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Clinicopathologic and Diagnostic Imaging Characteristics of Systemic Aspergillosis in 30 Dogs

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Background: Systemic aspergillosis is a serious disease of dogs for which the clinical characteristics are poorly described.

Objective: To describe the clinical and diagnostic imaging characteristics of dogs with systemic aspergillosis.

Animals: Thirty dogs with systemic aspergillosis.

Methods: Retrospective case review. Medical records were reviewed for signalment, clinical features, and results of clinicopathologic testing and diagnostic imaging. Diagnosis was confirmed by culture of *Aspergillus terreus* (n = 13), *Aspergillus deflectus* (n = 11), or other *Aspergillus* spp. (n = 6).

Results: Compared with the background hospital population, German Shepherd dogs and female dogs were overrepresented (odds ratio [OR] 43, 95% confidence interval [CI] 20–91, $P < .0001$, and OR 2.9, 95% CI 1.2–6.7, $P = .02$), respectively, with 20 of the 30 dogs being German Shepherd dogs and 77% (23 of 30) of the dogs being female. The median age was 4.5 years (range 2–8 years). Anemia, leukocytosis, hyperglobulinemia, azotemia, hypercalcemia, and hypoalbuminemia were present in 8, 21, 12, 9, 8, and 6 dogs, respectively. Diskospondylitis, osteomyelitis and thoracic lymphadenomegaly were present in 16, 10, and 5 dogs, respectively. Sonographic findings were enlarged hypoechoic lymph nodes (n = 12), mottled and irregular kidneys with or without masses (n = 12), pyelectasia, and an aggregate of echogenic material in the renal pelvis (n = 9). Thirteen dogs were treated with antifungal drugs, with survival times ranging from 0 to 25 months after diagnosis.

Conclusions and Clinical Importance: Systemic aspergillosis typically involves young to middle-age female German Shepherd dogs, and there are characteristic abdominal ultrasound findings with the disease process. Infection with *A. deflectus* was as common as *A. terreus*, and in rare cases, long-term survival was associated with antifungal therapy.

Key words: Abdominal ultrasound; Diskospondylitis; Fungus; Infection; Mold.

Aspergillus species are saprophytic organisms that are ubiquitous in the environment and are opportunistic pathogens. *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger*, and *Aspergillus deflectus* cause disease in dogs. *A. fumigatus* and *A. flavus* usually cause localized disease in the nasal cavity. *A. terreus* is most frequently associated with systemic aspergillosis in dogs, but *A. deflectus* and *A. niger* cause disease in some dogs.[1–19]

Because German Shepherd dogs appear to be predisposed to systemic aspergillosis, it has been suggested that a hereditary immune defect might exist.[8–12] Dogs with systemic aspergillosis usually have nonspecific clinical signs, including anorexia, weight loss, weakness, lethargy, and vomiting. Laboratory abnormalities include neutrophilia, azotemia, increased total serum protein concentrations, and isosthenuria.[11] Radiographic changes consistent with diskospondylitis and osteomyelitis have also been described.[11,14]

The objectives of this study were to describe the clinical, laboratory, diagnostic imaging, and necropsy findings in a group of dogs with systemic aspergillosis, and to determine whether clinical presentation varies with infecting *Aspergillus* species.

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Materials and Methods

The electronic medical record system at the Veterinary Medical Teaching Hospital (VMTH), University of California, Davis was searched for all dogs with disseminated or systemic aspergillosis diagnosed between January 1990 and March 2007. Dogs were included only if a diagnosis was confirmed by identification of the infecting organism by fungal culture ante- or postmortem from a normally sterile site. Dogs without a culture-confirmed diagnosis were excluded.

The medical records were reviewed by 2 authors. Descriptive information regarding signalment, history (date of examination, presenting complaint, duration of illness, and previous immunosuppressive disease or drug therapy), physical examination findings, initial clinicopathologic data (CBC, serum biochemistry panel, and urinalysis), cytology reports of cerebrospinal fluid and aspirates, culture results, treatment regimes, and findings on biopsy or necropsy were abstracted. Follow-up data, consisting of treatment duration and response, morbidity, and case fatality rate, were obtained from the medical records and telephone calls to referring veterinarians. Because diagnostic testing and patient management were based on the primary clinicians' recommendations, and also client-related factors, there was considerable variability between dogs with respect to diagnostic testing and treatment protocols.

All available radiographs, ultrasound, CT, and MRI images were reviewed by the primary author and 1 board-certified radiologist, and qualitatively characterized in regard to pulmonary, lymph node, skeletal, abdominal organ, vascular, or central nervous system abnormalities. The type and extent of lesions, including size, shape, number, opacity, margination, location, echotexture, echogenicity, color Doppler findings, intensity, and contrast enhancement, were assessed.

