal disease cryptococcosis is caused by a yeast species that is classically thought mainly to grow in accumulations of bird and bat guano, as well as occasionally in tree trunk hollows. Until the 1980s, this yeast, *Cryptococcus neoformans*, was mainly known for causing cryptococcal pneumonia and meningitis in small numbers of persons who were heavily exposed to guano dust, usually during cleanup operations, plus occasional immunocompromised patients who had contact with birds. When the HIV virus became widespread, numbers of cryptococcosis cases rose by over 200X traditional rates, and this disease was classified as an AIDS-defining secondary infection. Mainly in Australia, however, a distinct second type of cryptococcal infection was found, caused by a closely related yeast long known as *C. neoformans* var. *gattii*, recently upgraded to full species status as *Cryptococcus gattii*. This organism mainly infected persons living or working in the Australian bush, few of whom had any history suggestive of heavy dust exposure. Even during the HIV outbreak, incidence of *C. gattii* infection did not increase significantly, and though the infection was not unknown in AIDS patients, there was no marked association. *Cryptococcus gattii* was found to be strongly associated with sylvan environments, in particular with certain *Eucalyptus* species. Apart from being found in Australia, it could be found worldwide in areas where these originally Australian eucalypt species had been planted. In addition, it was found in tree holes (wounds, natural cavities) of numerous other tree species in Brazil, Colombia, India and other tropical regions, and occasional cases of human and animal infection were also reported in these countries. This picture appeared relatively stable until the summer 2001, when an unprecedented, major outbreak of sylvan cryptococcosis occurred in Vancouver Island, British Columbia, Canada.

Causal agent

Cryptococcus gattii and *C. neoformans* belong to a tightly knit species complex in the order Tremellales of the phylum Basidiomycota. Unlike *Blastomyces* and *Histoplasma*, then, these species are more closely related to the common mushrooms than to the common moulds or commercial yeasts. There has been 30 years of debate over precisely how many species should be recognized in the *C. neoformans* species complex, which consists of four relatively distinct lineages (best known as serotypes A and D in *C. neoformans* and serotypes B and C in *C. gattii*) plus some lineages apparently descended from hybridizations among these groups (Fraser et al. 2003, 2005). At present, however, there is a reasonable degree of consensus that the two species recognized here fit modern species concepts reasonably well (Kwon-Chung & Varma 2006). Within *C. gattii*, a number of subsidiary lineages have been recognized through DNA typing with restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP) and multigene sequencing (Kidd et al. 2004). The Canadian isolates from the Vancouver Island outbreak belong to just two restricted genetic groups, VGII/AFLP6 and VGI (Kidd et al. 2004). The predominant outbreak type, VGII/AFLP6, is hypervirulent in a mouse model and is generally very rare, being known only from B.C. outbreak strains and from a clinical strain isolated in Seattle, WA, USA, in 1975, and a eucalypt-derived environmental strain isolated in San Francisco, CA, in 1992 (Fraser *et al.* 2005).

The minor outbreak type VGI shows very low virulence in the mouse and is identical to a common group of isolates in Australia.

Cryptococcus gattii is a sexual species mating to form the teleomorph *Filobasidiella bacillospora*. All outbreak strains are of mating type α and should not be able to reproduce sexually according to classic models, but as Fraser *et al.* (2003, 2005) have shown, these isolates appear to be capable of α-α same-sex mating, and are likely to be producing basidiospores in the environment. This is highly significant epidemiologically, as basidiospores in their transverse axis are just 1 – 2 µm in diameter, readily respirable, and are produced in delicate chains adapted for airborne dispersal, whereas cryptococcal yeast cells are larger (ca. 3-8 µm) and are produced in highly mucoid masses not specifically adapted for aerial dispersal. In particular, Fraser et al. (2005) discovered that one of the 95 outbreak isolates they investigated from Vancouver Island was diploid, meaning it very likely possessed the ability to spontaneously differentiate as the filamentous teleomorphic stage and to produce basidiospores spontaneously.

