Surveillance for *Cryptococcus gattii* in horses of Vancouver Island, British Columbia, Canada

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In the last decade *Cryptococcus gattii* has emerged as an important human and animal pathogen in southwestern British Columbia (BC), Canada. When the disease initially emerged it was identified in humans and multiple animal species on the east coast of Vancouver Island. From fall 2003 until summer 2004, active surveillance was initiated to look for horses exposed to or infected with the organism by performing nasal cultures and serum antigen testing in horses residing within 10 km of known areas of environmental reservoirs of the fungus. Surveillance efforts were facilitated by local equine practitioners who were also encouraged to report clinical cases. Nasal colonization was identified in four of the 260 horses tested but none had a serum cryptococcal antigen titer. All positive horses were from the same geographic area near Duncan, BC. During the study period, a single horse was diagnosed with systemic cryptococcosis and euthanized; clinical and post mortem information is described. As this organism continues to disseminate in the Pacific Northwest it is important for veterinarians to be familiar with the disease as early diagnosis may enable more effective treatment.

**Keywords** *Cryptococcus gattii*, horse, surveillance

Introduction

Cryptococcosis has been described in a wide variety of host species affecting many, and often multiple, organ systems. In horses, there are case reports describing pneumonia, rhinitis, meningitis, sinusitis and abdominal cryptococcal granulomas [1–6]. *Cryptococcus* has also been isolated from the equine reproductive tract and reported to cause abortion in mares [7–9]. The majority of literature focuses on individual clinical cases while less is known about the epidemiology of the disease in horses.

*Cryptococcus gattii* has emerged as a significant cause of morbidity and mortality of humans and animals in southwestern British Columbia (BC) [10–12]. Previously only *C. neoformans* had been isolated, while *C. gattii* was thought to be restricted to the tropics and sub tropics [13,14]. Clinical illness has been identified in numerous animal species in BC including cats, dogs, ferrets, llamas, porpoises and birds [11,12]. Cases initially clustered on the east coast of Vancouver Island, largely within the coastal Douglas fir (CDF) biogeoclimatic zone where *C. gattii* had been isolated from soil, air and vegetation since 2001 [10,15]. Since its initial detection on Vancouver Island, cases involving this *Cryptococcus* species have been increasingly diagnosed on mainland of BC and USA [16].

Given the airborne nature of the organism, it may be assumed that many host species residing within endemic areas are exposed, but during the initial investigation into *C. gattii* in BC no equine cases were identified. Asymptomatic carriage of *C. gattii* has been recognized in companion animal species of BC [17] with most of the identified individuals remaining asymptomatic but others progressing to disease [18]. Environmental exposure and asymptomatic colonization of the respiratory tract has been proposed to...
Results

Cross-sectional study

Nasal swabs and serum samples were collected from 260 horses residing within 10 km of a site where the fungus had been isolated from the environment. No horses were positive on the antigen test, but C. gattii was isolated from the nasal passages of four horses. The positive surveillance cultures ranged from 1+ to 2+ growth using the described a semi-quantitative scale and were positive for antibody 1 (cryptococcal antigen) and antibody 5 (serogroup B) but negative for antibody 7 (C. neoformans var. grubii) and antibody 8 (C. neoformans var. neoformans). All positive horses resided in the area of Duncan, BC, Canada.

All positive horses, except for one, had lived on Vancouver Island their entire lives. The exception was a horse that had moved to the island approximately six months prior to testing and resided only in the Duncan area during that time. The median duration of residence on Vancouver Island was 5 years (minimum 3 months, maximum 9 years) for the cryptococcal positive horses and 8 years (minimum 3 months, maximum 32 years) for the negative horses; a difference that was not statistically significant (P = 0.20). The median duration of the residency of positive horses on the property where testing occurred (2.25 years, minimum 3 months, maximum 6 years) did not differ significantly from that of negative horses (3 years, minimum 3 months, maximum 23 years). The four positive horses were 4, 6, 9 and 10 years of age (median age of 7.5 years) which did not differ significantly from the horses that proved to be negative for the presence of Cryptococcus (12 years, minimum 3 months, maximum 35 years, P = 0.20). The proportion of positive horses fed on the ground did not differ statistically from...
the negative horses \((P = 0.26)\). Owners of positive horses did not report any illness or historical medical problems.