χ^2 analysis was used to examine the breed and sex distribution in the hospital population with that of the group of dogs with aspergillosis. Odds ratios (OR) and 95% confidence intervals (CI) were

determined for each comparison. The relationship between infecting species (*A. terreus*, *A. deflectus*, or other *Aspergillus* species) and the following variables was determined: breed, sex, age, previous glucocorticoid therapy, involvement of specific sites (kidneys, central nervous system [CNS], eye, spleen, lymph nodes, bone, intervertebral disks), results of the CBC (including HCT and white cell, neutrophil, band, lymphocyte, monocyte, and platelet counts), and results of the serum chemistry panel (including serum creatinine, albumin, globulin, calcium, and BUN concentrations, and activities of ALT and ALP). χ^2 analysis was also used to compare categorical variables among infecting species. For continuous variables, the D'Agostino and Pearson omnibus normality test was used to determine if values were normally distributed. For data that were normally distributed, an unpaired *t*-test was used to compare continuous variables between every combination of 2 groups (*A. deflectus* and *A. terreus*, *A. deflectus* and other species, *A. terreus* and other species). For data that were not normally distributed, the Mann-Whitney test was used to compare continuous variables between groups. All analyses were performed with statistical software.[a]

Results

Thirty-nine dogs were initially identified with a diagnosis of systemic aspergillosis, and of these a subset of 30 dogs met the inclusion criteria. Nine dogs were excluded because the presumptive diagnosis was based on the results of histopathology or cytology combined with suggestive clinical findings. During the same period, 169,600 dogs were examined, yielding a frequency for dogs with a diagnosis of systemic aspergillosis of 0.02% of all dogs examined. Of the 30 dogs included in the study, there were 20 German Shepherd dogs, 3 Labrador Retrievers, 2 Rhodesian Ridgebacks, and 1 each of English Setter, Pug, Labrador Retriever cross, Hound cross, and a Whippet. German Shepherd dogs constituted 4.5% of dogs examined, compared with 67% of the dogs with systemic aspergillosis (OR 43, 95% CI 20–91, $P < .0001$). Rhodesian Ridgebacks were also overrepresented (2 of 30 compared with 839 of 169,570, OR 14, 95% CI 3–60, $P < .001$), but Labrador Retrievers were equally represented in the aspergillosis group and the background hospital population. Twenty-three of the dogs were female, and, of those, 18 were spayed. Females were overrepresented compared with the background hospital population (90,320 of 169,570, OR 2.9, 95% CI 1.2–6.7, $P = .02$). Seven dogs were male and of these 6 were neutered. The median age of affected dogs was 4.5 years (range 2–8 years).

Clinical Findings

Historical data were available for all dogs. The presenting signs and history varied widely. The median duration of illness before admission was 1 month (range 2 days to 9 months). Only 1 dog had evidence of immunosuppression before the development of clinical signs directly related to aspergillosis. This dog had been treated with immunosuppressive doses of cyclosporine and prednisolone for 3 and 5 weeks, respectively, for potential immune-mediated thrombocytopenia before the diagnosis of systemic aspergillosis. Dogs had signs attributable to the musculoskeletal (12), neurologic (6), respiratory (4), or gastrointestinal systems (2). Musculoskeletal signs were pain (8) or lameness (4). Neurologic signs could be divided into ataxia (5), paresis (5), obtundation/mental dullness (2), vision impairment (2), head tilt (1), circling (1), and seizures (1). Respiratory signs in these dogs were coughing (3) and respiratory distress (1), and gastrointestinal signs were attributable to chronic vomiting (2). Six dogs had nonspecific signs, including anorexia and weight loss.

The most common initial physical examination finding was muscle wasting and thin body condition (12), which could usually not be differentiated based on information in the medical records. Fever was also common (8), with a median rectal temperature of 103.4°F (range 102.7–104.2°F). Musculoskeletal abnormalities on physical examination included spinal pain (5) and lameness (4). Neurologic signs included vestibular abnormalities (5), ataxia (3), mental dullness (2), paraparesis (3), vision impairment (2), hemiparesis (2), circling (1), and seizures (1). Respiratory abnormalities included cough with harsh lung sounds (3), and respiratory distress (1). Five dogs had moderately enlarged peripheral lymph nodes. Four dogs had ocular abnormalities, which included bilateral chorioretinitis (2), hyphema (1), and panophthalmitis (1). Two dogs were recumbent and 2 had no clinically important abnormalities detected on physical examination. One dog had an arrhythmia, but no heart murmurs or pulse abnormalities were noted on initial examination.

