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Cryptococcosis in domestic animals in Western Australia: a retrospective study from 1995–2006

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A retrospective study of cryptococcosis in domestic animals residing in Western Australia was conducted over an 11-year-period (from 1995 to 2006) by searching the data base of Murdoch University Veterinary Teaching hospital and the largest private clinical pathology laboratory in Perth. Cryptococcosis was identified in 155 animals: 72 cats, 57 dogs, 20 horses, three alpacas, two ferrets and a sheep. There was no seasonal trend apparent from the dates of diagnosis. Taking into account the commonness of accessions to Murdoch University, cats were five to six times more likely to develop this disease than dogs, and three times more likely than horses, while horses were almost twice as likely as dogs to become infected. Amongst the feline cohort, Ragdoll and Birman breeds were over-represented, while in dogs several pedigree breeds were similarly overrepresented. Dogs and horses tended to develop disease at an early age (one to five years), while cats were presented over a much wider range of ages. In cats and dogs the upper respiratory tract was the most common primary site of infection, while horses and alpacas tended to have lower respiratory involvement. The most striking finding of the study was the high frequency with which *C. gattii* was identified, with infections attributable to this species comprising 5/9 cats, 11/22 dogs, 9/9 horses and 1/1 alpaca, where appropriate testing was conducted. Preliminary molecular genotyping suggested that most of the *C. gattii* infections in domestic animals (9/9 cases) were of the VGII genotype. This contrasts the situation on the eastern seaboard of Australia, where disease attributable to *C. gattii* is less common and mainly due to the VGI genotype. *C. gattii* therefore appears to be an important cause of cryptococcosis in Western Australia.

Keywords *Cryptococcus gattii*, *Cryptococcus neoformans*, cryptococcosis, animals, VGII

Introduction

Cryptococcosis is the most common systemic mycosis in Australia [1], affecting humans and a wide range of domestic and native animals. The disease is caused by members of the *Cryptococcus neoformans* species complex, which are basidomycete yeasts that are encapsulated, capable of growth at 37°C, produce melanin and cause disease in mammalian hosts. The infectious propagules are believed to be sexually or asexually produced spores, which transform into budding capsulated yeasts during the infectious process [2]. Until recently, *Cryptococcus* species of veterinary importance were classified as *C. neoformans* var *neoformans* (serotypes A, D and, AD) and *C. neoformans* var *gattii* (serotypes B and C) [3,4]. This nomenclature has been revised with *C. neoformans* var *gattii* being

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proposed as a new species, *C. gattii* (teleomorph *Filobasidiella bacillispora*), including serotypes B and C [5]. *C. neoformans* has been divided into varieties *C. neoformans* var. *grubii* (serotype A) and C. *neoformans* var. *neoformans* (serotype D) [2]. Both of these varieties are worldwide in distribution and are associated with pigeon guano and soil enriched with avian excreta [1]. *C. neoformans* has also been shown to be present in decaying plant matter of certain trees [6,7]. The distribution of *C. gattii* was initially thought to be restricted to tropical or subtropical climates [8] and associated with eucalyptus trees, especially *E. camaldulensis* [7]. Recently, however, *C. gattii* has been identified as the cause of disease outbreaks in temperate parts of Canada [9–12]. Indeed on close inspection of the literature, *C. gattii* has a very wide geographical distribution including Europe, the Indian subcontinent and many South American countries [4].

There is some variation in the histopathological features of the host response to cryptococcosis in different animals. The route of infection is similar amongst host species, with *Cryptococcus* spp. acquired from the environment primarily through the inhalation of basidiospores, ingestion of desiccated yeast cells [13], or more rarely,direct cutaneous inoculation [2,14]. Direct transmission between mammals has not been observed, and therefore the disease is not considered to be contagious or zoonotic [2,4].

In humans, infection is thought to begin in the lower respiratory tract. In many individuals the infection is self limiting, presumably due to development of effective adaptive immunity [15,16]. However, in some individuals, either soon after infection or after a highly variable lag period, haematogenous spread, especially to the meninges and central nervous system (CNS), may occur. Symptoms (headache, neck pain visual disturbances) are largely referable to involvement of the brain, whereas the primary pulmonary lesion is often clinically silent [1]. The lungs also appear to be the primary site of disease in horses and goats, with cryptococcal pneumonia being the predominant form of disease in these species [17–24]. However, deforming rhinitis has also been reported [23,25–27]. In horses with cryptococcosis, as in humans, the infection can spread from the lower respiratory tract, with the development of meningitis [28–31], osteomyelitis [32] and abdominal disease [22,26]. There is very little data available regarding cryptococcosis in South American camelids [11,33–35], but it appears these may be infected by a mechanism similar to horses and goats.

In contrast, cats and dogs appear to display a different pathogenesis, with initial localization of infection in the nasal cavity and paranasal sinuses. In these animals, cryptococcosis is often confined to the upper respiratory tract, although the infection may extend locally to contiguous structures, into the CNS (typically via the cribriform plate) or to the lower respiratory tract [36]. It may also spread haematogenously from the primary focus to other organs, although in cats this is generally seen only in the setting of immunodeficiency [2,36,37]. The reason for the variability in pathogenesis between different species is unknown but may be related to the variation in size and complexity of nasal turbinate structures [2], efficiency of the mucociliary clearance mechanisms and the cough reflex, length of the trachea or behavioral exposure to different types of infectious propagules [37]. The koala [38] and ferret [39] can have protean manifestations of cryptococcal disease, although both species often have primary infection of the upper or lower respiratory tract as the first disease feature. Cattle, on the other hand, tend to develop cryptococcal mastitis as a result of ascending infection of the mammary gland via the teat canal [40–42].