In the laboratory, *C. gattii* grows as a mucoid yeast, with colonies composed of a mixture of globose cells 3-10 µm in diameter and oval to lemon-shaped cells 3 – 5 X 3 – 7 µm (Kwon-Chung & Bennett, 1992). The tapered cell shapes are not typically found in the related *C. neoformans*. Individual yeast cells are surrounded by a broad mucous capsule composed of glucuronoxylomannan. Though this mucoid appearance suggests adaptation to aqueous circumstances, in fact the yeast cells are highly desiccation-tolerant (Kwon-Chung & Bennett 1992). They may become aerosolized when material they have been growing on becomes dried and friable. In their moist condition, they may be splashed into the air by heavy rainfall and other impacting water.

When mated, the *F. bacillospora* state that is produced features hyphae with clamp connections and single basidia produced on elongated upright stalks. The basidia initially produce four meiotic basidiospores; these then undergo mitosis to give rise to long, delicate, acropetally extending chains of rod-shaped secondary spores 3 – 8 X 1 – 1.8 µm.

Geographic distribution

Cryptococcus gattii is dispersed all over the world in areas of tropical, subtropical or Mediterranean-type climate where associated *Eucalyptus* species (family Myrtaceae), especially *E. camaldulensis* and *E. tereticornis*, have been planted (Randhawa *et al.* 2003). In addition, it has a wide variety of potential host species in tropical trees, particularly in the plant families Myrtaceae (*Angophora, Syzygium*, *Syncarpium*), Moraceae (*Ficus*), Combretaceae (*Terminalia*) and the leguminoid families Fabaceae (*Erythrina*) and Caesalpiniaceae (*Cassia*) (Randhawa *et al.* 2003). Distribution has been particularly well studied in Australia, Brazil, Colombia and India. Within Canada, *C. gattii* has primarily been isolated from the environment on the central and southeast coasts of Vancouver Island, along with some adjacent Gulf Islands, areas on the east end of Alberni Sound and a small number of locations in the lower Fraser valley on the B.C. mainland (Kidd et al. 2007b).

Spectrum of disease

Cryptococcus gattii infection, like *C. neoformans* infection, classically manifests as meningoencephalitis and/or pneumonia, with immunocompromised patients occasionally showing dissemination to the skin, skeletal system, eyes and internal organs (Kwon-Chung & Bennett 1992; Chayakulkeeree & Perfect 2006). Meningitis cases present with typical signs of cerebral infections such as headache, dizziness, cognitive impairment, ataxia, nausea, double vision, etc. Cases in the Vancouver Island outbreak appeared mainly to present with pulmonary symptoms, though the central nervous system (CNS) was involved in circa 25% of cases, and skin involvement was seen in at least one reported case (Hoang et al. 2004; MacDougall & Fyfe 2006). This is an unusual pattern for *C. gattii*, which often causes meningoencephalitis: for example, nearly 70% of *C. gattii* cases in the Northern Territories of Australia involved non-immunosuppressed patients with meningitis (Kwon-Chung & Bennett 1992).

Ecology

Unlike *B. dermatitidis* and *H. capsulatum*, *C. gattii* offers the advantage that it is easily selectively isolated from the environment by means of Staib's niger seed agar (e.g., see Randhawa *et al.* 2003, Kidd *et al.* 2007). Thus considerable understanding can be gained directly about its ecology. The classic ecological picture of *C. gattii* is that it is associated with certain eucalypt species and the organic litter derived from them, and that it may disperse synchronously with the flowering of the trees (Ellis & Pfeiffer 1990). It also, as mentioned above, known from a wide variety of tropical trees (Lazera *et al.* 2000;

