Clinicopathologic features of an unusual outbreak of cryptococcosis in dogs, cats, ferrets, and a bird: 38 cases (January to July 2003)

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Objective—To determine clinical and pathologic findings associated with an outbreak of cryptococcosis in an unusual geographic location (British Columbia, Canada).

Design—Retrospective study.

Animals—1 pink-fronted cockatoo, 2 ferrets, 20 cats, and 15 dogs.

Procedure—A presumptive diagnosis of cryptococcosis was made on the basis of serologic, histopathologic, or cytologic findings, and a definitive diagnosis was made on the basis of culture or immunohistochemical staining.

Results—No breed or sex predilections were detected in affected dogs or cats. Eleven cats had neurologic signs, 7 had skin lesions, and 5 had respiratory tract signs. None of 17 cats tested serologically for FeLV yielded positive results; 1 of 17 cats yielded positive results for FIV (western blot). Nine of 15 dogs had neurologic signs, 2 had periorbital swellings, and only 3 had respiratory tract signs initially. Microbiologic culture in 15 cases yielded 2 isolates of Cryptococcus neoformans var grubii (serotype A) and 13 isolates of C neoformans var gattii (serotype B); all organisms were susceptible to amphotericin B and ketoconazole. Serologic testing had sensitivity of 92% and specificity of 98%.

Conclusions and Clinical Relevance—Serologic titers were beneficial in identifying infection in animals with nonspecific signs, but routine serum biochemical or hematologic parameters were of little value in diagnosis. Most animals had nonspecific CNS signs and represented a diagnostic challenge. Animals that travel to or live in this region and have nonspecific malaise or unusual neurologic signs should be evaluated for cryptococcosis. (J Am Vet Med Assoc 2004;225:1716–1722)

Cryptococcosis is considered a sporadic and not uncommon fungal infection in humans and animals throughout the world and is the most common systemic mycosis in cats. The nomenclature of the various cryptococcal species has been subjected to considerable change based on culture characteristics, serologic identification, and recently, DNA sequencing of various genes. The clinically important cryptococcal organisms are considered to be Cryptococcus neoformans var neoformans and C neoformans var gattii (the later also identified as the separate species C bactillisporus). On the basis of serologic testing, C neoformans var neoformans includes the serotypes A, D, and AD. Varieties within this group now include C neoformans var grubii (serotype A) and C neoformans var neoformans (serotype D). Cryptococcus neoformans var gattii includes serotypes B and C. Results of genetic studies now support the contention that C neoformans var gattii and the C neoformans group are separate species, although taxonomic changes have yet to be finalized.

Cryptococcus neoformans var gattii has a strong association with eucalyptus trees and tropical or subtropical regions in Australia and South America and 1 focus in California. Cryptococcus neoformans var neoformans has been historically associated with pigeon droppings. This variety is the most common variety isolated from humans and has been implicated as a secondary pathogen in immune-compromised patients.

Cryptococcus neoformans is routinely identified in the North American literature without further definition of the variety or serotype. In contrast, reports from Australia commonly identify varieties and have established the prevalence of both varieties in many mammalian hosts. The Australian studies have linked C neoformans var gattii (serotype B) with koalas (Phascolarctos cinereus), and this species is both an indicator host and amplifier of the organism in the environment. The spectrum of disease in koalas varies from carrier to subclinical to severe systemic involvement. The disease incidence in cats and dogs is variable; a recent study from a large metropolitan region of Australia identified 194 cases during a 20-year period, with identification of both cryptococcal varieties, and a study encompassing southern California and the southwestern states identified 47 cats during an 8-month period but included no identification of the specific cryptococcal varieties involved.

The presumptive diagnosis of this disease is based on cytologic or histopathologic findings because the morphology of the organisms is generally distinctive; a large polysaccharide capsule surrounds narrow-necked budding yeast bodies. Typically, the organism occurs in large numbers within lesions, often with scant inflammation. Serologic tests for cryptococcal capsular pro-
teins have been helpful in establishing the diagnosis as well as monitoring the response to treatment. Cryptococcosis has been a sporadic problem along the east coast of Vancouver Island and the greater Vancouver regional district of British Columbia, Canada. These regions are climatically similar, with a temperate climate and large tracts of northern rain forest interspersed with agricultural areas and dense urban areas. The climate is such that it is a tourist destination for those interested in outdoor activities including fishing, hiking, and camping. Analysis of visitors to the area indicates that approximately 3.6 million US residents entered British Columbia in June 2003 with approximately 32% traveling to Vancouver Island. Most of these visitors arrive by car, motor home, or similar transport and may bring pets with them.

