Successful treatment of cryptococcal pneumonia in a pony mare

LM BEGGa, KJ HUGHESb, A KESSELLb, MB KROCKENBERGERc, DI WIGNEYc and R MALIKd

A 20-year-old Welsh Mountain Pony (212 kg) mare was initially presented for a chronic cough, fever, weight loss and low grade abdominal pain. She later developed dyspnoea, tachypnoea and exercise intolerance. The presence of multiple masses (up to 17 cm diameter) in the pulmonary parenchyma was established using lateral thoracic radiography and transthoracic ultrasonography. Encapsulated, budding yeasts were observed in smears made from transtracheal washings and needle aspirations of the pulmonary lesions. Cryptococcus gattii (synonym: Cryptococcus neoformans variety gattii; Cryptococcus bai-lisporus) was cultured from the transtracheal washings and aspirates of the lung masses. The pony was successfully treated using daily intravenous infusions of amphotericin B (typically 0.5 mg/kg in 1 L 5% dextrose in water over 1 h, following premedication with 50 mg flunixin intravenously) over a 1 month period, until a cumulative dose of 3 g had been administered. Treatment was considered to be successful on the basis of progressive improvement in clinical signs, reduction in the size of pulmonary cryptococcomas, 48 kg weight gain and a reduction in the cryptococcal antigen titre from 4096 to 256, 1 year after cessation of treatment.

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ALP Alkaline phosphatase
AST Aspartate aminotransferase
bpm Beats/breaths per minute
DSW 5% dextrose in water
GGT γ-glutamyl transferase
HR Heart rate
IM Intramuscularly
ICPMR Institute for Clinical Pathology and Medical Research, Westmead Hospital
IV Intravenously
LCAT Latex cryptococcal antigen agglutination test
MIC Minimum inhibitory concentration
RR Reference range
UVCC University Veterinary Centre Camden
WA Western Australia

Cryptococcosis is the most common systemic mycosis of people and animals that occurs in Australia.1 All domestic species, including cats, dogs, ferrets, cattle, sheep, goats and horses have been shown to be susceptible to this infection.2,3 Although cryptococcosis has been well documented as a cause of disease in horses, infection in this species would appear to be less common than in small companion animals based on published reports4 and our own unpublished observations. Interestingly, a disproportionately large number of the published equine cases have been recorded by Australian investigators,5 in agreement with our impression that this disease is more common in Australia than either North America or Europe.

Horses can suffer from one or a combination of clinical syndromes, of which invasive nasal cavity disease,6-9 nasopharyngeal disease,4,6 pneumonia10-12 and meningoencephalitis5,13-17 are the most common. These syndromes are associated with sinusosal or retro-orbital deformity, nasal discharge that is often unilateral, intussusception, respiratory difficulty and neurological dysfunction such as cranial nerve deficits, seizures, and paresis/ataxia. Less common forms of equine cryptococcal infection include localised granu-losmas of the skin,18 keratitis,19 uterine infections resulting in foetal pneumonia and abortion or neonatal pneumonia,20,21 intestinal polyoid granulomas,6,22 and mesenteric lymph node abscessation.12 In cattle, mastitis is by far the most common clinical manifestation of this infection.2 Extrapolating from human data, large pulmonary cryptococcal granulomas (also called cryptococcomas) sometimes associated with pleural effusion. These lesions are often cavitating, and tend to be situated in the dorsocaudal lung lobes, a portion of the lung where inhaled particles are preferentially deposited, presumably including infectious propagules of Cryptococcus.12 Less commonly, there are miliary (1 to 3 mm diameter) interstitial granulomas distributed evenly throughout both lungs, suggesting haematogenous spread from a primary focus elsewhere. In both presentations, the presence of cryptococcal granulomas in mesenteric lymph nodes is a common pathological finding, compatible with the intestinal tract providing the primary focus of infection in some cases.