Clinicopathologic Abnormalities

Eight dogs had a normocytic normochromic nonregenerative anemia (Table 1). Leukocytosis was observed in 21 dogs, 10 with a left shift and 4 with toxic neutrophils. Common abnormalities in the serum biochemical profile included hyperglobulinemia, azotemia, hypercalcemia, and hypoalbuminemia (Table 1). Urine specific gravity in all azotemic dogs was isosthenuric. Four of the 8 dogs with hypercalcemia were azotemic. Urinalyses were performed in 25 dogs. In 2 of the dogs fungal hyphae were seen in the urine sediment, hematuria was present in 11, and pyuria in 5. Cerebrospinal fluid was obtained for cytologic analysis in 8 dogs. Suppurative inflammation was noted in 4 samples and mononuclear reactivity in 1 sample. These 5 dogs had neurologic signs. The remaining cerebrospinal fluid samples contained no cytologic abnormalities. Cytologic evaluation of aspirates revealed pyogranulomatous inflammation with fungal hyphae from 8 lymph nodes, 2 kidneys, and 1 each from pleural effusion, lung, bone, joint fluid, and transtracheal wash. No cytologic abnormalities were noted in 2 splenic and 1 lymph node, liver, and bone marrow aspirates.

Diagnostic Imaging

Thoracic radiography was performed in 26 dogs, spinal radiography in 17 dogs, long bone survey radiography in 4 dogs, and cervical radiography in 1 dog. No abdominal radiographs were performed. Radio-

Table 1. Results of hematologic (26 dogs) and serum biochemistry (25 dogs) testing in dogs with systemic aspergillosis.

Variable	Median (Range)	No. (%) with Low Values	No. (%) with High Values	Reference Range
Hematocrit (%)	42% (14–46%)	8 (31%)	0	40–55%
Total WBC (cells/μL)	16,535 (9,600–33,900)	0	21 (81%)	6,000–13,000
Neutrophils (cells/μL)	12,643 (7,584–28,321)	0	21 (81%)	3,000–10,500
Band neutrophils (cells/μL)	0 (0–3,051)	0	10 (38%)	Rare
Monocytes (cells/μL)	1,608 (499–3,760)	0	16 (62%)	150–1,200
Lymphocytes (cells/μL)	1,566 (115–3,998)	4 (15%)	0	1,000–4,000
Platelets (cells/μL)	262,000 (86,000–400,000)	1 (4%)	0	150,000–400,000
Albumin (g/dL)	2.7 (1.3–3.6)	6 (24%)	0	2.9–4.2
Globulin (g/dL)	4.4 (2.3–5.8)	0	12 (48%)	2.3–4.4
Creatinine (g/dL)	1.1 (0.5–7.8)	0	9 (36%)	0.5–1.6
SUN (mg/dL)	19 (8–196)	0	9 (36%)	8–31
Calcium (mg/dL)	10.8 (9.9–13.9)	0	8 (32%)	9.9–11.4
Alanine aminotransferase (IU/L)	37 (19–609)	0	3 (12%)	19–67
Alkaline phosphatase (IU/L)	78 (21–1,920)	0	3 (12%)	15–127
Aspartate aminotransferase (IU/L)	37 (22–158)	0	5 (20%)	21–54

graphic abnormalities included evidence of diskospondylitis in 16 dogs, which had a median of 2 sites affected (range, 1–9 sites) (Fig 1), productive and destructive bone changes consistent with osteomyelitis in 10 dogs, 8 of which had multiple lesions (Fig 2), enlarged tracheobronchial lymph nodes (5), pleural effusion (4), pulmonary alveolar infiltrates (4), enlarged sternal lymph nodes (3), cranial mediastinal mass (3), and a cavitary pulmonary nodule (1). The productive and destructive bone lesions involved the vertebral bodies exclusive of the end plates (5), humerus (3), multiple sternebrae (3), rib (2), and 1 each of the scapula and tibia.

Twenty-three dogs (77%) had an abdominal ultrasonographic examination. Abnormalities were most commonly detected in the kidneys (16) and included pyelectasia (13), distorted mottled architecture (12), decreased corticomedullary distinction (11), an aggregate of echogenic debris in a dilated renal pelvis and dilated proximal ureter (9), distinct nodules or masses (7), papillary blunting (5), hydronephrosis (3), and renomegaly (1) (Fig 3). Splenic abnormalities were noted in 14 dogs and included hypoechoic nodules or masses (7), splenomegaly (6), hypoechoic lacy regions with sharp demarcation and absent Doppler signal consistent with infarction (6), mottled parenchyma (2), and splenic venous thrombosis (2) (Fig 4). Abdominal lymphadenomegaly was seen in 12 dogs and was generalized in 7 dogs. Diffuse hepatic hypoechogenicity was present in 5 dogs. Two dogs had an enlarged hypoechoic pancreas with surrounding hyperechoic mesentery, 2 had thickened gastric walls, 2 had ascites and 1 dog each had venous thrombi of the caudal vena cava or renal vein. Echocardiography was performed in 1 patient because of