In 2000, there was a dramatic increase in the diagnosis of cryptococcosis in this region and isolates cultured from humans and animals were most often C. neoformans var gattii (serotype B). The multispecies outbreak involved birds, ferrets, cats, dogs, llamas, dolphins, and humans. The organism was cultured from trees in the region including fir, alder, Garry oak, maple, cedar, and pine, both at the base and extending up the bark of the living tree, as well as from air samples collected among tree stands located along the east coast of Vancouver Island. These findings are in contrast to the current state of knowledge regarding the distribution and environmental associations of this organism.

The purpose of the study reported here was to increase awareness of this geographic location as an endemic region for cryptococcosis and evaluate characteristics of this unique outbreak of cryptococcosis. We hypothesized that certain features of the disease were different from those commonly reported in other regions.

Criteria for Selection of Cases

Laboratory records of all cases that involved submission to the laboratory for cytologic, histologic, serologic, immunohistochemical, or culture examinations for which a diagnosis of Cryptococcus spp infection was obtained from January 2003 through June 2003 were retrieved for evaluation. Diagnoses obtained via immunohistochemical testing or culture were considered definitive diagnoses, whereas those made via other tests were considered presumptive.

Procedures

Diagnoses were made primarily from cytologic or histologic specimens. Cytologic specimens included nasal flushes or exudates, CSF, and impression smears or aspirates of skin masses; histologic specimens included those from complete necropsies and biopsy specimens from many selected tissues such as intestinal tract, lung, and skin nodules. Cytologic specimens were stained with Wright-Giemsa, and histologic sections were stained routinely with H&E as well as silver stains and Mayer's mucicarmine stain. Specimens from 15 animals underwent fungal culture by use of media including Sabouraud dextrose agar and Niger (Guizotia abyssinica) seed agar. Differentiation between C. neoformans and C. neoformans var gattii was performed on canavanine-glycine-bromthymol blue agar (CGB), and C. neoformans var gattii was melanin-positive in the presence of caffeic acid. Serologic identification of the organisms was determined by use of antibodies against cell antigens. This test incorporates 5 antisera, and the isolates were identified as serotype A (C. neoformans var grubii), serotype D (C. neoformans var neoformans), or serotype B (C. neoformans var gattii). Minimum inhibitory concentrations (MICs) were determined on all isolates with a commercial method by use of RPMI 1640 broth with morpholinepropanesulfonic acid and 2% glucose to form an agar media and included the antifungal agents, fluconazole, itraconazole, ketoconazole, and amphotericin B.

Hematologic and serum biochemical values were determined with the same instrumentation used to validate reference ranges for the species in this report. Feline viral status was determined with a variety of ELISA and indirect fluorescent antibody procedures. Cryptococcal antigen testing was performed by use of a kit. Immunohistochemical procedures for Cryptococcus spp were performed by use of a described method.

Results

Signalment—Twenty affected cats were identified, ranging in age from 2 to 13 years, with 11 neutered males and 9 spayed females. The age distribution was wide, but 70% of the cats were 7 years or older. Most cats were identified as domestic shorthair, and no breed predilection was identified.

There were 15 dogs, ranging in age from 9 months to 8 years of age, of which 8 were spayed females, 5 were neutered males, and 2 were sexually intact males. Thirteen of the dogs were <5 years of age. There were 2 American Cocker Spaniels, both from the same household. The remaining dogs were either large mixed-breed dogs or individual purebreds, including Miniature Dachshund, Boston Terrier, Jack Russell Terrier, Irish Setter, and Blue Heeler. There were 2 ferrets and 1 pink-fronted cockatoo.