Unlike other systemic mycoses, cryptococcosis is more common in cats than in dogs [37,43]. Documented feline cases have shown a preponderance of males [43,44], an over-representation of certain pedigree breeds [36,44] and a broad range of ages [44]. Cats predominantly have sino-nasal disease, either alone or together with local spread to the skin/subcutis of the nasal planum/nasal bridge, regional lymph nodes [44] and less commonly to the CNS and optic nerves via the cribriform plate [37]. Response to treatment in cats has been reported to be good [37,44], especially in cases without CNS involvement. Canine cryptococcosis usually affects young, large breed dogs with Great Danes, Dobermans, German Shepherds [36,45,46] and American Cocker spaniels [47] over-represented. There is no gender predilection, and like cats, dogs often present with sino-nasal disease [36,45]. Canine cryptococcosis, in contradistinction, has a propensity to disseminate early, resulting in a larger percentage of neurological, ocular or disseminated infections [36,45–47]. Accordingly, there is a poorer prognosis and response to therapy. Disease in horses is reportedly less common than in other companion animals, mainly affecting the young, large breeds such as Thoroughbreds and Standardbreds [22]; lower respiratory [18–22], CNS [22,28–31,48] and disseminated disease [22,26,32] predominates in this species, at least in Western Australia, and infection is often associated with poor outcomes [19,20,22,25,26,28,32,49].

Anecdotally and from limited case reports, cryptococcosis appears to be common in both humans and animals in Western Australia, occurring in novel species

(goats [23], sheep [50], potoroos [51] and horses [19,22,32]) and with a number of unique disease features and associations [50]. This report is a retrospective study designed to identify the clinicopathological and epidemiological factors associated with naturally-occurring cryptococcosis in domestic animals in Western Australia and to compare the findings within and between species. The results are compared with previous reports of cryptococcosis in domestic animals from the east coast of Australia.

Material and methods

Evaluation of cases

Case records of animals presenting to Murdoch University Veterinary Hospital (MUVH) between 1995 and 2006 (inclusive), and sample submissions to a commercial diagnostic laboratory used extensively by WA practitioners (Vetpath Laboratory Services [VLS]), between 1998 and 2006, were identified retrospectively. Animals were included in the study if they had (i) a presumptive diagnosis of cryptococcosis based on cytological or histological evidence of infection (capsulated yeasts demonstrating narrow neck budding) or (ii) immunohistochemical identification of cryptococcal epitopes in biopsy specimens, (iii) positive fungal culture from a normally sterile site (iv) positive latex cryptococcal antigen agglutination test (LCAT) on serum or cerebrospinal fluid (CSF).

The dates of presentation and diagnosis, age, breed and gender of the patient, anatomical distribution of lesion(s), species of *Cryptococcus*, molecular genotype (when cultures were available), geographical location where the animal resided, treatment details and clinical outcome were entered into a spreadsheet (Excel™; Microsoft) and compared within and between species. Retroviral status in cats and latex cryptococcal antigen agglutination test (LCAT) results were also tabulated. The data from cases identified at MUVH were generally complete, whereas case notes retrievable through VLS records were limited; in particular, outcomes were difficult to determine.

Clinicopathological tests

Samples collected from affected animals were submitted for cytology, mycology, histopathology and serology. These included (i) nasal secretions collected on pre-moistened swabs, (ii) specimens collected from nasal, tracheal and bronchoalveolar washings, (iii) needle aspirates from cutaneous and subcutaneous masses, enlarged lymph nodes and pleural or peritoneal effusions, (iv) biopsies taken from affected tissues antemortem or at necropsy (v) cerebrospinal fluid (CSF) collected from the cerebello-medullary cistern or the lumbosacral space and (vi) serum collected for determination of an LCAT or feline retroviral status.

Cytological evaluation was performed on smears stained with Diff Quik, Leishman's or May-Gruenwald Wright's stains and examined for the presence of inflammatory cells and/or encapsulated yeasts. Samples were inoculated onto Sabouraud's dextrose agar plates containing 0.1 g/l gentamicin and 0.05 g/l chloramphenicol and incubated at 25°C and 37°C for a minimum of 10 days. If the sample was from a normally sterile anatomical site (e.g., CSF, body cavity centesis), fungal culture was performed by inoculation onto Sabouraud's dextrose agar [1]. In recent years samples were also inoculated onto bird seed agar plates and incubated at 28°C [52]. Identification of the species of *Cryptococcus* was performed by demonstration of typical colony morphology, capsule formation, urease production at 25°C, the ability to grow at 37°C, brown-colour-effect on bird seed agar and microscopic examination [1]. Commercial identification systems (API 32C, Biomerieux, France) and caffeic acid agar [53] were used in some cases to confirm the identification of *C. neoformans* and *C. gattii*. Growth on CGB (L-canavanine, glycine and 2-bromothymol blue) agar [54] and immunohistochemistry on tissue sections [55] was used for species differentiation. Biopsy samples were processed in a routine manner and stained with haematoxylin and eosin (H&E). Mayer's mucicarmine stain and/or Periodic Acid Schiff were also used to highlight capsulated organisms in tissues. Commercially available test kits were used to detect cryptococcal capsular polysaccharide antigens in serum using latex agglutination following pretreatment of serum by heat and pronase. Retroviral status of cats was determined using rapid immunomigration (RIM) and enzyme linked immunosorbent assay (ELISA) kits (Crypto-LA test, Walpole laboratories, Princeton; Cryptococcal Antigen Latex Agglutination System; Meridian Diagnostics, Cincinnati, OH) which detect feline leukaemia virus (FeLV) core antigen and serum antibodies to certain feline immunodeficiency virus (FIV) proteins.

Molecular typing

DNA extraction was based on the Novozyme 234 dodecyltrimethylammonium bromide and 234 hexadecyltrimethylammonium bromide method described previously [56] with modifications as described by Saul *et al*. [57]. Molecular type analysis was performed using a modified version as described by Saul *et al*. [57] of the

restriction digest of the *URA5* gene as described by Myer *et al*. 2003 [58].

Classification of cases

In this study, disease was categorized by the anatomical location of lesions and their extent and severity, as outlined in Table 1, based on a scheme proposed by O'Brien *et al*. [36]. The animals' place of domicile was classified as being (i) within the metropolitan area of Perth (a radius of approximately 25 km from the central business district) or (ii) from elsewhere in the state, classified as rural Western Australia (WA). For cases accrued through the private laboratory, these data were based on the location of the attending veterinary practice, rather than the actual place of domicile of the animal, as the latter was not determinable. Outcome was categorized as either favourable (animals treated and recovered), or unfavourable (where patients died or were euthanased because of refractory or progressive disease). Animals euthanased for emotional or financial reasons, or lost to follow-up, were not included in outcome analyses.