Extrapolating from human data, large pulmonary cryptococcomas most likely represent a vigorous cell-mediated immune response of an immunocompetent host to a primary pathogen. Immunological studies have failed to demonstrate defective humoral or cell-mediated immunity,12 at least in horses from WA infected with C gattii. Cryptococcosis in horses has been postu-lated to occur secondary to injuries sustained during passage of a stomach tube,7 following viral or bacterial upper respiratory tract infections,10 and as a sequela of exercise induced pulmonary haemorrhage,12 although it may occur without these predispositions in cats and dogs.23,24 The present report records clinical findings in a pony with pulmonary cryptococcosis. Although the patient had severe lesions when the diagnosis was made, timely therapy using an IV amphotericin B protocol similar to that described previously for treating horses with pythiosis22,26 and histoplasmosis27 was successful in effecting a favourable outcome.
Case report

History
A 20-year-old Welsh Mountain Pony mare housed at stables adjacent to Centennial Park in Sydney presented initially with low grade abdominal pain. The horse had been domiciled at this location for at least 2 years. She was febrile (39.0°C; RR 37.0 to 38.5°C) and mildly lame in both front feet. The mare had a history of a chronic cough, but because she was in good body condition, had a good appetite and no evidence of exercise intolerance, the cause of the cough had not been investigated further. On thoracic auscultation, wheezes were audible diffusely over both lung fields. The mare was treated for 5 days with phenylbutazone (2.5 mg/kg orally once daily) and procaine penicillin (20,000 IU/kg IM twice daily). Colic and fever resolved, as did the foot pain and she was sent to a paddock for a spell. Approximately 3 weeks later she again developed a fever and was sent to the UVCC for further evaluation.

Physical findings
On examination (March 2002), the pony was thin (212 kg) and febrile (38.7°C), with a HR of 60 bpm (RR 26 to 40). At rest, she showed some evidence of dyspnoea and tachypnoea (respiratory rate 60 breaths per minute; RR 8 to 16 bpm), which were exacerbated by exercise. On thoracic auscultation, loud wheezes were present over both lung fields, with the exception of the right cranial thorax. In this region, bronchovesicular sounds were absent and there was decreased resonance on percussion. An ultrasonographic examination of the chest with an ATL Ultramark 9-HDI using 3 to 5 MHz and 4 to 7 MHz phased array transducers demonstrated a large pulmonary mass (17.4 cm diameter) in the right cranial thorax, a second mass (10 cm diameter) in the left mid-thorax, and a third smaller mass (6 cm diameter) in the left cranial thorax (Figure 1). All mass lesions were well demarcated from the surrounding pulmonary parenchyma by a hyperechoic capsule up to 1 cm in thickness and displayed a variable internal echogenicity. No pleural fluid was detected in either hemithorax. Standing right-lateral radiographs of the craniodorsal and caudodorsal lung fields (82 kVp; 36 mAs) confirmed the existence of the masses and failed to demonstrate a pleural effusion (Figure 2). A trans-endoscopic tracheal aspirate was performed using a guarded microbiological aspiration catheter (Mila International; Kentucky, USA). The fluid retrieved was thick, contained flocculent material, and clotted. A transthoracic needle aspirate from the right cranial lung lesion yielded yellow gelatinous material. The pony was maintained on phenylbutazone (2.2 mg/kg orally twice daily) during hospitalisation because she continued to be mildly lame and had increased digital pulses in her forefeet.

Laboratory findings
Cytological evaluation of smears from the trans-tracheal washing and pulmonary aspirate both demonstrated degenerate neutrophils and numerous encapsulated yeasts showing narrowed budding, a morphology consistent with a Cryptococcus sp isolate as determined by fingerprinting studies. The isolate was sensitive to amphotericin B (MIC 0.25 mg/L), itraconazole (MIC 0.125 mg/L) but resistant to fluconazole (MIC 8 mg/L) using the ‘Yeast One’ Sensititre test. The baseline serum latex cryptococcal antigen titre was 4096.13