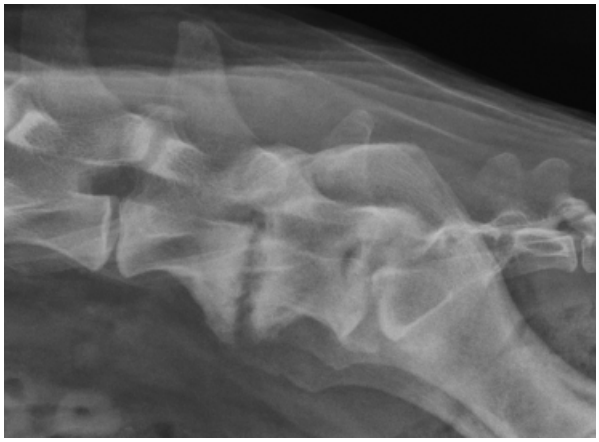

Fig 1. Lateral radiograph of the lumbar spine from a German Shepherd dog with systemic aspergillosis, showing the typical radiographic appearance of *Aspergillus* spp. diskospondylitis. There is irregular lysis with surrounding sclerosis of the end plates of the 6th and 7th lumbar vertebrae. Spondylosis deformans is also present on the ventral aspect of the affected vertebrae.

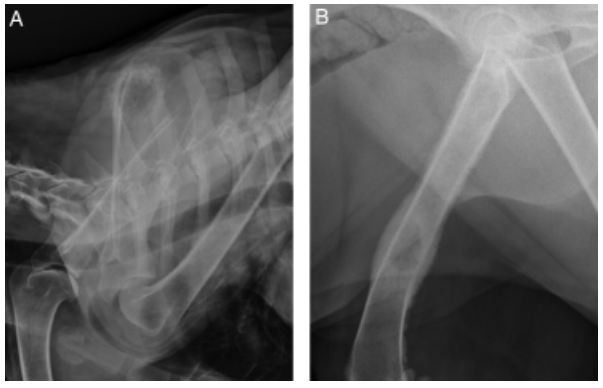

Fig 2. Bone survey lateral radiographs from a German Shepherd dog with systemic aspergillosis. (**A**) An aggressive mixed productive and destructive lesion is present involving the proximal aspect of the body of one of the superimposed scapula. There is also periosteal production and increased medullary opacity of the proximal diaphysis of the caudally positioned humerus. (**B**) An aggressive mixed productive and destructive lesion is present in the medullary cavity of the femur. In addition, there is an extensive mixed smooth to palisading periosteal reaction along the femoral diaphysis. The histologic diagnosis was granulomatous osteomyelitis.

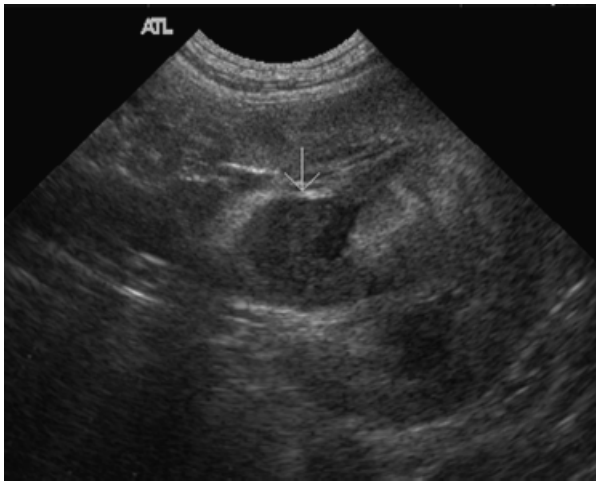

Fig 3. Transverse ultrasound image of the left kidney from a 4-year-old female spayed German Shepherd dog with systemic aspergillosis. There is moderate pyelectasia and proximal ureteral dilation. The renal pelvis is filled with an aggregate of echogenic debris (white arrow) and the papilla is blunted. The renal architecture is also distorted and mottled. The histologic diagnosis was fungal pyelonephritis with renal parenchymal granulomas.

ventricular bigeminy, and revealed mildly enlarged and irregular mitral leaflets. Echocardiography in 2 other dogs was unremarkable.

One dog had a thoracic CT scan that confirmed severe cranial mediastinal and tracheobronchial lymphadenomegaly, peribronchiolar, and alveolar infiltrates. Three dogs that presented for circling or vestibular disease had an MRI of the brain. All 3 dogs had asymmetric multifocal contrast enhancing lesions in the cerebrum on postcontrast T1-weighted images (Fig 5). The multifocal

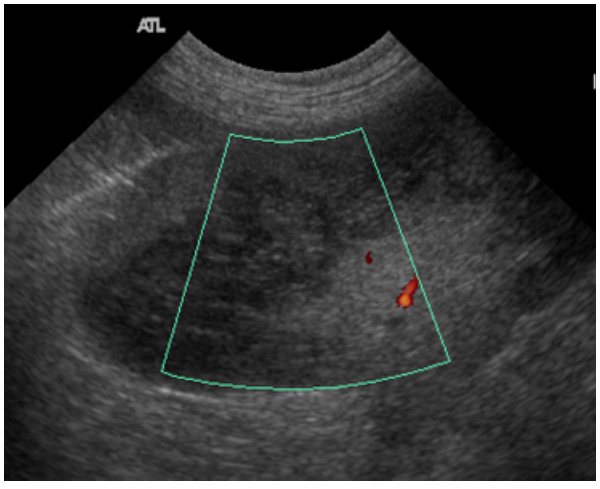

Fig 4. Sagittal ultrasound image of the spleen with color Doppler window from a German Shepherd dog with systemic aspergillosis. There are hypoechoic lacy regions with sharp demarcation from normal splenic tissue and absent Doppler signal, consistent with infarction. Evidence of blood flow is present within the Doppler window in the normal appearing splenic tissue. The histologic diagnosis was splenic granulomas with infarction.