Statistical analyses

The absolute numbers of canine, feline and equine cases presenting to MUVH between the years 1995 and 2006, were determined from the hospital's database. Likewise, the computer database of VLS was searched using the terms *Cryptococcus*, *neoformans*, *gattii* and cryptococcosis. Information regarding the individuals' breeds and gender were recorded. Statistical analyses were then performed to determine association between *Cryptococcus* species, anatomical location of disease, geographic place of domicile and outcome by creating a series of 2×2 and 2×3 contingency tables. Odds ratios (OR), Fisher's exact (two-tailed) and Chi-squared tests were used, as indicated. P values <0.05 were considered significant. The database of MUVH was also used to generate a reference population of dogs, cats and horses without cryptococcosis to enable categorical comparisons (in relation to gender and breed) to be made between hospital cases without cryptococcosis and the cohort of cryptococcosis cases reported here.

Results

Species breakdown, seasonality and prevalence over time

Cryptococcosis was identified in 155 animals comprising 72 cats, 57 dogs, 20 horses, three alpacas, two ferrets and one sheep from the databases of MUVH ($n=57$) and VLS ($n=98$). There did not appear to be a significant seasonal trend in terms of when the condition was diagnosed, although fewer cases were diagnosed in winter and more were diagnosed in spring compared to summer and autumn (Fig. 1).

With reference to cases from MUVH, there appeared to be a trend towards an increased number of new cases diagnosed in successive years, with the highest number identified in 2005 and 2006 (Fig. 2). This increased incidence rate was only statistically significant in horses ($P=0.0464$). During the study period, the number of dogs, cats and horses presenting to MUVH was 39,447 (70%), 8,990 (16%) and 7,730 (14%), respectively. Taking into consideration the number of individual cases, cats were five to six times more likely to develop disease than dogs, and three times more likely to develop disease than horses. Horses were almost twice as likely to develop disease as dogs.

Cryptococcosis in cats

Seventy two cases of feline cryptococcosis were identified, 19 at MUVH and 53 by VLS. Males and females accounted for 30 and 38 cases, respectively (gender was unrecorded in four instances). Age was recorded for 68 cats and ranged from one to 15 years, with a mean of 7.5 years and a median of seven years (Fig. 3). Breed data was recorded for 59 cats, comprising 23 pedigree cats and 46 domestic crossbreds. Strikingly, pedigree

Table 1 Disease categories used to classify patients with cryptococcosis for the purpose of statistical analysis

Category	Clinical signs
Sino-nasal disease only	Signs referable to the nasal cavity or nasopharynx including sneezing, nasal discharge, stertor or granulomatous growths protruding from the nostrils.
Sino-nasal disease with local dissemination, excluding the CNS	Includes disease involving the nasal planum, bridge of the nose, soft or hard palpate, retrobulbar space, salivary glands and regional lymph nodes.
Lower respiratory tract disease	Disease involving the trachea, bronchi, pulmonary parenchyma and pleural space
CNS disease	Disease of the meninges or neural tissues from direct spread through the cribriform plate or via haematogenous dissemination. Includes ocular and optic nerve lesions.
Disseminated disease	Multiple areas affected or generalized lymph node involvement
Atypical disease	Single lesion in an atypical site such as the skin, subcutis or intestine

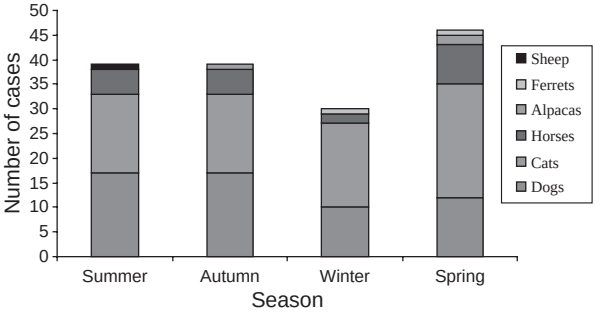

Fig. 1 Time of year in which a diagnosis of cryptococcosis was made. The time of initial infection was impossible to determine with certainty in the vast majority of instances.

cats were almost three times more likely to be diagnosed with cryptococcosis than domestic cats (OR 2.68, confidence interval [CI] 1.61–4.45). Ragdoll and Birman breeds were over-represented amongst the purebreds ($P = 0.003$ and 0.0018, respectively) (Table 2). Retroviral status was determined for 18 cats: one patient was FIV-positive, another was FeLV-positive. Anatomical location of disease is outlined in Table 3 for the 71 cats in which this data was retrievable.

Species identification was established for nine cats, and showed an approximately equal number of *C. gattii* (5) and *C. neoformans* (4) isolates, with molecular type determined for only two isolates of *C. gattii*, both VGII (Table 4). Clinical outcome was known for 11 patients: 8 (73%) cats recovered, while the others were euthanased or died of cryptococcosis. Of those that recovered, 75% had sino-nasal or atypical disease, with the remainder having CNS or disseminated disease. Place of domicile of affected animals was recorded in 69 instances: 54 cats resided in the Perth metropolitan area, while 15 lived in rural WA. The distribution of *Cryptococcus* species amongst the metropolitan cats was *C. gattii* (4): *C. neoformans* (4), with no animals from rural WA having culture or species identification

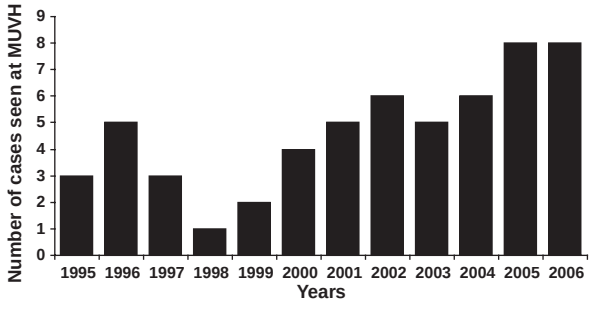

Fig. 2 Number of new cases of cryptococcosis diagnosed each year at MUVH over the study period. Data from VLS was not available for the entire study period; for this reason, this data is not included here.