Haematological examination demonstrated anaemia (PCV 0.27 L/L; RR 35 to 0.44 L/L) and leukocytosis (total white cell count 13.6 x 10⁹/L; RR 6.5 to 12.0 x 10⁹/L) due to a mild to moderate mature neutrophilia (11.8 x 10⁹/L; RR 2.47 to 6.96 x 10⁹/L). These changes were consistent with chronic inflammation. Serum biochemical analyses revealed hyperproteinenaemia (81 g/L; RR 60 to 70 g/L), hyperglobulinaemia (57 g/L; RR 26 to 40 g/L), hypoalbuminaemia (24 g/L; RR 29 to 35 g/L) and hyperfibrinogenenaemia (6.1 g/L; RR 2 to 4 g/L). Hypoalbuminaemia was caused by exercise. On thoracic auscultation, loud wheezes were present over both lung fields, with the exception of the right cranial thorax. In this region, bronchovesicular sounds were absent and there was decreased resonance on percussion. An ultrasonographic examination of the chest with an ATL Ultramark 9-HDI using 3 to 5 MHz and 4 to 7 MHz phased array transducers demonstrated a large pulmonary mass (17.4 cm diameter) in the right cranial thorax, a second mass (10 cm diameter) in the left mid-thorax, and a third smaller mass (6 cm diameter) in the left cranial thorax (Figure 1). All mass lesions were well demarcated from the surrounding pulmonary parenchyma by a hyperechoic capsule up to 1 cm in thickness and displayed a variable internal echogenicity. No pleural fluid was detected in either hemithorax. Standing right-lateral radiographs of the craniodorsal and caudodorsal lung fields (82 kVp; 36 mAs) confirmed the existence of the masses and failed to demonstrate a pleural effusion (Figure 2). A trans-endoscopic tracheal aspirate was performed using a guarded microbiological aspiration catheter (Mila International; Kentucky, USA). The fluid retrieved was thick, contained flocculent material, and clotted. A transthoracic needle aspirate from the right cranial lung lesion yielded yellow gelatinous material. The pony was maintained on phenylbutazone (2.2 mg/kg orally twice daily) during hospitalisation because she continued to be mildly lame and had increased digital pulses in her forefeet.

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agar and produced a brown-colour-effect on bird seed agar.1 Molecular typing at ICPMR (using primer M13 for microsatellite fingerprinting studies)29 and immunocytochemistry using a range of monoclonal antibodies (Figure 3B)30 further identified the isolate as Cryptococcus gattii. The isolate was sensitive to amphotericin B (MIC 0.25 mg/L), itraconazole (MIC 0.125 mg/L) but resistant to fluconazole (MIC 8 mg/L) using the ‘Yeast One’ Sensititre test. The baseline serum latex cryptococcal antigen titre was 4096.13

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considered secondary to hyperglobulinaemia. Enzyme activities of GGT, ALP and AST were 68 U/L, 272 U/L, and 203 U/L, respectively (RR 9 to 27, 120 to 202, 215 to 347, respectively). Urea and creatinine concentrations were within reference ranges (Table 1). Urinalysis demonstrated a specific gravity of >1.035 (RR 1.025 to 1.050), pH of 8.0 (RR 7.0 to 9.0), very occasional granular casts, and some calcium carbonate crystals, but was otherwise unremarkable. No cryptococci were seen in the urine. Immunoelectrophoresis demonstrated normal concentrations of all immunoglobulin classes.

**Antifungal therapy**

The patient was treated on an outpatient basis by the senior author (LMB). A 14 g polyurethane over-the-needle catheter (Milacath; Mila International, Kentucky, USA) was placed in a jugular vein after aseptic preparation of the overlying skin. Amphotericin B (Fungizone, Bristol Myers Squibb) was used for single agent therapy, as cost considerations precluded combination therapy incorporating either fluconazole or a triazole. A starting dose of 0.35 mg/kg amphotericin B diluted in 1L of D5W was given over approximately 1 hour. The pony’s temperature remained within normal limits and her HR was 64 bpm at the completion of the procedure.