lesions were hyperintense or heterogeneous on T2-weighted and FLAIR images and hypo- to isointense on T1-weighted images. In 2 of these dogs, the brainstem was involved in addition to the cerebral hemispheres. Two dogs had mild or severe meningeal enhancement. Two dogs had hyperintense regions on T2-weighted and FLAIR images that were consistent with perilesional and periventricular white matter edema. In addition, 1 of the 3 dogs that had an MRI performed had a mixed intensity heterogenous mass in the cerebrum that was contrast enhancing and caused a moderate shift of the falx cerebri.

Microbiology

The definitive diagnosis of systemic aspergillosis was determined by culture of a variety of normally sterile tissue and body fluid samples. *Aspergillus* spp. were most commonly isolated from the urine. Fourteen of 27 dogs (52%) with urine cultures performed were positive for *Aspergillus* spp. Other samples from which *Aspergillus* spp. were isolated included lymph node aspirates (6 of 10 cultures were positive), blood (5 of 15 cultures were positive), pleural effusion (3 of 4 cultures were positive), cerebrospinal fluid (2 of 6 cultures were positive), bone biopsies (2 of 2 cultures were positive), intervertebral disc aspirates (2 of 3 cultures were positive), and 1 kidney, splenic, bone, and inguinal mass aspirate and 1 joint fluid culture were positive (from 1–3 cultures per each of these additional sites). *Aspergillus* spp. were isolated from 12 of the dogs at necropsy, and in 2 of these dogs, antemortem fungal cultures were negative.

The most common species isolated were *A. terreus* (13) and *A. deflectus* (11). *A. fumigatus* (3), *A. niger* (2), and *A. flavipes* (1) were isolated less frequently. Dogs infected with *Aspergillus* species other than *A. deflectus* and *A. terreus* were more likely to be male than dogs infected with *A. deflectus* or *A. terreus* ($P = .02$) (Table 2). There was a similar sex distribution between dogs infected with *A. deflectus* and *A. terreus* ($P = 1.00$), and when these numbers were combined and compared with dogs infected with other species, the difference was still apparent ($P = .016$). Dogs infected with *A. deflectus* had a higher mean serum calcium than dogs infected with *A. terreus* (11.3 ± 0.3 versus 10.4 ± 0.2 mg/dL, $P = .04$); otherwise there was no difference in clinical presentation between dogs infected with *A. deflectus*, *A. terreus*, and *Aspergillus* species other than *A. deflectus* and *A. terreus* (Table 2).

Aspergillus serology using an agar gel antibody immunodiffusion test was performed in 5 dogs. In 4 dogs the test was negative. Only 1 dog, which was infected with *A. fumigatus*, had a positive result.

Histology

The results of light microscopic evaluation of biopsy specimens were recorded for 3 dogs. One was a cavitated pulmonary lesion after lobectomy from which *A. fumigatus* was cultured, and the other 2 were bone biopsies that contained fungal organisms. Microscopic evaluation of necropsy specimens were recorded for 18 dogs. The