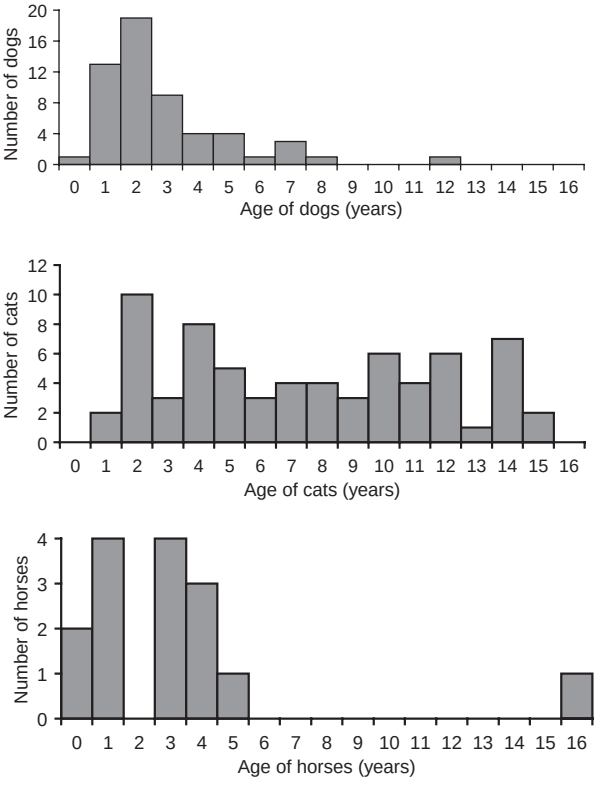

Fig. 3 Age distribution of domestic species with cryptococcosis from Western Australia from 1995 to 2006. Data from dogs (a), cats (b) and horses (c) are presented. Too few alpacas, sheep and ferrets were seen to justify graphical treatment.

performed. One cat with species identification did not have a place of domicile recorded. There was no correlation between *Cryptococcus* species and anatomical location of disease ($P = 0.8936$) (Table 5). An association with *Cryptococcus* species and clinical outcome could not be assessed as *C.neoformans* was not cultured in this subset of cats.

Cryptococcosis in dogs

Fifty seven cases of canine cryptococcosis were identified, 25 at MUVH and 32 by VLS. Males and females accounted for 26 and 30 cases, respectively; the gender of one dog not being recorded. Age (available for all but one patient) ranged from 11 months to 12 years, with a mean of 2.5 years and a median of 2 years (Fig. 3). There was a preponderance of young adult dogs amongst this cohort, with only 10% of dogs in excess of five years. Breed included 24 large, 23 medium and eight small breed dogs (data irretrievable for two dogs); 47 of these were purebreds. Staffordshire bull terriers, British bulldogs, Dalmatians, Greyhounds and Irish setters were over-represented in relation to the reference

Table 2 Prevalence of cryptococcosis in different species according to breed

Breed	No. of animals with cryptococcosis	Percentage of animals with cryptococcosis	Percentage of overall hospital population	Odds ratio
Dogs				
Staffordshire bull terrier*	10	17.5%	7.96%	1.26
Labrador	4	7%	4.72%	0.79
German shepherd dog	4	7%	4.20%	0.9
Dalmatian*	3	5.2%	0.87%	3.51
Kelpie	3	5.2%	6.02%	0.44
Jack Russell terrier	3	5.2%	4.51%	0.61
Australian cattle dog	2	3.5%	3.78%	0.48
Boxer	2	3.5%	2.23%	0.85
Greyhound*	2	3.5%	0.48%	4.2
Cocker spaniel	2	3.5%	1.88%	1.02
English bulldog*	2	3.5%	0.04%	55.93
Saint Bernard	1	1.75%	0.16%	6.06
Irish setter*	1	1.75%	0.77%	13.65
Alaskan malamute	1	1.75%	0.09%	10.92
Cats				
Birman*	6	8.3%	1.79%	5.58
Ragdoll *	6	8.3%	2.03%	4.87
Siamese	5	6.9%	3.17%	2.43
Persian	2	2.7%	2.48%	1.17
Burmese	2	2.7%	5.8%	0.48
Scottish Fold	1	1.4%	0.07%	30.24
Chinchilla	1	1.4%	0.76%	1.94
Purebreds*	23	31.9%	16%	2.68
Domestic breed	46	63.8%	74%	0.68
Horses				
Thoroughbred	7	35%	46.7%	1.01
Standardbred	3	15%	24.1%	0.79
Clydesdale* – two siblings that shared the same paddock	2	10%	1.11%	14.79
Arab	2	10%	6.4%	2.27
Welsh mountain pony	1	5%	1.93%	3.7
Connemara pony*	1	5%	0.15%	69.63

*Significant $P < 0.05$ – shaded grey for emphasis.

hospital population ($P = 0.002$, 0.014, 0.032, 0.0004, 0.046, respectively) (Table 2).

Anatomical location of disease is outlined in Table 3. Cryptococcal species identification, available in 22 instances, showed an equal number of *C. gattii* (11) and *C. neoformans* (11) isolates, with molecular type determined for four *C. gattii* isolates, all being VGII (Table 4). Clinical outcome, determinable for 22 patients, was disappointing. Only six dogs (27%) recovered, the remainder either dying from the disease

Table 3 Anatomical distribution of tissues affected in dogs, cats, horses and alpacas with cryptococcosis seen in Western Australia between 1995 and 2006

Anatomical location	Dogs No. (%)	Cats No. (%)	Horses No. (%)	Alpacas No. (%)
Sino-nasal disease	11 (19%)	26 (36%)		
Sino-nasal plus local disease	16 (29%)	24 (34%)		
Lower respiratory tract disease	1 (2%)	2 (3%)	14 (70%)	2 (67%)
Central nervous system	11 (19%)	4 (6%)	1 (5%)	
Disseminated disease	10 (17%)	6 (8%)	5 (25%)	1 (33%)
Atypical disease	6 (11%)	9 (12%)		
Unknown	2 (3%)	1 (1%)		
Total	57	72	20	3

No., number.