The same infusion was given the following day, however approximately 4 h later the pony developed muscle tremors, tachycardia (HR 88 bpm) and pyrexia (41.0°C). Fever and tremors subsided following flunixin administration (250 mg IV). Accordingly, the pony was pretreated with flunixin (100 mg IV) prior to receiving the next infusion the following day; her temperature was 39.1°C prior to treatment and remained within normal limits after amphotericin B. A free-catch urine sample was collected. It had a specific gravity of 1.015 and was negative for blood, glucose and protein using a multi-reagent test strip.

On day 4 of therapy the pony had a normal rectal temperature and was pretreated with flunixin (100 mg IV) and given a 1 L infusion containing 0.4 mg/kg of amphotericin B over 1 hour. On day 5, the pony's temperature was 38.6°C and she was given the same drug regimen as on the preceding day. On day 6, the amphotericin B dose was increased further to 0.5 mg/kg IV, following pretreatment with flunixin (100 mg IV). On day 7, this protocol was repeated. On day 8, the IV dose of flunixin was reduced to 50 mg to minimise interference with autoregulation of renal blood flow, while the dose of amphotericin B remained at 0.5 mg/kg IV. This treatment protocol was subsequently repeated daily on days 9 to 31. The pony received a cumulative dose of 3g of amphotericin B over a month. The cost of amphotericin B, other drugs and consumables was approximately A$1,800.

From day 10 onwards, the rectal temperature returned to within normal limits and the HR gradually normalised to 42 bpm. The mare gradually became brighter and maintained a good appetite. Her water consumption increased progressively until she was drinking approximately 10% of her body weight daily. On days 16 and 23 urine was collected and found to have a specific gravity of 1.006 and 1.005, respectively. On both occasions, the urine was negative for blood, glucose and protein on dipstick analysis, but unfortunately a sample was not submitted for microscopic examination. Urea and creatinine concentrations in blood were determined on days 15 and 33; neither was increased significantly (Table 1). On the second last day of treatment the mare became very sore in the near fore foot. A foot abscess subsequently drained out of the coronary band.

**Figure 2. Standing right-lateral radiographs of the caudodorsal lung fields (82 kVp;36 mAs) of a pony mare with pulmonary cryptococcosis before (A), during (B) and ten months after (C) a month-long course of daily amphotericin B. Note the marked reduction in the size of the pulmonary cryptococcal granuloma as a result of therapy.**
reported. One horse with a localised intestinal granuloma that treatment of a horse with systemic cryptococcosis has been
to the best of our knowledge, this is the first time that successful
monitoring the titre every 6 months, at least until the titre
the serum cryptococcal antigen titre was 256. The authors intend
repeated 10 months following completion of treatment revealed
the size of the pulmonary lesions (Figure 2B). The mare subse-
sequently appeared to make an unremarkable recovery, putting on
40 kg in weight and regaining stamina. Thoracic radiographs repeated 10 months following completion of treatment revealed
almost complete resolution of the cryptococcomas (Figure 2C).
The patient is considered to have normal water consumption,
low-normal urine specific gravity of 1.016 and no evidence of
significant azotaemia (Table 1). The pony is currently asympto-
matic and ridden over jumps at pony club. At the time of writing,
was used successfully to treat a horse with mucormycosis caused
by Absidia corymbifera. The use of this route of administration
does not occur. The equine kidney seems tolerant of ampho-
tericin B were used at first and increased progressively, as
outlined above. The equine kidney seems tolerant of ampho-
tericin B delivered in this fashion and although reversible loss of
renal concentrating ability may occur, azotaemia rarely
develops.26,27 According to recommendations for human patients,
nephrotoxicity can be further minimised by supplementing the
diet with sodium chloride. Based on data from cats and dogs, an
effective cumulative dose of amphotericin B required to treat
invasive cryptococcosis successfully in horses is likely to be in
the order of 10 to 20 mg/kg.32 Interestingly, and almost heretically,
orally administered amphotericin B (40 mg/kg daily for 3 weeks)
was used successfully to treat a horse with mucormycosis caused
by Absidia corymbifera. The use of this route of administration
may deserve reappraisal.