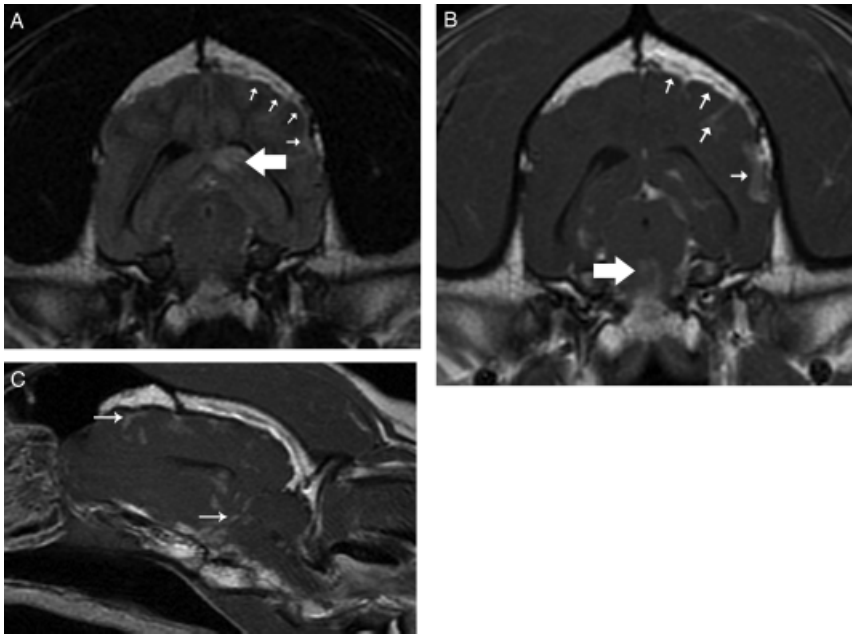

Fig 5. MR images of the brain and brainstem from a German Shepherd dog with systemic aspergillosis. The dog's left is to the right of the transverse images. (**A**) Transverse FLAIR image of the brain and brainstem reveals asymmetric hyperintensity in the left hippocampus (large white arrow) and meninges (small white arrows). (**B**) Transverse contrast enhanced T1-weighted image of the brain and brainstem revealing contrast enhancing lesions in the brainstem (large white arrow) and meninges (small white arrows). The plaque-like appearance of the ventral contrast enhancing meningeal lesion is caused by the image being acquired in the plane of a sulcus. (**C**) Sagittal contrast enhanced T1-weighted image of the cerebrum, cerebellum, and brainstem demonstrating multifocal contrast enhancing lesions involving the meninges (white arrows). The lesions appear deep in the brain parenchyma because the image plane was acquired in plane with the falx cerebri. The histologic diagnosis was fungal meningoencephalitis.

necropsies were all performed within 2–6 days of the diagnostic imaging tests. All 18 dogs had infiltration of multiple organ systems with fungal hyphae and granulomatous inflammatory cells. Organs involved included the kidney (13), spleen (13), lymph nodes (12), bone (9), vertebral endplates and disk (8), liver (6), heart (6; myocardium 4, endocardium 1, epicardium 1), pancreas (4), brain (3), lungs (3), eye (3), bone marrow (2), and the meninges, pleura, small intestine, skin, spinal cord, thyroid, prostate, and trachea/larynx (1 each). Renal abnormalities included granulomas (12) and pyelonephritis (6). In addition to granuomatous splenitis (13), splenic infarctions (7) were common. In general, the necropsy results correlated well with imaging findings, although the necropsy identified granulomatous inflammation in 3 spleens, 1 liver, 1 kidney, and 1 pancreas from dogs that had no abnormalities of these organs noted in the abdominal ultrasound reports.

Treatment

Seventeen of the 30 dogs (57%) were euthanized within a week of examination (median, 3 days). Fifteen of these 17 dogs had severe central neurologic signs, severe pain, or respiratory distress at the time of euthanasia. Antifungal drug therapy was initiated in 3 of these 17 dogs and consisted of amphotericin B in 1 dog with neurologic signs (1 dose), itraconazole in a dog with granulomatous enteritis and lymphadenitis (3 doses), and ketoconazole (3 doses) in another dog with neurologic signs. One dog had been treated with ketaconazole by the referring veterinarian for 8 months before presentation. The diagnosis was made at necropsy in 8 of the 17 dogs, and 6 dogs were euthanized immediately after the diagnosis was made.

Three of the 30 dogs were discharged without treatment shortly after diagnosis and were lost to follow-up.

Ten of the 30 dogs were treated with antifungal drugs for $\geq$ 6 days after presentation to the VMTH. All of these dogs lacked severe CNS signs, pain, or respiratory distress at the time treatment was initiated. Five of the 10 dogs were treated with itraconazole (5 mg/kg q24h).[b] One dog with *A. terreus* meningoencephalitis, diskospondylitis, and renal failure was lost to follow-up 2 days after diagnosis. Another dog with *A. terreus* diskospondylitis and pneumonia was lost to follow-up 2 months after diagnosis. One dog with *A. flavipes* diskospondylitis was lost to follow-up 1 month after diagnosis, when radiographic abnormalities remained static. One dog with a localized pulmonary infection with *A. fumigatus* was treated with itraconazole in combination with lung lobectomy, and was alive at the time of writing, 9 months after discharge. One dog with *A. terreus* chorioretinitis, pyelonephritis, and diskospondylitis was euthanized 3 months after diagnosis for progressive clinical deterioration and inability to stand. Five dogs were treated with multiple antifungal drugs in combination. One of these 5 dogs had an *A. terreus* infection associated with chorioretinitis, pyelonephritis, and osteomyelitis. Administration of 11 doses of lipid complex

Table 2. Comparison of selected categorical variables in dogs systemically infected with *Aspergillus terreus*, *Aspergillus deflectus*, and other *Aspergillus* species.