History and clinical signs—Among the 20 cats, clinical signs typically indicated involvement of more than 1 organ system. Eleven had CNS signs that included generalized weakness, ataxia, blindness, or seizures. Seven had skin nodules including 2 with subcutaneous masses along the body wall in the thoracic region, 2 with involvement of the eyelids, 2 with involvement of the bridge of the nose, and 1 with a lesion in the deep tissues of the neck. In 3 of these cats, seizures were included in the initial complaint. Four of these cats had only skin nodules as the initial complaint. Nasal discharge was detected in 5 cats, and upper respiratory tract signs were primarily related to nasal congestion. One cat had dyspnea and developed CNS signs.

In the dogs, 9 had CNS signs including ataxia, blindness, nystagmus, and seizures; 2 of these dogs also had nasal discharges, whereas 3 dogs had nasal...
discharge and 2 had periorbital swelling alone. One dog had signs of acute abdominal pain and a palpable mass within the abdominal cavity.

Thirteen of 35 (37%) dogs and cats were blind either initially or during the course of the disease, whereas 12 of 35 (34%) had seizures. Other manifestations of CNS disease including ataxia, signs of neck pain, nystagmus, signs of depression, and malaise were included in the initial complaint in 2 others. On the initial examination, signs attributable to the CNS were detected in 21 of 35 (60%) animals.

The bird had a fluctuant skin mass in the craniodorsal portion of the pelvic region. One ferret had respiratory tract signs including nasal discharge, whereas the other had signs of acute abdominal discomfort and a palpable abdominal mass.

**Environment**—Three cats were confined indoors, but all had access to potted plants containing soil from the same geographic area. The remainder of the animals had indoor and outdoor access, and the history of many of the dogs included hikes through parks and forested areas. The bird was caged but often the cage was placed outdoors. The ferrets were frequently walked on leashes outdoors. All animals were from suburban or metropolitan areas with 4 of the 38 animals from the greater Vancouver regional area. Thirty-three animals were from the eastern coast of Vancouver Island, and 1 was from the gulf islands. The animals from Vancouver Island also included those from metropolitan regions as well as suburban or small-acreage plots. There were 6 cases during January, 4 during February, 11 during March, 4 during April, 7 during May, and 6 during June. There were only 2 cases in dogs in May and none in June.

**Clinical pathology**—Hematologic tests, serum biochemical panels, or both were performed on samples from 8 dogs and 13 cats. Routine hematologic testing (CBC and differential cell counts) revealed no specific abnormalities. Among the cats, 3 had marginal nonregenerative anemia (PCV range, 0.275 to 0.298 L/L; reference range, 0.40 to 0.55 L/L); 2 had leukocytosis characterized by neutrophilia (neutrophil range, 16.900 to 29.900 × 10⁹ cells/L; reference range, 5.000 to 12.000 × 10⁹ cells/L); 6 had lymphopenia (lymphocyte range, 0.325 to 0.710 × 10⁹ cells/L; reference range, 1.176 to 6.564 × 10⁹ cells/L); and 2 cats had monocytosis (monocyte range, 1.070 to 1.460 × 10⁹ cells/L; reference range, 0.000 to 0.870 × 10⁹ cells/L).

Three of 8 dogs had lymphopenia (lymphocyte range, 0.539 to 0.724 × 10⁹ cells/L; reference range, 0.960 to 2.500 × 10⁹ cells/L), and 4 of 8 had monocytosis ranging from 1.050 to 2.198 × 10⁹ cells/L (reference range, 0.000 to 0.980 × 10⁹ cells/L). None of the dogs were anemic.

Routine serum biochemical panels included the analytes glucose, BUN, creatinine, sodium, potassium, calcium, phosphorus, total protein, albumin, total bilirubin, alkaline phosphatase, alanine aminotransferase, gamma glutamyl transferase, chloride, total CO₂, and creatine kinase and were performed in 13 cats and 8 dogs, whereas in 8 cats analysis of serum total thyroxine concentration was also performed. Among the cats, 1 had hypercalcemia (3.03 nmol of calcium/L; reference range, 2.26 to 2.74 mmol/L); 2 had hyperthyroidism and high liver enzyme activities; and 1 had high serum concentrations of total protein and globulin and an altered albumin-to-globulin ratio. Two cats had hyperphosphatemia (2.00 and 2.14 mmol of phosphorus/L; reference range, 1.02 to 1.98 mmol/L).