Fortes *et al.* 2001; Randhawa *et al.* 2003; Kidd *et al.* 2007b), mainly from trunk hollows (for illustration of typical trunk hollows and fissures involved, see Randhawa *et al.* 2003). In British Columbia, however, a very different picture was found, suggestive of ruderal (weed-like) invasion of a new habitat (Kidd *et al.* 2004, 2007a, 2007b). *Cryptococcus gattii* in affected areas was more reliably isolated from soil than from trees, though numerous trees appear to be colonized consistently or intermittently. Douglas fir, alder, arbutus, red cedar, and Garry oak were the tree species most likely to be involved, but most other species native to the area yielded positive samples, apart from western hemlock. Soils yielding *C. gattii* tended to be acidic, relatively dry, low in nutrient content, and associated with open areas such as parking lots and beach fronts (Kidd et al. 2007b). Two biogeoclimatic zones were predominantly involved, the "coastal Douglas-fir" zone, and the slightly wetter "coastal western hemlock xeric maritime" zone (Kidd et al. 2007a, 2007b). Lakes and streams in the affected areas tended to yield *C. gattii*, as did seawater samples. The organism was able to survive for extended periods in both fresh and marine water in laboratory tests (Kidd *et al.* 2007b). Isolation by air sampling was common; the pattern of positive isolations showed an association with dry summer conditions but no association with the flowering of any tree species (Kidd *et al.* 2007b). In multi-stage Anderson sampling studies partitioning the particles yielding colonies into various particle sizes, particles between 3.3 and 7.0 µm were the most common sources of *C. gattii* colonies, but highly respirable particles under 3.3 µm were also common (Kidd *et al.* 2007b).

Epidemiology

Cases of *C. gattii* infection are almost entirely acquired via inhalation (Kwon-Chung & Bennett 1992). The majority of patients involved worldwide are non-immunocompromised; only in southern California, where the host tree *Eucalyptus camaldulensis* commonly occurs, are cases in HIV+ patients relatively common (Chaturvedi et al. 2005). Cases connected with the Vancouver Island outbreak have been almost entirely in non-immunocompromised patients and have shown no strong predisposition for any particular human subpopulation (Hoang et al. 2004; MacDougall & Fyfe 2006). A large number of animal species in and around Vancouver Island have been affected, particularly cats and dogs but also native marine mammals such as porpoises (especially Dall's porpoises, *Phocoenoides dalli*) and various non-native terrestrial species such as eastern gray squirrels, llamas, ferrets and horses (Stephen *et al.* 2002; Duncan *et al.* 2006a). Nasal colonization not associated with detectable disease symptomatology has been found in wild eastern gray squirrels (Duncan *et al.* 2006a), as well as dogs and cats (Duncan *et al.* 2005). Such nasal colonizations are well known from koalas in the principal endemic areas of *C. gattii* in Australia (Connolly *et al.* 1999) and clearly have the potential to transmit the organism to new areas (Duncan *et al.* 2006a).

As mentioned above, the transmission of the outbreak to infected humans and animals appears to be mainly carried out by dispersal of *C. gattii* propagules in the air and on fresh and marine waters (Kidd *et al.* 2007b). Infectious particles in the respirable range may be abundantly detected at affected environmental sites (Kidd *et al.* 2007b). Isolation of *C. gattii* from automobile wheel wells and from footwear has shown that the organism could very easily be transported by persons travelling from one place to another (Kidd *et al.* 2007a). The numbers of infectious particles in the air were significantly increased by forestry and parks maintenance-related activities such as felling and limbing trees, and in particular by wood chipping procedures (Kidd *et al.* 2006). A study of infected dogs showed that development of cryptococcosis appeared to be associated with "residing within 10 km of a logging site or other area of commercial soil disturbance ... hunting by the animal, and owners hiking or visiting a botanic garden" (Duncan et al. 2006b).

Recommendations on prevention of infection

Though currently restricted in geographic distribution, *C. gattii* poses a difficult new problem for homeowners and property occupants in the affected areas. To date, no control strategies have been proposed or tested, and the only obvious preventive measures are unsavoury, e.g., to depart the area or to cease normal outdoor recreational activity. A few reasonable control measures, however, can be proposed based on the findings presented above.

1. Minimize dust generation in all activities involving the soil or wood in the affected areas. For example, to the extent reasonably possible, moisten surfaces of felled trees that are to be chipped or limbed, and lightly to moderately moisten soil that is to be disturbed in construction, forestry, agricultural or gardening activities.

2. Since the fungus is strongly favoured by acidic soils, consider, where reasonable, liming cultivated soils near heavily affected areas to bring the pH higher than 6.5. Nearly

all soils associated with heavy *C. gattii* content are below this pH level (Kidd *et al.* 2007b). Liming to reduce soil pH could also be considered for soils around parking lots and other areas heavily affected by human activity, not just around homes but also in parks and recreational areas situated within the outbreak zone. It is also possible that increasing the organic content of soils, preferably with a material other than wood or bark chips generated within the endemic zone, may decrease the risk of *C. gattii* proliferation in soil (see Kidd *et al.* 2007b).