Variable	*Aspergillus terreus* (n = 13)	*Aspergillus deflectus* (n = 11)	Other *Aspergillus* spp. (n = 6)	*P* Value[a]
Breed				
GSD	10	8	2	.15
Non-GSD	3	3	4	
Sex				
Female	11	10	2	.02
Male	2	1	4	
CNS involvement				
Present	5	3	2	.34
Absent	8	8	4	
Renal involvement				
Present	9	9	3	.39
Absent	4	2	3	
Splenic involvement				
Present	7	7	1	.17
Absent	6	4	5	
Lymph node involvement				
Present	7	8	4	.62
Absent	6	3	2	
Osteomyelitis				
Present	8	3	1	.10
Absent	5	8	5	
Diskospondylitis				
Present	8	6	1	.18
Absent	5	5	5	

[a] *P* values represent the results of a χ^2 analysis across all 3 groups of species.

amphotericin B (ABLC)[c] (2 mg/kg IV, every other weekday) together with itraconazole (5 mg/kg PO q24h) was associated with gradual clinical improvement. Fungal culture of the urine 1 month after discontinuation of ABLC yielded a single colony of *A. terreus*, despite continued therapy with itraconazole. The dog was euthanized 4 months after diagnosis because of acute onset of respiratory distress. Tracheal mural granulomas with intralesional fungal hyphae were found using bronchoscopy, and disseminated fungal disease was found at necropsy. The 2nd dog was treated with 7 doses of ABLC before deterioration of renal function necessitated discontinuation of therapy. This dog presented with abdominal lymphadenomegaly, which resolved during ABLC treatment. Treatment with itraconazole was initiated after discontinuation of ABLC, but there was progression of renal failure. The dog was diagnosed with diskospondylitis, and 6 months after diagnosis the dog was lost to follow-up. The 3rd dog had an *A. niger* infection, which was associated with hilar and mesenteric lymphadenomegaly. This dog was treated with 12 doses of ABLC in combination with itraconazole, which was associated with initial improvement in the lymphadenomegaly, but a recheck ultrasound 1 month after starting treatment showed recurrence of lymphadenomegaly and splenomegaly. Treatment was changed to terbinafine[d] (15 mg/kg PO q24h) and itraconazole, with no clinical improvement. The dog was lost to follow-up 3 months after diagnosis.

The dog with the longest survival time had an *A. deflectus* infection involving the lungs and thoracic and abdominal lymph nodes. This dog was treated initially with itraconazole (5 mg/kg PO q12h) and 10 doses of ABLC, with marked improvement and resolution of pulmonary infiltrates. Development of renal failure necessitated discontinuation of ABLC treatment. Six weeks after diagnosis, treatment with subcutaneous deoxycholate amphotericin B[b] (0.8 mg/kg in 22 mL/kg 0.45% NaCl and 2.5% dextrose) was initiated because of worsening hilar lymphadenomegaly. A sterile injection site abscess developed following the 3rd treatment. Because of persistent hilar lymphadenomegaly, itraconazole was replaced with voriconazole[e] (5 mg/kg PO q12h) and IV ABLC therapy was reinstituted, which was associated with partial remission. A serum voriconazole level 1 month after initiating therapy was in the desirable therapeutic range (2.1 μg/mL).[20] Treatment with ABLC was continued for 7 weeks (20 doses). One month after discontinuing ABLC, the dog's condition deteriorated and abdominal ultrasound showed generalized abdominal lymphadenomegaly. Voriconazole was discontinued and terbinafine (10 mg/kg PO q24h) and posaconazole (5 mg/kg PO q24h)[f] were initiated, and the dog was treated twice with ABLC. Because of disease progression in the face of this therapy, 2 weeks later treatment with caspofungin[g] (1 mg/kg IV in 250 mL 0.9% NaCl over 1 hour, q24h) was initiated. Rapid clinical improvement and resolution of lymphadenomegaly was noted 6 weeks after starting therapy, at which time caspofungin was administered 3 times weekly for 2 months, then on 3 consecutive days every 3 weeks for 4 months. Urine culture 1 month after discontinuing therapy showed small numbers of *A. deflectus* that were sensitive to caspofungin. Twice weekly caspofungin therapy was reinstituted for 2 weeks, at which time urine fungal culture was negative, then on 3 consecutive days every 3 weeks. One year later, the dog developed lethargy, inappetence, vomiting, and diarrhea, and aspirates of enlarged mesenteric lymph nodes revealed fungal hyphae with marked necrosis. Treatment with anidulafungin[h] (3 mg/kg IV in 250 mL 0.9% NaCl over 1 hour, q24h) was instituted. Because of the development of a severe diffuse urticarial reaction, diphenydramine was administered (2 mg/kg IV, once) and the rate of infusion was slowed. Because there was no response after 8 treatments, treatment with ABLC (2 mg/kg IV q24h, 6 treatments) and micafungin[i] (3 mg/kg in 250 mL 0.9% NaCl over 1 hour, q24h, 3 treatments) was instituted, with no response. The dog was euthanized, 25 months after diagnosis.

The final dog was diagnosed with *A. terreus* diskospondylitis and treated with hemilaminectomy and voriconazole (5 mg/kg PO q12h). Marked clinical and radiographic improvement was noted at a 1 month recheck. Multiple new diskospondylitis lesions were documented 6 months after diagnosis, and therapy was changed to posaconazole (5 mg/kg PO q24h), which again was associated with clinical improvement but only static to mildly improved radiographic changes 2 months later. A recheck 4 months later showed progression of

diskospondylitis, and the dog was euthanized 13 months after diagnosis.