Table 4 Molecular genotypes of *Cryptococcus gattii* isolated from domesticated animals and human patients in Western Australia[1]

Source	Genotype	Year isolated	Geographical origin
Isolates from this study			
Horse[2]	VGII	1995	Rural WA
Horse[2]	VGII	1995	Unknown
Dog[2]	VGII	1996	Metropolitan Perth
Dog[2]	VGII	1997	Metropolitan Perth
Horse[2]	VGII	1997	Metropolitan Perth
Cat	VGII	2003	Metropolitan Perth
Dog	VGII	2003	Metropolitan Perth
Dog	VGII	2005	Rural WA
Cat	VGII	2006	Metropolitan Perth
Previous reported isolates of *C. gattii* from WA			
Sheep (case 24)[3]	VGII	1993	Rural WA
Sheep (case 25)[3]	VGII	1993	Rural WA
Sheep (case 26)[3]	VGII	1993	Rural WA
Horse (case 27)[3]	VGI	Before 1992	WA
Horse (case 28)[3]	VGII	Before 1992	WA
Human patient WA-8[4]	VGII	1985	WA
Human patient WA-9[4]	VGI	1965	WA
Human patient WA-10[4]	VGI	1987	WA
Human patient WA-11[4]	VGI	–	WA
Human patient WA-12[4]	VGII	1990	WA
Human patient WA-13[4]	VGI	1965	WA
Human patient WA-14[4]	VGI	1994	WA

[1]A number of avian isolates (mainly parrots) and isolates from wildlife (e.g., potoroos), mainly from Perth Zoo, have been found to be VGI [[87]].
[2]Campbell [60], Sarah Kidd unpublished observations.
[3]Sorrell *et al.* [50].
[4]Sorrell *et al*. [88].

or being euthanased. Of those that recovered, all had sino-nasal disease (with or without local extension), except one dog with a subcutaneous mass that was cured by simple surgical excision. No dog with disseminated or CNS disease was treated successfully. Place of domicile was available for 56 dogs: 43 lived in the Perth metropolitan area, while 13 resided in rural WA. The distribution of *Cryptococcus* species (*C. gattii*: *C. neoformans*) was 8:9 for metropolitan dogs and 3:1 for rural dogs (1). There was no correlation between *Cryptococcus* species and anatomical location of disease, domicile or outcome ($P = 0.807$, $P = 0.586$, $P = 0.659$) (Table 5).

Cryptococcosis in horses

Twenty cases of equine cryptococcosis were diagnosed, 11 at MUVH and 9 by VLS. For the 17 cases where gender was recorded, females ($n = 12$) were over-represented compared to males ($n = 5$) ($P = 0.021$). For the 15 horses where data was available, age ranged

Table 5 Relationship between *Cryptococcus* species, animal species and outcome for dogs and cats from Western Australia with cryptococcosis. The nine horses were not tabulated here as all were *C. gattii* infections

Cryptococcus species	Metropolitan	Rural	Domicile combined	Treated with success	Died or euthanased	Lost to follow-up	Outcome combined
Dogs							
C. gattii	8	3	11	2	7	2	11
C.neoformans	9	1	10	1	5	5	11
Not cultured	26	9	35	3	4		
Total	43	13	56	6	16		
Cats							
C. gattii	4	0	4	1	3	1	5
C.neoformans	4	0	4	0	0	4	4
Not cultured	46	15	61	7	0		
Total	54	15	69	8	3		

from 6 months to 16 years, with a mean of 3.3 and a median of 3.0 years (Fig. 3). There was a preponderance of young horses in this cohort, with all but one case under four years-of-age. Although the majority of cases were large breed horses, only Clydesdales and Connemara ponies were over-represented ($P=0.01$, $P=0.03$) (Table 2). Anatomical distribution of disease ($n=20$) in horses differed conspicuously to dogs and cats, with a preponderance of lower respiratory involvement, as outlined in Table 3.

Where infection was identified within the lungs, there appeared to be two distinct patterns of disease: (i) focal granulomas confined to the caudo-dorsal lung lobes (Fig. 4) and (ii) a diffuse miliary pattern (Table 6). Species identification was determined in nine of the 20 horses; all nine were due to *C. gattii*, with 3/3 isolates subjected to molecular testing being of the VGII genotype (Table 4). Outcome ($n=13$) was poor, with only two horses being treated successfully, the remainder being euthanased due to poor response to treatment or for financial reasons. Geographic location of affected animals was known for 16 horses: six resided in the Perth metropolitan area, while 10 were from rural WA. One of the equine cases included in this study has been reported as an individual case study [32].

Cryptococcosis in alpacas

Three alpacas were diagnosed with cryptococcosis based on cytological and histological evaluation of specimens obtained at necropsy. Gender was known for 2 of these (both female), but age was recorded for only one (18-months-old). Disease was localized to the lower respiratory tract in two alpacas, while the other had disseminated and respiratory disease (Table 3). A culture was obtained from a single animal and typed as *C. gattii*. The sites of domicile for all three animals were known: two from rural WA and one from the metropolitan area. Treatment was not attempted in any instance.

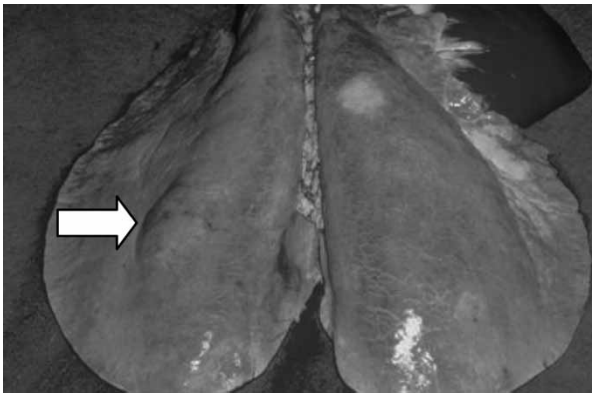

Fig. 4 Necropsy photograph from a horse euthanased because of signs referable to pulmonary cryptococcosis. Note the large cryptococcal granulomas within the caudo-dorsal lung (the largest lesion is highlighted by an arrow).

Miscellaneous species

Two ferrets and one sheep were diagnosed with cryptococcosis based on characteristic histology of tissue specimens obtained at necropsy. Both ferrets had cryptococcal pneumonia, while the sheep had CNS involvement. Information regarding signalment was not recorded and culture was not performed in any of these animals.

Discussion

General comments

Cryptococcosis is one of the most common systemic mycoses diagnosed in domestic animals worldwide. It is by far the most common life-threatening fungal infection of animals in Australia. Until recently, the veterinary cryptococcosis literature consisted predominantly of single case reports and small case series. Since the 1990s, larger studies of naturally-occurring disease in North America [9–12] and the east coast of Australia [36] have appeared, reflecting advances in small animal medicine and increased interest by mycologists regarding insights from spontaneous disease in animals.