The *C. gattii* outbreak in Vancouver Island may be a transient phenomenon; it remains to be seen if the organism will continue to proliferate unchecked or if local microbiota will rapidly evolve antagonistic reactions that limit its growth. It is interesting that many sites found positive by Kidd et al. (2007a) in 2004 were negative in 2005. The main research need that emerges in connection with the situation of homeowners and occupants is for follow-up studies on the environmental prevalence of the organism, and for studies on the natural development or, if necessary, potential introduction of antagonistic biocontrol microbial populations in the environment.

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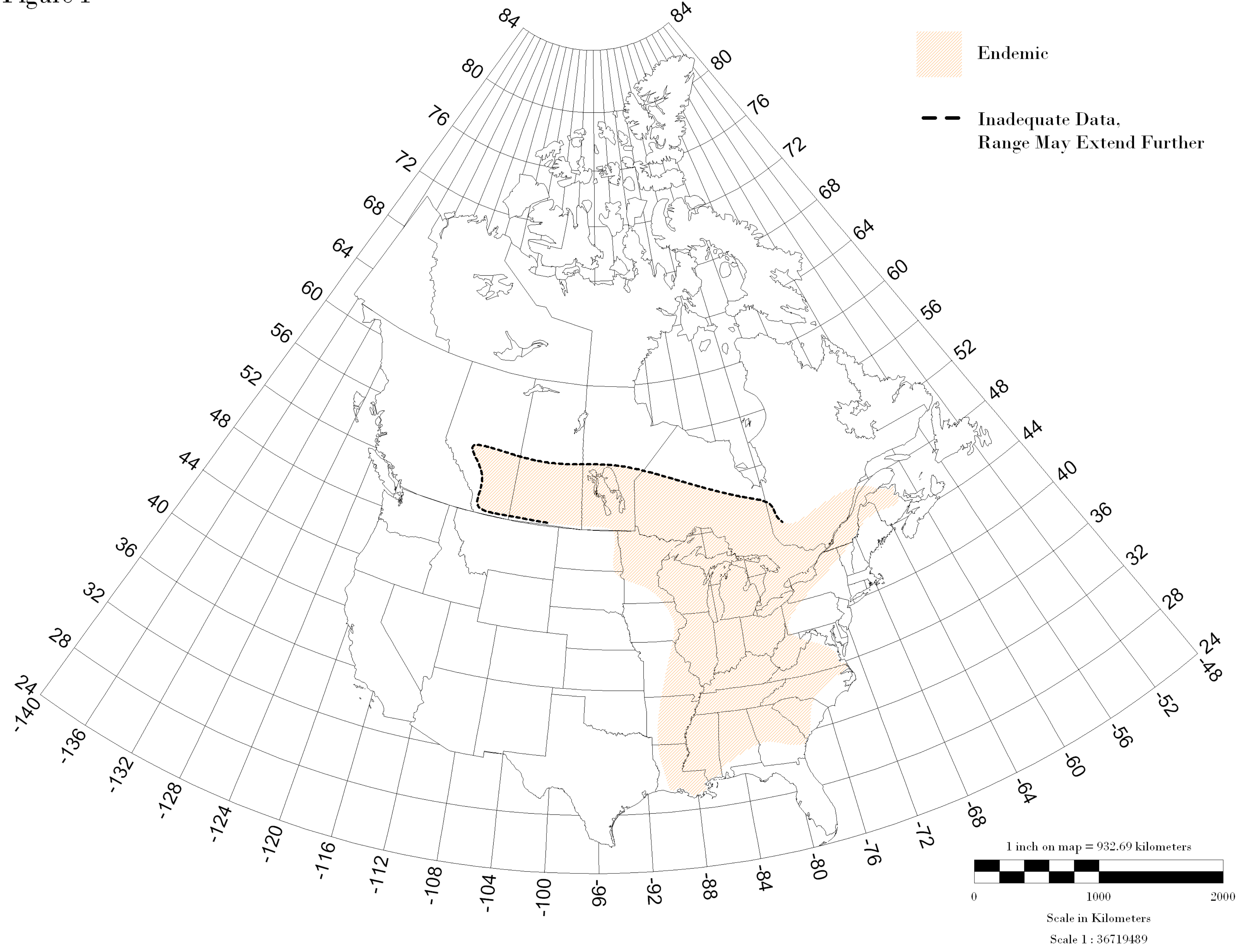
Figure 1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Inadequate Data,
Range May Extend Further
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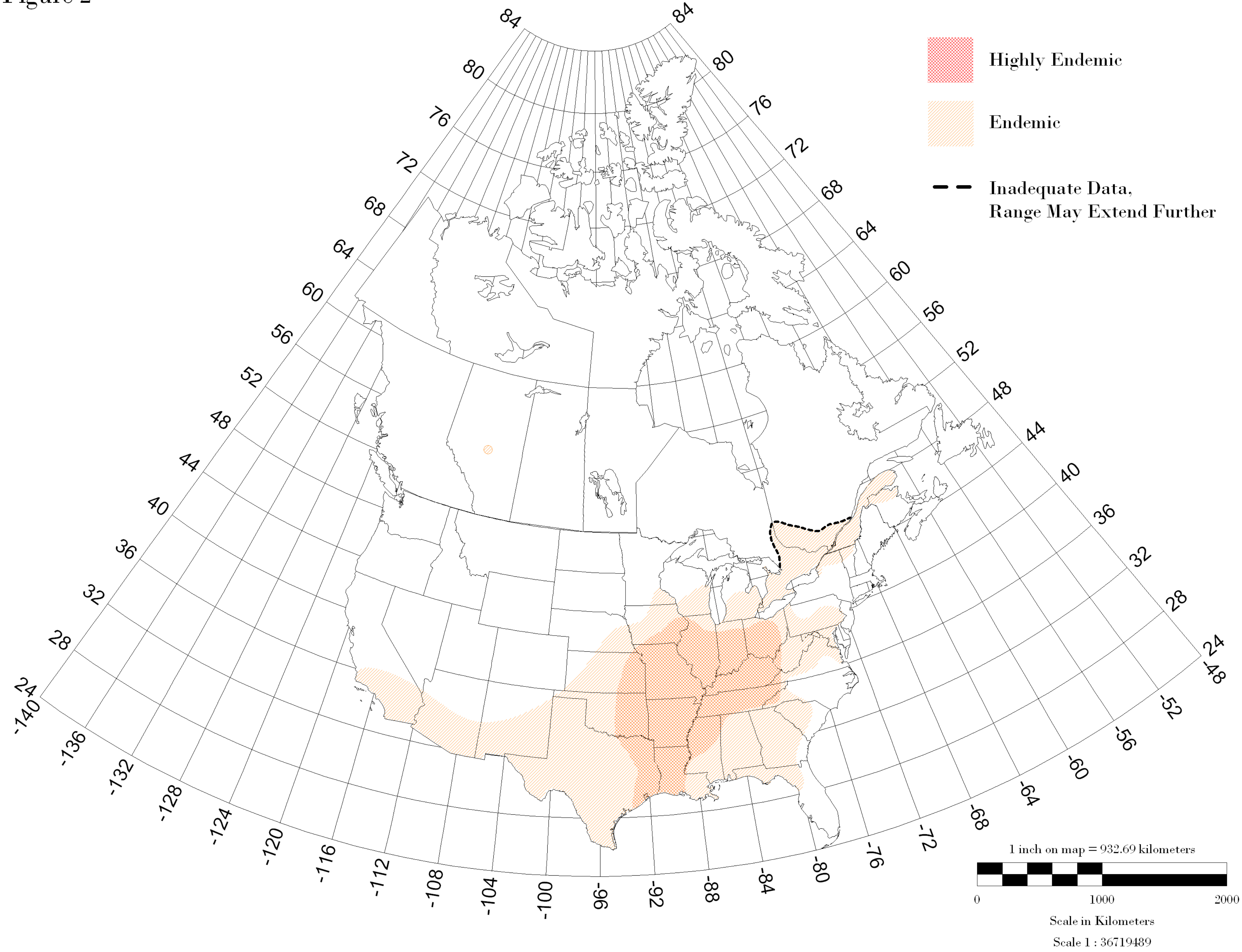
Figure 2