Among the dogs, 2 with CNS signs had high total CO₂ concentration (24 mmol/L; reference range < 1 year of age, 16 to 23 mmol/L) and 31 mmol/L (reference range > 1 year of age, 13 to 26 mmol/L). One dog that had received corticosteroids had high serum activities of liver enzymes.

The bird had nonregenerative anemia (PCV, 0.28 L/L; reference range, 0.40 to 0.55 L/L). All 17 cats tested for FeLV yielded negative results. Sixteen of the 17 cats tested for FIV yielded negative results. An equivocal result was recorded for 1 cat because of interference from a high globulin concentration. Western blot confirmation testing on this cat yielded positive results for FIV. The remaining 3 cats were of unknown status. One cat had a low-positive IgG toxoplasmosis titer. This cat had also received corticosteroids.

**Cytologic examinations**—Most of the organisms were readily identified as large structures with clear halos and central yeast bodies with narrow budding. The central yeast bodies varied in diameter from 3 to 12 µm. Two cytologic specimens had organisms with scant capsules and variably sized yeast bodies with narrow budding. Both of these yielded *C neoformans* var *grubii* (serotype A) via culture (Figure 1), and both also failed to develop the large characteristic capsule. Although organisms in the remainder of the cytologic specimens had typical cryptococcal morphologic features, variably sized organisms were commonly seen, and the size variation often reflected the amount of capsular material that surrounded the yeast bodies (Figure 2). Variable numbers of WBCs were evident in the aspirates and included macrophages, lymphocytes, and neutrophils.

![Figure 1](image-url)
Histopathologic findings—Histologic examination revealed that the organisms appeared similar to those seen cytologically; only 1 specimen had marked variations in the capsule thickness. Among 6 animals from which multiple tissues were collected at necropsy, 3 cats had extensive lung involvement, whereas only 2 of 3 animals (1 dog and 1 cat) in which the sinus and nasal cavity were evaluated had extensive sinusitis. Cryptococcal meningitis with variable extension into the brain parenchyma was detected in 5 of these 6 animals (brain tissue was not received for examination in 1 animal). The organisms were in highest concentrations in the region of the olfactory bulbs and along the ventral portion of the cerebrum, extending past the optic chiasma and to the midbrain; dorsal extension along the meninges to the cerebellum was also observed. None of the animals had other disease processes in the multiple organ systems evaluated. Two cases involved the upper portion of the small intestine, with a fungal granuloma in ulcerated areas of intestinal mucosa and extending through the intestine to the serosal surface. The 4 skin-associated granulomas had occasional small epidermal ulcerations with the bulk of the granuloma in the deep dermis and panniculus and variable extension into underlying muscle bundles.

Immunoperoxidase testing with a panel of monoclonal antibodies was performed on specimens from 10 animals, 3 of which had yielded Cryptococcus neoformans var gattii (serotype B); immunoperoxidase testing identified organisms as Cryptococcus sp (serotype A) in all 10 animals.

Serologic testing—Antigen tests were performed on 22 of 38 animals. One cat with respiratory tract signs and treatment with fluconazole at the time of testing and 1 dog with CNS signs yielded negative results. Titers varied from 1:2 to 1:20,000 (the maximum dilution performed). One of the animals with a titer of 1:20,000 was a ferret. Overall sensitivity of serologic testing was 91.6%. Specificity was calculated by use of bacteriologic culture of the nasal cavity in 50 animals without clinical signs and performing antigen testing on sera from blood collected at the same time. The specificity was 98% when positive results of culture were considered diagnostic. The 1 false-positive result was positive only at a dilution of 1:2. Preliminary information regarding animals receiving treatment indicated that the titer may increase initially after treatment (within the first month) and then decline, although titers did not become negative during treatment (the longest period of treatment was 5 months and continuing, at the end of this study).

Microbiologic culture—Microbiologic cultures were performed in 1 bird, 8 cats, and 6 dogs. Two animals yielded Cryptococcus neoformans var grubii serotype A, whereas the others including the bird yielded Cryptococcus neoformans var gattii serotype B. The serotype A isolates were from animals from the metropolitan Vancouver area. All serotype B isolates were from Vancouver Island.