The current investigation was conceived to retrospectively investigate cryptococcosis in domestic animals in W.A. in the light of new insights stemming from taxonomic changes with the *C. neoformans* species complex, molecular epidemiology and in particular the ongoing 'outbreak' of *C. gattii* disease in Vancouver Island, Canada. The current study is by far the largest conducted in WA and builds on smaller case series from Murdoch University concerning cats, goats, sheep and horses [19,20,22,23,59]. Overall, our impression is that there has been an increasing prevalence of cryptococcal disease in WA, with larger numbers of cases being diagnosed in more recent years.

Although only 41 (26%) of cases included culture records, it appears that there is a relatively high prevalence of *C. gattii*, with 26/41 (63%) cultured cases caused by this species. This is in striking contrast to the situation in the eastern states of Australia, where *C. gattii* makes up 27% of isolates from canine and feline cryptococcosis patients [26,36,37]. This is extremely interesting and has some parallels to the situation encountered in the Vancouver Island outbreak [9–11]. Factors which may have contributed to an increased

Table 6 Distribution of lesions in the lungs and corresponding necropsy findings of horses diagnosed with cryptococcosis at MUVH

Case	Signalment	Distribution of lung lesions	Necropsy findings	Outcome
1	16-year-old Thoroughbred mare*	Pulmonary granulomas (N)	*Cryptococcus* lesions confined to the lungs	Euthanased
2	4-year-old Arab mare	Pulmonary granulomas in caudo-dorsal lung fields (R & N)	Cryptococcal osteomyelitis of pedal bone and rib in addition to pulmonary granulomas	Euthanased
3	1-year-old Thoroughbred filly	Diffuse military lung lesions (N)	Cryptococcal lesions in mediastinum, mesenteric lymph node and adrenal glands	Euthanased
4	9-month-old Palomino filly	Diffuse military pattern (R)	Not performed	Euthanased
5	3-year-old Thoroughbred gelding	Pulmonary granulomas in caudo-dorsal lung fields (R & N)	*Cryptococcus* lesions confined to the lungs	Euthanased
6	4-year-old Standardbred mare	Pulmonary granulomas in caudo-dorsal lung fields (R)	Not performed	Improved but lost to follow up
7	3-year-old Welsh mountain pony filly	Pulmonary granuloma in caudo-dorsal lung fields (R)	Not performed	Recovered
8	18-month-old Arab colt	Diffuse miliary lung lesions (N)	Mesenteric lymph node involvement in addition to miliary lung lesions	Euthanased
9	6-month-old Connemara pony filly	Diffuse military lung lesions (N)	Mesenteric lymph node involvement in addition to miliary lung lesions	Euthanased
10	5-year-old Standardbred gelding	Pulmonary granuloma in caudo-dorsal lung fields (N)	*Cryptococcus* lesions confined to the lungs	Euthanased
11	3-year-old Thoroughbred filly	Pulmonary granuloma in caudo-dorsal lung fields (R & N)	*Cryptococcus* lesions confined to the lungs	Euthanased

Pulmonary lesions observed at necropsy (N) or radiographically (R) or both (R & N).
Mare – intact female, Filly – young mare, Colt – young male, Gelding – castrated male.
*Concurrent *M. avium* pneumonia.

prevalence of *C. gattii* infections in WA may include: (i) environmental presence of organisms in the local area, ii) climatic conditions, and the diversity of eucalyptus and other native trees [32] or (iii) an increased virulence of endemic *C. gattii* strains for mammalian hosts.

Preliminary data (Table 4) is also suggestive of an increased prevalence of VGII isolates in this region, as well as the better known eucalyptus-associated VGI isolates (David Ellis, Nathan Saul, Mark Krockenberger unpublished results). This is supported by previous data that looked at 20 veterinary isolates and found 70% to be of the VGII genotype [60] (S. Kidd and D. Ellis, unpublished results). Little is known about the epidemiology or environmental niche of VGII in Australia, although it appears to be much more prevalent in Western Australia and the Northern Territory than in the eastern states, and it is unlikely to be associated with *E. camaldulensis* [61]. In the eastern states, the vast majority of *C. gattii* infections are with eucalyptus-associated VGI strains, with VGII strains making up only a small proportion of *C. gattii* infections [62]. Although, molecular genotyping was only performed in a limited number of our cases, all isolates that underwent molecular typing ($n=9$) were of the VGII genotype, in addition to the isolates from sheep (3) horses (1) and human patients (2) reported previously [50] (Table 4).

In all species, except cats, there was a marked trend for young adults to be affected. This suggests that colonization and subsequent infection of immunologically naïve animals by infectious propagules may result in the development of clinical disease shortly after exposure. Although it is likely that many cats are also infected whilst young, presumably they either completely eliminate the infection at this time, or mount a sufficiently effective immune response that the organism is restricted to a small clinically quiescent focus, from which it may or may not later reactivate, perhaps as a result of intercurrent disease (e.g., lymphoma, long standing FIV infection), stress or immune senescence. There is compelling circumstantial evidence that such subclinical infections occur in people [63], and they have been proven to occur also in koalas [64,65] and in a rat model [66]. Such a scenario would account for the broader age range encountered in feline cryptococcosis.

Cryptococcosis in cats

Historically, cats are reportedly more often affected by cryptococcosis than other domestic species [9–11,36,45,63]. Consistent with that finding, cats comprised the largest group of patients in this study. The ratio of cats to dogs in our data-set suggested a true increased susceptibility to infection, especially considering that they are less commonly kept than dogs as a

companion animal in the Perth region [67], and less commonly presented for veterinary attention. It was of interest that most cases of feline cryptococcosis were diagnosed by general practitioners using a private laboratory, suggesting practitioners readily recognize the disease in cats and referral is not necessary.