Highly Endemic
Endemic
Inadequate Data,
Range May Extend Further
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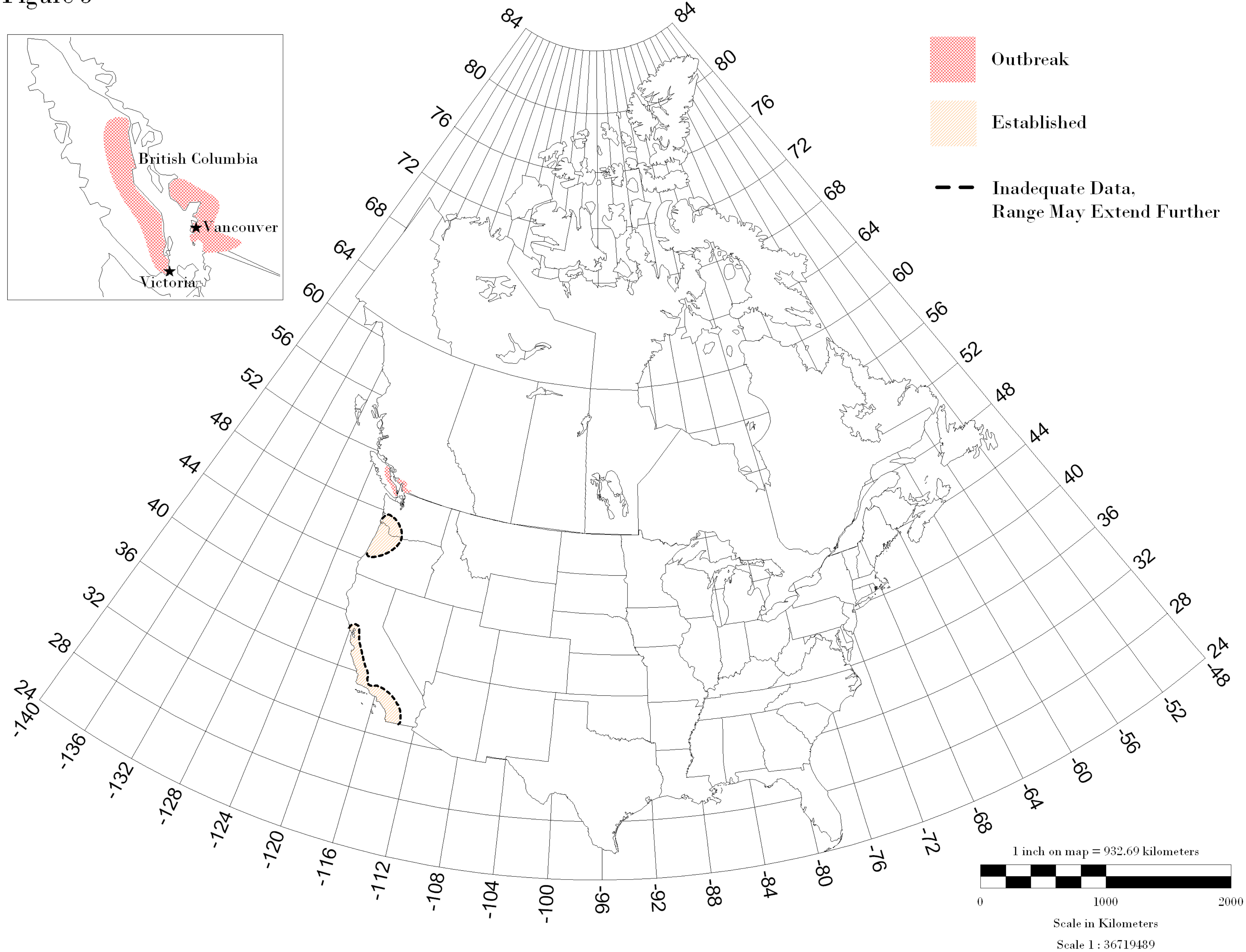
Figure 3


British Columbia
Vancouver
Victoria
Outbreak
Established
Inadequate Data,
Range May Extend Further
1 inch on map = 932.69 kilometers
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