Results of minimum inhibitory concentration determinations on the 15 isolates indicated all were susceptible to amphotericin B and ketoconazole. The susceptibility pattern for fluconazole was 68% susceptible, 26% intermediate susceptibility, and 6% resistant. The susceptibility pattern for itraconazole was 67% susceptible and 33% intermediate susceptibility. The susceptibility pattern for flucytosine was 67% susceptible, 20% intermediate susceptibility, and 13% resistant.

Survival status—At the time of this report, 19 of 38 animals were alive and had been treated via surgical resection of cutaneous granulomas and various combinations of antifungal drugs. Twelve of the 19 animals that died were euthanatized as a result of neurologic signs. Six animals were given corticosteroids, and 5 of these 6 animals developed rapid progression of neurologic signs and were euthanatized.

Discussion

The clinical signs of affected animals in southwestern British Columbia were most often associated with the CNS, which is different than those reported for C neoformans var gattii (serotype B) infection in Australia and C neoformans var neoformans infection in other parts of North America. Only 20% of the cats in our study had upper respiratory tract signs, compared with a large Australian study11 in which 61 of 153 cats had only respiratory tract signs. The dogs in our study consistently had clinical signs attributed to neurologic disease, whereas in the Australian studies,11,23 only 31% of dogs had signs of disseminated disease that included neurologic signs and 61% predominantly had clinical signs of nasal disease. The ages of dogs and cats in our study were similar to ages of dogs and cats in other studies,10,11,12 but affected dogs in our study were almost as numerous as cats, which is not typical. This may reflect the unique environment of the region or be related to the influence of regional genetics on the animal populations.

Among 6 animals for which lung tissue was submitted for histologic examination, 3 (all cats) had invasion of the lung parenchyma by the organisms. In Australian studies,11,23 pulmonic lesions were found in only 3 of 133 cats. Pulmonary involvement is considered rare and has been attributed to the size of the organisms, which makes inhalation and pulmonary
colonization difficult. One report from North America did describe 6 of 9 cats with lung lesions, which is consistent with the finding in our study. In humans, pulmonary colonization is an integral part of the pathogenesis of the disease.

Environmental factors that differ between the habitats of C. neoformans var. gattii (serotype B) in British Columbia, compared with Australia, not only relate to the different trees involved but also to growth substrate. In Australia, the organism exists in highest concentration in dead plant material in the hollows of eucalyptus trees, whereas in British Columbia, the organism is consistently found in the living bark of trees as well as in the air. Because the organisms are readily cultured from air samples, it seems possible that particles are smaller or substantially greater in number, compared with those in Australia, which may make inhalation a more common process.

It is of interest that C. neoformans var. grubii was cultured from animals in the greater Vancouver metropolitan area, whereas C. neoformans var. gattii has not been cultured from any animals within this region. This suggests that there may be unique environmental factors in 1 locale, although climatic conditions are not substantially different. Furthermore, results of current research in Australia do not support differences in temperature, climate, or latitude as influencing the growth of C. neoformans var. gattii (serotype B).

In Australia, the inner city isolates are typically C. neoformans var. grubii, whereas the rural isolates are typically C. neoformans var. gattii. The population base in the metropolitan Vancouver region is higher than that on Vancouver Island, but cases of C. neoformans var. gattii infection were identified within the metropolitan area of a major city on Vancouver Island.

Disease in most mammalian species does appear to be directly related to the environmental concentrations of the organisms, but environmental factors that favor increased numbers are still uncertain. In Australia, koalas may function as a reservoir and maintain environmental numbers of C. neoformans var. gattii, but no wildlife reservoir population has been identified in British Columbia. Environmental studies are in progress to assess temperature, precipitation, other organisms, and competition between species that may contribute to this increased incidence.

Increased numbers of cryptococcal infections were first detected in August 2000 and reported to the British Columbia Centre for Disease Control in May 2001. The number of cases has continued to increase with 38 cases in the 6-month period of our study, compared with 45 cases diagnosed between late 2000 to March 2002. The reason for the sharp increase in cases is unknown.