**Clinical disease**

During the period of study, a 4-year-old Quarter Horse mare from the area of Duncan, BC, was referred with a 5-month history of respiratory disease. The horse had been treated with trimethoprim/sulfamethoxazole and ampicillin/sulbactam benzathine with no improvement. Results of a transtracheal wash performed four days prior to presentation revealed a highly cellular sample with the majority of cells being non-degenerate neutrophils and low numbers of macrophages. There were moderate numbers of pleomorphic fungal yeasts with thick capsules consistent with *Cryptococcus* spp. (Fig. 2). The yeast was cultured and further identified as *C. gattii* which was found to be sensitive to amphotericin B, fluocytosine and ketoconazole and intermediate sensitivity to itraconazole and resistant to fluconazole.

On admission the horse was in poor body condition with partial anorexia. It exhibited tachycardia (48 beats/min), tachypnea (50 breaths/min) and a rectal temperature of 38.3°C. There was severe respiratory distress with marked wheezes and crackles over both lung fields and fluid tracheal sounds. A complete blood count showed a marked leukocytosis \((29.1 \times 10^9/l, \text{reference } 4.2–11.0)\) with a neutrophilia \((25.3 \times 10^9/l, \text{reference } 1.8–7.5)\) and monocytosis \((2.0 \times 10^9/l, \text{reference } 0.0–0.46)\) and fibrinogen (semi-quantitative) of 7.0 g/l \((0.5–4.0 \text{ g/l})\). Serum chemistry revealed a low AST \((145 \text{ iu/l, reference } 170–435)\) and low serum iron \((14 \text{ umol/l, reference } 17–37)\). The horse had a positive CALAS test with a titer of 1:8000. Thoracic radiography was performed and majority of the lung fields were obliterated by diffuse nodular opacities. Inhaled salbutamol treatment did not improve the respiratory distress. Given the poor prognosis and severity of disease, the owner elected euthanasia on humane grounds.

On post mortem examination the horse was in poor body condition with generalized muscle atrophy. Within the thoracic cavity there was massive, bilateral enlargement of the lung lobes and prominent rib impressions (Fig. 1). Change was more severe on the left side, which weighed 28.5 kg, relative to the right at 12.8 kg. On cut surface of the lobe there was replacement of the parenchyma, with numerous variably sized pale to bright yellow mucoid, finely encapsulated masses which compressed intervening lung parenchyma. There was moderate enlargement of hilar lymph nodes which were pale grey brown, glistening and occasionally nodular. Along varying levels of the bronchi and to a much lesser extent within the trachea, there are small to intermediate size mucoid brown yellow deposits. Portions of the lungs were cultured and *C. gattii* genotype VG IIb identified by URA5 RFLP was isolated.

Histologically the lung masses were consistent with aggregates of pyogranulomatous inflammation admixed with sheets of fungal yeasts morphologically consistent with *Cryptococcus* spp. (Fig. 2). Hilar lymph nodes were expanded by similar inflammation, fungi and reactive lymphoid follicles. The meninges of the spinal cord were expanded by fibrin, mixed inflammation and fungal organisms. Fungal elements were observed within the vasculature of the small intestine, kidney and brain.

There was a mild, acute gastritis that was associated with intralesional yeast morphologically consistent with candidiasis. Throughout both the small and large intestine the lamina propria was expanded by subacute, eosinophilic inflammation with scattered crypt abscessation and hyperplastic gut associated lymphoid tissue. Small foci of

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**Fig. 1** Left lung field with multifocal to coalescing areas of pyogranulomatous inflammation and intralesional yeast.

**Fig. 2** Histopathology of nodule from lung. There is a rim of pyogranulomatous inflammation surrounding sheets of fungal yeasts (H&E, 2X). Insert: Yeast range from 4–8 μm in diameter with a thick (5–20 μm) negative staining capsule. Histiocytic cells and minimal supporting stroma are also present (H&E, 20X).
chronic, lymphoplasmacytic inflammation were observed within the renal interstitium and the vaginal mucosa. Within the liver there was mild biliary hyperplasia with bridging fibrosis. Sarcocystis spp. were identified within skeletal muscle but were devoid of associated inflammation.