This is the first horse in NSW for which the causal species of Cryptococcus has been recorded. Even though the horse was domi-
ciled close to Sydney’s central business district at the time the
infection was diagnosed, it was also situated adjacent to
Centennial Park, a eucalypt rich environment in which infectious
propagules of C gattii are likely to be present. Although it was
originally suspected that cryptococcosis was precipitated by a

Thoracic radiographs were repeated on day 18 and at the comple-
tion of treatment, and demonstrated a progressive reduction in
the size of the pulmonary lesions (Figure 2B). The mare subse-
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40 kg in weight and regaining stamina. Thoracic radiographs repeated 10 months following completion of treatment revealed
almost complete resolution of the cryptococcomas (Figure 2C).

Discussion
To the best of our knowledge, this is the first time that successful treatment of a horse with systemic cryptococcosis has been
reported. One horse with a localised intestinal granuloma that resulted in an intrususception was treated successfully by surgical
reseion of the lesion.27 Attempted treatment of a horse with cryptococcal meningitis was ultimately unsuccessful, although the
horse improved during therapy.13 Topical treatment of crypto-
coccal rhinitis was unsuccessful in the few horses in which it was attempted.4,6 The authors are aware of an additional horse with
cryptococcal pneumonia that was successfully treated using
amphotericin B by an equine practitioner in Perth. This patient, a
standardbred race horse with small cryptococcomas in the dorso-
caudal lung lobes, was treated with IV amphotericin B infusions
over a period of about 2 months until pulmonary lesions resolved
and transtracheal washings were consistently negative. This horse
went back into work and did not suffer recurrence, with treat-
ment costs in the order of A$10,000 (John Bolton, personal
communication).

The physical findings and results of clinical and laboratory inves-
tigations were in accord with those recorded previously for C gattii infections in horses from WA. Based on one of the author’s
(RM) experiences treating cryptococcosis in cats and dogs,32 and
a wealth of data from the human literature,2 amphotericin B was considered the most effective antifungal agent available for
therapy. Furthermore, in a species as large as the horse, this drug
has considerable cost advantages over azoles (ketoconazole, itra-
conazole, fluconazole) and flucytosine. A search of electronic
databases helped us find practical protocols for administering
amphotericin B as daily IV infusions. These protocols had been
developed principally for treating cutaneous pythiosis (‘swamp
cancer’).25,26 but have also been utilised successfully to treat other
invasive mycoses of horses such as histoplasmosis.27

A modified version of this protocol was developed by the senior
author for use in the pony mare of the present report. The daily
dose of amphotericin B was increased over several days, as is
recommended for human patients, then a daily dose of 0.5 mg/kg
was given on consecutive days to give a total treatment period of 1
month. The total cumulative dose of amphotericin B adminis-
tered, based on the horse’s final body weight, was 12 mg/kg, a
dose likely to have fungicidal effect on cryptococci in vivo based
on previous animal studies.32 Doses of amphotericin B cited in
the literature have varied from 0.3 mg/kg to as high as 0.9 mg/kg,
as a 1 hour IV infusion daily. In some cases, twice this dose was
given every other day, for convenience. Typically, lower doses of
amphotericin B were used at first and increased progressively, as
outlined above. The equine kidney seems tolerant of ampho-
tericin B delivered in this fashion and although reversible loss of
renal concentrating ability may occur, azotaemia rarely
develops.26,27 According to recommendations for human patients,
nephrotoxicity can be further minimised by supplementing the
diet with sodium chloride. Based on data from cats and dogs, an
effective cumulative dose of amphotericin B required to treat
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by Absidia corymbifera. The use of this route of administration
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ciled close to Sydney’s central business district at the time the
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Centennial Park, a eucalypt rich environment in which infectious
propagules of C gattii are likely to be present. Although it was
originally suspected that cryptococcosis was precipitated by a
following inapparent infection was the rule. This notion is supported by the observation that pulmonary infection in humans is asymptomatic in a proportion of diagnosed cases. Furthermore, recent studies of humans and koalas have confirmed that many individuals become infected, but develop self-limiting, inapparent forms of disease associated with small granulomas in either the upper or lower respiratory tract. A preliminary serological survey suggested that perhaps 10% of horses in the Hunter Valley of NSW had antibody concentrations in serum 'pigeon plague', pigeons, and more specifically their guano, are linked epidemiologically with \textit{C. neoformans} infections rather than \textit{C. gattii} infections. Although the history suggests that the disease process was chronic rather than acute, it is impossible to say if the infection had a monophasic course or reflected recrudescence of a residual focus from an earlier infection.