For cats with cryptococcosis a gender predisposition was originally reported, with males being more likely to develop disease than females [43,44,68]. It was hypothesized that males were more likely to be outdoors and roam, with attendant risk of increased exposure to infectious propagules [43]. More recent studies [9,69] have not confirmed this, and our study also reports a lower proportion of male cats. The age distribution and trend for purebred cats to be affected is similar to previous reports [36], with Ragdoll and Birman cats overrepresented in this study. This may be associated with an underlying genetic predisposition [37], a higher prevalence of a predisposing co-morbidity (such as viral rhino-sinusitis) or because owners of pedigree cats may be more likely to seek veterinary attention than owners of domestic crossbreds. There was insufficient data to comment on the influence, if any, of retroviral status on the establishment or evolution of disease in cats, or their response to therapy. Some previous studies have found an increased incidence of FeLV and FIV infection in cats with cryptococcosis compared with the prevalence in the hospital population [43], with seropositivity for FeLV and FIV being associated with less favourable outcomes [68]. Australian studies [36,44], in contrast, have found that the prevalence of FIV infection in cats with cryptococcosis was not significantly different from a population of "sick" cats from the same area, and further studies of FIV seroprevalence have supported this contention [70]. Furthermore, it was found that many cats with concurrent FIV had comparable CD4 counts to cats with cryptococcosis [71], could be successfully treated and did not relapse when therapy was discontinued [37].

In this and other studies [36,44], sino-nasal infection, often with local extension and involvement of the skin and subcutis of the nasal bridge and planum nasale, was the most common clinical presentation. This supports the presumed aetiopathogenesis, whereby inhalation of a heavy inoculum of basidiospores results in nasal colonization followed by subsequent invasion of the mucosal barrier and establishment of a primary site of infection in the nasal cavity. Atypical disease in cats largely consisted of solitary lesions of the skin and subcutis in areas such as the hock, axilla, hip and inguinal region. Such lesions are suggestive of direct inoculation [72], rather than haematogenous spread from a primary site of infection. In contrast to dogs and horses, there were no cases of intestinal cryptococcosis detected in our feline cohort, suggesting that ingestion was an unlikely portal of entry in this species.

Cryptococcosis in dogs

The preponderance of large purebred dogs identified in this series is consistent with earlier reports [36,45,46,73–75], and has been postulated to be due to these breeds leading a more active outdoor lifestyle, with increased potential for exposure in the environment, for example as a result of vigorous sniffing of soil and dirt containing cryptococcal organisms or basidiospores [46]. Genetic susceptibility, similar to the predisposition of Cavalier King Charles spaniels and miniature dachshunds to develop *Pneumocystis* pneumonia [76,77], may also play a part in certain breeds, including German Shepherd dogs (certain lines of which are known to be at risk for developing disseminated aspergillosis [78]) and American Cocker spaniels. Our study also identified a number of medium sized dog breeds which were over-represented, in accord with another report from North America [47].

The propensity for symptomatic cryptococcosis to develop in young adult dogs is marked. Similar observations have been made in British Columbia, where VGII isolates predominate, and in the eastern states of Australia where *C. neoformans* varieties predominate. In these studies, as well as in our data, over 80% of dogs with cryptococcosis were less than 5 years at diagnosis. Similar findings from disparate study cohorts strongly suggest dogs develop clinical disease soon after primary infection, and that reactivation disease is unusual in this species. While the anatomical location of disease in dogs reported here was initially categorized as sino-nasal alone, or sino-nasal with spread to contiguous tissues (49%), disease often progressed to involve the lower respiratory tract, CNS or other organs (40% of the canine cohort)-more readily than in feline patients (17% of the feline cohort). This is similar to recent reports from eastern Australia [36,45], but contrasts with studies from North America [9–11] and earlier cases from Australia [46], where CNS and disseminated disease predominated.

Cryptococcosis in horses

An intriguing finding of this study was the relatively high prevalence of cryptococcal infection in horses, this representing the largest number of cases of equine cryptococcosis reported in a single investigation. This supports the earlier contention of Riley and colleagues [22] that the epidemiology of cryptococcosis in Western

Australian horses is unique. Horses also appear to be at increased risk of infection in comparison to the eastern states. Equine cases are seen on a yearly basis in the Perth region, where there has only been a single reported case of equine cryptococcosis from the eastern states of Australia in the last 20 years [18].

Affected individuals were predominantly young adults, with only one horse older than four-years. The latter atypical case had been diagnosed with chronic sinusitis secondary to tooth root abscessation 12 months earlier and represented with ongoing nasal discharge, weight loss and limb swelling. A necropsy was performed and identified a number of abnormalities including a pituitary and thyroid adenoma, secondary hypertrophic osteopathy, maxillary sinus abscess, pulmonary mycobacteriosis (*M. avium* complex infection based on PCR of formalin-fixed lung tissue) and cryptococcosis; the combination of lesions suggested that the horse was immunocompromised, with concurrent opportunistic pneumonic infections with organisms known to be problematic in individuals with poor cell-mediated immunity. A preponderance of young adult horses was likewise identified in the seminal study from WA [22], with all cases under the five years. This is in contrast to a number of single case reports from eastern Australia and North America involving older animals, 10–20 years of age [18,26,49]. There also appeared to be a gender predilection for mares in our study, not observed previously. In addition to Thoroughbreds and Standardbreds [22], a number of other breeds were affected also.

Like human patients, but unlike cats and dogs, horses had a strong propensity toward lower respiratory tract involvement; indeed, the appearance of the pulmonary granulomas in horses was quite reminiscent of that described recently in aboriginal patients in the Northern Territory of Australia with *C. gattii* infections [79]. Dissemination was observed in only 5 of 20 cases, with a small number of horses presenting with CNS disease or with meningoencephalitis at necropsy. Interestingly, no horses presented with sino-nasal disease.

When pulmonary involvement takes the form of multiple cryptococcal granulomas in the lung, inhalation of large number of infective propagules from an environmental niche is the most logical pathomechanism for the initiation of infection, although the preferential location of lesions in the caudo-dorsal lung is yet to be explained. It may be that this is the path of least resistance, a theory which is supported by an evaluation of blind bronchoalveolar lavage technique [80] where the caudo-dorsal lung lobes were shown to be the areas consistently lavaged. An alternative aetiopathogenesis has been proposed for cases in which diffuse miliary lesions are observed through the entire pulmonary parenchyma, with primary localization in the mesenteric lymph nodes and subsequent spread via the lymphatics and/or haematogenously to the lungs and other tissues (Table 6) [18,22,32].