Discussion

In this cross-sectional study, *C. gattii* was present on 1.5% of equine nasal swabs. As cryptococcal antigen was not identified in serum samples collected from any of the four horses, identification of *C. gattii* in the nasal passages of the animals is likely the result of environmental exposure or nasal colonization but not systemic infection. Investigations into subclinical infections in companion animals identified 4.3% of cats and 1.1% of dogs residing within the CDF zone of Vancouver Island had *C. gattii* in their nasal cavity [17]. The prevalence of nasal colonization observed in horses in this study is similar to that of companion animals but only this one described clinical case of equine *C. gattii* was reported on Vancouver Island during the time of this surveillance. This discrepancy may reflect differing species susceptibility to clinical disease or failure to diagnose clinical cases because they are not being seen by veterinarians or the diagnosis is being missed. In companion animals, feline cases outnumber those in canines by over 50% suggesting a variation in species susceptibility [21]. The paucity of equine cases in BC relative to those in companion animals differs from a retrospective review of cases in Western Australia where horses were twice as likely as dogs to be infected with *C. gattii* [6]. The reason for this discrepancy is unclear, but given the non-reportable nature of the disease in both countries along with inherent biases involving cross species comparisons using a hospital diagnosis inclusion criteria, generalizations should be made with caution.

All four asymptomatic horses as well as the clinical case were from the same geographic location. The city of Duncan is central in the region in which clinical cryptococcosis cases have been reported and a cross-sectional study in dogs and cats identified Duncan to have a higher proportion of colonized or sub-clinically infected animals [17]. One of the horses lived directly adjacent to a site in which two *C. gattii* positive squirrels were trapped [22]. Environmental *C. gattii* has not been recovered ubiquitously on Vancouver Island [10] and investigations have indicated increased environmental concentrations of *C. gattii* in soil samples collected from the Duncan area relative to most other parts of Vancouver Island [15].

Age of horse, breed, underlying health problems, duration at sampling location and on Vancouver Island, average time spent outside per 24-h period, source of hay and feeding methods were not identified as statistically significant risk factors for nasal colonization with *C. gattii* in this study. It is important to note however that there were not enough positive horses in this study to make significant conclusions regarding risk factors. While geographic location relative to environmental organism is likely the most significant variable influencing exposure, it is important to identify other risk factors, if present, such that owners and veterinarians can attempt to mitigate risk where possible.

Clinical disease and post mortem findings in the described single clinical case was severe and focused on the respiratory tract suggesting infection was likely through aerosolization, invasion and proliferation within the lung and subsequent hematogeneous dissemination. Serial, 2–3 cm wide sections of the entire length of the nares failed to reveal any upper respiratory tract involvement and there was no apparent infection localized to the retropharyngeal or mesenteric lymph nodes. The reactive lymph nodes and peripheral leukocytosis are attributed to persistent antigenemia (fungemia). The interstitial nephritis is non-specific, chronic and unrelated to the associated yeast within arcuate arteries. Although there were no parasites detected in fecal floatation, the eosinophilic enterocolitis is likely due to intestinal helminths. The weight loss and associated muscle atrophy could be attributed to generalized illness associated with the mycosis; complete gross and histologic evaluation of multiple tissue types failed to reveal any pre-existing diseases which may have predisposed this animal to infection.

The clinical case described in this report is thought to be one of the first cases of equine *C. gattii* infection recognized on Vancouver Island. As with companion animal cases, the initial presenting complaints were non-specific and more invasive diagnostics were required to make a definitive diagnosis by which time the organism was widely disseminated throughout the pulmonary parenchyma and associated pathology was severe. While there is a published report of successful treatment of pulmonary cryptococcosis in a pony [23], little is known about treatment of these cases and the cost of medication may be cost prohibitive in large horses. While cryptococcosis remains an uncommon equine disease in British Columbia, the expanding geographic and environmental niche of the organism necessitates awareness on the part of horse owners and regional equine practitioners such that early diagnosis is facilitated and more information on therapeutic strategies is available.

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References


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