The actual number of cases within the animal population is likely higher than the 38 cases reported here because the organisms have characteristic morphologic features, and many cases are diagnosed with in-clinic cytologic examination or by other laboratories in this region. The increase could also reflect increasing awareness of the disease and its manifestations or an altered virulence pattern of the organism.

Until recently, cryptococcosis has not been a prominent differential diagnosis for animals with neurologic disease, and screening tests for cryptococcal antigen were uncommon. Many animals may have been euthanatized for neurologic disease without further diagnostic testing; in our case series, 2 young dogs had respiratory tract and neurologic signs consistent with canine distemper. However, the earlier report of the disease increase in British Columbia only included 2 animals with CNS signs.

The increase in CNS signs could also reflect virulence factors of the organism. The virulence of Cryptococcus spp is related to a number of factors, of which the capsule is probably the most important. Opsonins and a cell-mediated immune response are required to bind the organism’s capsule to macrophages, and without these there is decreased clearance of the organism by the body. Changes in the immune system’s recognition of the capsular antigens could alter response to the organism; thus, genetics and species differences may play a role in virulence. Further influences may involve the innate immune system and neutrophilic responses to the organism. In a rat-human model, there are differences between strains in the proinflammatory properties and neutrophilic response in lung tissue.

In contrast with our findings, the incidence of cryptococcosis in the human population has not risen as steeply as in animals; in a similar time frame, only a fourth the number of cases were reported in the human population. As with previous studies, there is no evidence that infected animals are involved in transmission of the disease to humans, but rather animals are responsible for the same environmental factors that also affect humans and thus serve as a sentinel species.

Colonization of the nasal cavity has been detected in animals and humans that do not have clinical disease, which could indicate that they are subclinical carriers or might develop disease in the future. Although the exact mechanisms of extension of infection to the CNS are unknown, it is suggested that extension is the result of invasion of the cribiform plate or along the optic nerves. Changes in hematologic and serum biochemical variables were nonspecific and did not aid in the diagnosis of the disease. In this study, the only cat with substantially high globulin concentration was infected with Cryptococcus neoformans var. grubii. This was also the only cat with a confirmed positive result of an FIV test. Two of the 4 cats with specific upper respiratory tract involvement, more typical of the classic disease in cats, were also infected with this organism.

Serologic testing was informative throughout this disease outbreak. The sensitivity of the antigen test was such that only 2 false-negative results were encountered. The cause of false negatives is unknown, but it has been speculated that low antigen concentrations in circulation attributable to localized lesions, timing of the sampling, prozone-type effects, or interfering factors that have yet to be determined may be involved. The test detects capsular antigen, and the cryptococcal organisms shed this capsular antigen after colonization occurs. The time required for the organism to begin shedding the antigen into the circulation appears variable, and colonization of the respiratory tract without
disease or antigenemia has also been detected.\textsuperscript{11,12,17} Specificity of the testing was such that 1 false-positive result was encountered in a healthy animal in which results of nasal culture were negative. These results are consistent with previous findings that indicate test sensitivity of 90% to 100% and specificity of 95% to 98%.\textsuperscript{6,22} Antigen titers are also used to measure responses to treatment, and declining titers or return to a negative titer are considered indicators of a good prognosis.\textsuperscript{5,6,13,24}

The ages of the dogs and cats in this cohort were consistent with reports\textsuperscript{12,13,14,23} in which it is suggested that younger dogs and older cats are more commonly affected. In British Columbia, this difference in ages is more likely to reflect behavior than unique species differences. Young active dogs are more prone than older dogs to habits that include digging, sniffing, and rolling in rotten vegetation. Cats may have an increased chance for aerosol infection when the dirt is drier in the later spring and summer months, compared with other seasons.

Although cryptococcosis in humans is often associated with immunocompromise, only 1 cat in our study had serologic evidence of a virus associated with immunocompromise. The cats were not antigenic for FeLV. Among animals for which multiple tissues were available, no other disease processes were recognized. The cat with an IgG titer for toxoplasmosis had a low titer, which is indicative of prior exposure rather than disease. The cat had been treated with corticosteroids, so the low titer may have resulted from reactivation of a latent infection, but paired titers or IgM titers were not evaluated. The behavior of Cryptococcus spp in this group of animals was as a primary pathogen rather than an opportunist.