As far back as 1965 it was speculated that the development of cryptococcal meningitis in man following exposure to a large inoculum of organisms was the exception, and that recovery following inapparent infection was the rule. This notion is supported by the observation that pulmonary infection in humans is asymptomatic in a proportion of diagnosed cases. Furthermore, recent studies of humans and koalas have confirmed that many individuals become infected, but develop self-limiting, inapparent forms of disease associated with small granulomas in either the upper or lower respiratory tract. 

### Table 1. Summary of treatment protocol using 1 hour IV amphotericin B infusions given daily to a pony mare

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Dose of amphotericin B (mg)</th>
<th>Dose of flunixin (mg)</th>
<th>Rectal temperature (°C)</th>
<th>Heart rate (bpm)</th>
<th>Comments</th>
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<td>1</td>
<td>75</td>
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<td>38.1</td>
<td>64</td>
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<td>2</td>
<td>75 (3pm)</td>
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<td>80</td>
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<td>100</td>
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<td>38.5</td>
<td>56</td>
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<td>Serum urea 5.6 mmol/L, serum creatinine 110 µmol/L</td>
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<td>50</td>
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<td>Repeat X-rays of chest (Figure 2B)</td>
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<td>38.3</td>
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<td>50</td>
<td>38.4</td>
<td>56</td>
<td>Urine sg 1.005; -ve for glucose and protein</td>
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<td>100</td>
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</tr>
<tr>
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(1-year follow-up)

urea RR 3.7 - 6.2 mmol/L; creatinine RR 87 - 150 µmol/L; sg - Specific gravity; LCAT - latex cryptococcal antigen agglutination test

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suggestive of previous exposure to *Cryptococcus*, and a proportion of these horses might be expected to have residual foci of infection within the sinonasal cavity or pulmonary parenchyma, reflecting the likely role of inhalation in the initiation of infection.

The absence of haematogenous dissemination to either meninges, brain or skin, despite a protracted clinical course, suggests that the present case represented primary infection of an immunocompetent horse. Interestingly, most human patients infected with *C. gattii*, including Australian aboriginals and Papuans, are also immunocompetent and many have mass lesions within the pulmonary parenchyma and/or brain. The presence of large granulomas is currently considered to be a marker of a well directed cell-mediated immune response against *Cryptococcus* sp, such as might be expected in an immunocompetent host.

In the present case, a diagnosis was made using routine investigative methods. The radiographic and sonographic findings were consistent with either pulmonary abscessation with pyogenic bacteria, metastatic pulmonary neoplasia or granulomatous pneumonia. Cytological findings from both tracheal washings and a transthoracic aspirate indicated the lesions were cryptococcal in origin, a finding further supported by the high initial serum antigen titre of 4096. Baseline measurement of the lesions using diagnostic imaging provided a non-invasive means for monitoring the response to treatment, as did serial determinations of the LCAT. Repeat tracheal aspirates or aspirates from lesions, combined with cytology and culture, would have provided additional information about the response to treatment. Such studies, however, would have been invasive and were not performed.