The exclusive isolation of *C. gattii* from the nine horses in this series where speciation was performed, and the fact that the majority of horses speciated by others [60] (Sarah Kidd, unpublished observations) were also *C. gattii*, is strongly suggestive that *C. gattii* is the most common cause of equine cryptococcosis in Australia. This is consistent with a case report from the New South Wales [18] and also a previous study from WA, where *C. gattii* was also isolated from 2/2 horses in which cultures were obtained [22]; one isolate was a VGI, while the other was a VGII. In the present study, all three horses from which cultures were available for testing were infected by the *C. gattii* molecular type VGII. The environmental niche of VGII is yet to be determined, but may be more common in south-western Western Australia and the 'top end' of the Northern Territory than in the eastern states of Australia. Currently it is not known why there is increased prevalence of equine cryptococcosis in WA, but it is possible that abiotic and biotic conditions in this region are especially conducive for sexual or asexual reproduction, resulting in more frequent or more successful elaboration of basidiospores [61].

Horses may also be at increased risk for pulmonary cryptococcosis because the large tidal volumes developed during and after vigorous exercise may reduce the efficiency of particulate matter filtering from inspired air by the nasal passages, resulting in deposition of infectious doses of organisms deep in the tracheobronchial tree. The high prevalence of cryptococcosis in koalas [81], goats [23] and horses [19,22] in the Perth region is likely referable to the high environmental presence there of the VGII genotype, which may be inherently more virulent either due to (i) inherent pathogenicity, or (ii) elaboration in large quantities from primary environmental niches. Cryptococcosis caused by VGII appears to be important in captive koalas in the region, associated with high environmental presence of this genotype (N. Saul, R. Malik and M. Krockenberger, unpublished results). The simultaneous occurrence of cryptococcosis in a large number of sheep from a single property near Busselton [50] further supports these contentions. Interestingly, a horse has also been reported to be affected by VGII strain in the Vancouver Island epizootic, possibly for similar reasons [82].

Cryptococcosis in alpacas

There is little data concerning cryptococcosis in South American camelids, but from reports published it appears the pathogenesis may be similar to horses, with pneumonia, neurological or widely disseminated disease predominating [11,33–35]. In a single case report of a mature female alpaca from NSW, Australia [34], *C. gattii* was isolated. The involvement of *C gattii* in both alpacas, that in other respects were normal, supports the notion that *C gattii* generally causes disease in immune competent individuals. The alpaca from NSW was infected by a VGI isolate and allegedly had meningitis in the absence of pulmonary disease, whereas pulmonary and disseminated disease was observed in the WA alpaca, infected with a strain of unknown genotype. The other cases recorded include two llamas with neurological signs [11], a 17-year-old male with neurological, pulmonary and disseminated disease [33], and a vicuna with meningitis and pneumonia [35].

Treatment outcomes

Although information on outcome in the patients reported here was quite limited, it appears that cats have a more favorable prognosis than dogs or horses. This may be due to the fact that they are: (i) small patients, and thus require smaller quantities of drugs, which are therefore more affordable and (ii) more likely to present early with disease localized to the upper respiratory tract or skin. In contrast, dogs and horses seem more predisposed develop lower respiratory, disseminated and neurological disease, the latter being a strong predictor of mortality [10]. The perceived severity of disease may also influence an owners' decision whether to euthanase or embark on a protracted and expensive course of therapy. Many of the antifungal medications used, in particular the azoles, are expensive and may be cost prohibitive in horses and large breed dogs. The recent availability of inexpensive formulations of fluconazole from compounding pharmacists (e.g. http://www.bovachemist.com.au) has, without doubt, made effective treatment regimens more affordable for owners in Australia [83]. The protracted course of treatment required, involving regular veterinary attention with serological and biochemical monitoring also contributes to the high cost of therapy, and mandates a significant emotional and time commitment from owners [2]. Finally, treatment failure or disease recurrence is not uncommon and may result from stopping therapy prematurely or abandoning therapy due to complications such as nephrotoxicity (amphotericin B) [84], hepatotoxicity (itraconazole) [85], cutaneous drug eruptions (flucytosine) [86], cutaneous vasculitis (itraconazole) and sterile subcutaneous abscesses (amphotericin B) [2,84]. For a variety of reasons, treatment was only attempted in two horses in this series. One was a pony which had complete resolution of clinical signs and a negative LCAT twelve months post-treatment (C. Secombe, personal communication), as has been reported recently in another pony with a *C. gattii* VGI infection [18]. The second horse was treated by the referring veterinary surgeon with amphotericin B and had a negative tracheal wash and radiographic improvement four months after initial diagnosis. Unfortunately this horse was then lost to follow-up.

Study limitations

A number of limitations were associated with this study, many of which related to its retrospective nature and the fact that much data was unobtainable for individual cases. This was particularly true for cases identified through the external laboratory, which relied solely on information supplied by referring practitioners during sample submission. In particular, the animals' geographical location was based on the submitting veterinary practices address, which may not have been exactly representative of the animals' actual place of domicile. Also, where population data such as breed, gender and age, were required for statistical analyses, they were obtained from the MUVH database due to the difficulty in obtaining this information from the external laboratory in relation to its reference population of companion animals. Although this data is likely to be reasonably representative of case signalment as seen in private practice throughout Perth, it is likely to have introduced some error. Finally, the number of isolates that underwent culture, species identification and especially molecular typing was limited, making definitive conclusions regarding the occurrence and significance of VGII difficult to establish with certainty. Prospective collection of representative cultures is ongoing, and should in time circumvent this deficiency.

Conclusions

This study provides a general overview of the epidemiology of cryptococcosis in domestic animals in WA over the last 11 years. Members of the *C. neoformans* species complex appear to act as primary pathogens of immune competent hosts most of the time, affecting predominantly young animals. Genetic make-up likely played some role in predisposing towards disease, as purebred individuals of all species appeared to be

over-represented. The anatomical distribution of lesions is consistent with the accepted notion that respiratory tract is the usual portal of entry for this organism, with subsequent spread to adjacent tissues, regional lymph nodes, and sometimes dissemination to other tissues, such as the CNS. Other routes of infection such as ingestion (in horses and dogs) and direct inoculation (in cats and dogs) were also considered possible. It is of great interest that *C. gattii*, with an apparently high prevalence of the VGII genotype, appears to be considerably more prevalent in this geographical region of Australia compared with the eastern states, having many similarities to the situation in Vancouver Island and the nearby mainland of British Columbia. The reasons for the high endemicity of VGII strains in south west Western Australia are not currently clear and without doubt are worthy of further investigation in relation to the natural environmental niche for this primary pathogen.

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