Results of the immunohistochemical procedure used in this study confirmed the identity of the yeast as Cryptococcus spp but were discordant with the culture results. The procedure does not include monoclonal antibodies against C. neoformans var. gattii; the diagnosis of infection with C. neoformans var. gattii is based on exclusion of the other varieties. In the 3 instances in which there was discrepancy between test results, both culture and serotyping confirmed the organism as C. neoformans var. gattii and these are considered the gold standard for identification of the varieties of Cryptococcus spp. The immunohistochemical method had previously been used only on organisms from Australia. In Australia, almost all of the C. neoformans var. gattii are classified as genomic group VG1,\textsuperscript{1} whereas those from British Columbia are VG11.\textsuperscript{5} In British Columbia, the organism also appeared to be a recombinant organism with a predominance of the α mating type.\textsuperscript{22} Small changes in the capsular antigens as a consequence of these differences could have an effect on the specificity of the monoclonal antibodies.\textsuperscript{12,24} Results of immunohistochemical testing, rather than being correct or incorrect, may substantiate the accumulating evidence of the difference between the organisms involved in British Columbia versus Australia.

In few of the animals were unusual sites affected at the time of evaluation. Two animals had primary intestinal lesions, which supports intestinal entry of the organism in limited numbers. Primary skin lesions were uncommon in this study, and there were no historical data to suggest primary penetrating wounds, so dissemination from other sites was considered the likely source for the skin granulomas.

The use of corticosteroids had a negative effect on all affected animals and resulted in euthanasia in 5 of 6 animals. Corticosteroid-induced interference with the immune response likely permitted widespread dissemination of the organisms throughout the CNS.\textsuperscript{5}

Two animals had cytologic findings inconsistent with typical cryptococcal organisms, and culture results were necessary to confirm the diagnosis. Although the literature mentions variations in capsular size, published reports that describe this unusual variant could not be found.\textsuperscript{19}

Antifungal treatment was reasonably effective in the treatment of these animals, although many had initial CNS signs. Treatment consisted mainly of administration of various imidazole or triazole derivatives. Ketoconazole and amphotericin B yielded the best in vitro susceptibility patterns, with no isolates resistant to either drug. To our knowledge, the use of MICs for these drugs has not been previously reported in animals and the clinical implications of MICs are still uncertain. The animals that received drugs for which MIC data were available received drugs that yield good in vitro susceptibility patterns. It is hoped that the information provided by the MICs will allow further investigation of the in vivo proficiency of these antifungal agents. Long-term administration appears necessary because animals in our study that received these drugs for up to 3 months still had serologic titers, although marked clinical improvement was evident.\textsuperscript{20} On the basis of the MIC tests, flucytosine was the least effective, but this drug was not used in our study. Flucytosine is a product for which innate and acquired resistance in Cryptococcus spp has been reported.\textsuperscript{30}

Flucytosine has also been reported as a cause of drug-induced cutaneous reactions in dogs.\textsuperscript{29} Cryptococcus neoformans var. gattii appears to have developed in an ecologic niche in southwestern British Columbia and particularly Vancouver Island. The clinical signs were different than those reported in other studies, being predominantly specific and nonspecific CNS signs. Veterinarians need to be aware of this disease in animals that live in or may have traveled to this region, just as they are aware of other specific locations in North America where fungal diseases are endemic, such as southern California for coccidioidomycosis or the Mississippi valley region for blastomycosis. The early use of serologic titers to detect the disease may increase diagnostic acumen as well as influence survival and response to treatment. From the conclusion of this study on June 30, 2003, to October 31, 2003, an additional 30 infected animals were detected; fungal culture identified C. neoformans var. gattii in 28 animals from Vancouver Island and C. neoformans var. grubii and C. neoformans var. neoformans in 1 animal each from the greater Vancouver region.

\textsuperscript{1}Iatron Laboratories, Mitsubishi Kagaku Iatron Inc, Tokyo, Japan.
\textsuperscript{2}CellDyn 3500, Abbott Diagnostics, Santa Clara, Calif.
\textsuperscript{3}Dimension RxL, Dade Behring, Newark, Del.
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