Monotherapy with amphotericin B appeared to be an effective, inexpensive and relatively straightforward treatment modality in the hands of an experienced equine practitioner. Although amphotericin B infusions are ideally given to human patients over 4 to 6 hours, the abbreviated protocol used in this case was much more practical for a horse, and recent data from human patients suggests that shorter infusion periods can be acceptable. The development of fever, tremors and tachycardia during amphotericin B infusions is often considered to be a marker of toxicity. However, in our present case, this reaction can generally be prevented by pretreatment with a low dose of an IV glucocorticoid such as hydrocortisone. Because of the safety, efficacy and low cost of fluconox, this non-steroidal agent was used prophylactically as part of the treatment regimen in the present case. Although it might be argued that a cyclo-oxygenase-1 inhibitor is relatively contraindicated by the co-administration of a nephrotoxic drug such as amphotericin B, the combination appeared safe, at least in the patient of this report, and had a significant cost advantage over hydrocortisone. The presence of concurrent chronic laminitis was a further reason for choosing fluconox rather than a glucocorticoid. Amphotericin B produces nephrotoxicity that is partially reversible upon discontinuation of therapy. The increased water consumption and reduced urine specific gravity observed in the present patient reflected transient loss of tubular concentrating ability, although the absence of clinically significant azotaemia either during or after therapy suggested there were sufficient functioning nephrons to meet the demands of the patient. Nephrotoxicity was at least partially reversible, with an improvement in urine concentrating ability at the 1 year follow-up.

The pony was given a cumulative dose of amphotericin B in the vicinity of 12 mg/kg over 1 month. This produced a pleasing and prompt clinical response, reflected by improved demeanour, appetite and exercise tolerance over the course of therapy, and subsequently, with a corresponding decrease in the size of the pulmonary lesions as assessed by thoracic radiography. Furthermore the antigen titre had declined from 4096 to 256. Without performing invasive procedures, it was not possible to determine whether a titre of 256 reflects dead and dying cryptococcal elements being cleared by the mononuclear phagocytes of the host, or viable, residual fungal organisms deep within the lesions. Because of this uncertainty, it is our intention to monitor the antigen titre periodically, and implement a further course of amphotericin B if the titre rises, or fails to continue to decline. This decision reflects a value judgement, and in a younger pony we may have elected to administer a further 2 to 4 weeks of therapy.

Recent work in France has suggested that preheating amphotericin B to 60 to 70°C for 10 minutes prior to administration increases clinical efficacy and decreases nephrotoxicity by changing the physicochemical properties of the constituent colloidal dispersion. This useful refinement in the amphotericin B protocol should be considered when treating horses with systemic mycoses using amphotericin B.

**Acknowledgment**

The authors wish to thank Dr Steve McClintock for referral of this challenging case. Dr Wieland Meyer for performing the molecular mycology studies at ICMPR and John Bolton for his comments on the manuscript.

**References**

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Addendum

In March 2004, two of the authors (LMB and RM) collected superficial nasal swabs from 29 horses in the stable complex in which the case of the present report was normally housed, including the actual patient. Swabs were plated out onto bird seed agar and incubated at room temperature for several days. No horse tested positive for either C neoformans or C gattii. The antigen titre of the patient in August 2004 (2 years and 5 months after completing therapy) was 128.

Antimicrobial resistance of human and canine strains of E coli

Susceptibility to 12 antimicrobial agents was determined for E coli isolates from 82 women with cystitis, 170 women with pyelonephritis, 45 canine faecal samples and 76 healthy human volunteers.

Forty five (12%) of the isolates were resistant to ampicillin, sulfisoxazole, trimethoprim and trimethoprim-sulphamethoxazole, the most common resistance pattern. Resistance was significantly more common and extensive among isolates from women with cystitis or pyelonephritis than among isolates from canine or healthy human samples. The only resistance phenotype that was more common among canine isolates than human isolates was to sulphisoxazole alone (8 of 45 isolates, 18%).

The authors of the study conclude that dogs are unlikely to be an important external reservoir of antimicrobial-resistant E coli strains causing infections in humans. On the contrary, it may be that dogs could conceivably acquire resistant E coli strains from humans.