Discussion

In this study, young adult female German Shepherd dogs comprised the majority of dogs with systemic aspergillosis and there were approximately 3 times the number of female as male dogs, although the female sex predisposition was not noted in dogs infected with species other than *A. deflectus* and *A. terreus*. Historical evidence suggested an immunosuppressed state in only 1 dog. Infection of the bone, intervertebral disks, central nervous system, eyes, pleural space, mediastinum, and pulmonary parenchyma led to presenting signs of pain and lameness, neurologic dysfunction, ocular disease, cough, and respiratory difficulty. Involvement of the renal parenchyma was associated with signs of weight loss, vomiting, and inappetence.

Hyperglobulinemia was the most common abnormality on serum biochemical analysis and has been reported previously in dogs with systemic aspergillosis.[11] Hypercalcemia was also common and may have resulted either from granulomatous inflammation or from renal failure, which was present in half of these dogs.[21] The availability of ionized calcium concentrations would have helped to differentiate between these causes.

Although necropsy findings of granulomatous nephritis and granulomatous splenitis with infarction have been reported in dogs with systemic aspergillosis, sonographic findings have not been reported.[3] The only differential diagnosis in the literature for the common finding of pyelectasia and proximal ureteral dilation with an aggregate of echogenic debris in the renal pelvis is a hematoma.[22] A differential diagnosis of systemic aspergillosis should be strongly considered in dogs with this finding in conjunction with the finding of distorted mottled renal architecture with or without discrete nodules. At necropsy these findings were correlated with renal granulomas and fungal pyelonephritis.

Given the common occurrence of splenomegaly and splenic nodules in dogs in general, the hypoechoic lacy regions in the spleen with sharp demarcation and absent Doppler signal was considered the most clinically important sonographic abnormality of the spleen. These were confirmed as infarctions on necropsy. Infarctions in the spleen in dogs of this study were seen with granulomatous infiltration, but could also represent altered blood flow and coagulation abnormalities.[23,24]

Radiographic findings in the dogs in this study consisted of multiple diskospondylitis lesions, productive and destructive bone lesions, and intrathoracic lymphadenomegaly. MRI features seen are similar to other infectious and noninfectious inflammatory brain diseases.[25,26]

The necropsy findings in this study confirmed the diagnostic imaging findings to be consistent with granulomatous organ infiltrate, fungal pyelonephritis, diskospondylitis, osteomyelitis, and infarction. The relative insensitivity of ultrasound was highlighted by the fact that several small lesions detected at necropsy were not detected antemortem. Because necropsies in these dogs were performed shortly after imaging, these lesions were most likely present at the time of ultrasound. The frequent finding of granulomatous lesions within the heart was unexpected, as physical examination findings did not suggest cardiac disease in these dogs. In 1 dog echocardiography revealed changes suggestive of fungal valvular endocarditis, although a necropsy was not performed. It is possible that lesions of the myocardium and valves may be additional sonographic findings in dogs with systemic aspergillosis.

An antemortem diagnosis was most commonly reached by isolation of fungus from urine culture, but positive cultures were also obtained from many other affected tissues. Therefore, in patients suspected to have systemic aspergillosis, fungal culture of urine, blood, and aspirates of abnormal appearing organs or lymph nodes identified via diagnostic imaging is recommended. Because of the lack of sensitivity noted in this study, *Aspergillus* serology using agar gel immunodiffusion does not appear useful. The clinical utility of PCR and other serologic tests for *Aspergillus* was not evaluated in this study.

Infection with *A. deflectus* in this case series was nearly as common as infection with *A. terreus*. Only 1 dog has been reported with systemic *A. deflectus* infection outside of California.[4] The previous reports of *A. deflectus* in 4 dogs and a Springer Spaniel were from California and included dogs seen before the dates of this study.[1–5] Therefore, even though *A. deflectus* was common in this case series, dogs with systemic aspergillosis in other geographic regions may be more commonly infected with *A. terreus*. To the authors, knowledge, only 2 other reports of systemic *A. niger* infections exist in the literature, and only 1 case each of systemic *A. fumigatus* and *A. flavipes* infection has been reported.[27–29] Although the number of cases was small, the results of this study suggested that the clinical presentation of dogs with systemic aspergillosis does not vary with infecting species. The lesion locations in this case series were consistent with hematogenous dissemination. The site of entry was not determined, but possibly occurred through respiratory or gastrointestinal routes because external wounds were not detected. However, intrathoracic disease, which may suggest the respiratory system as the route of inoculation, was detected in only 9 dogs and gastrointestinal involvement was detected in only 1 dog.

The survival times of the dogs in this study had a wide range. Euthanasia was often performed because of severe CNS signs, severe pain, or respiratory distress together with a diagnosis considered by the clinician to have a poor prognosis. Dogs presenting with less severe signs often had prolonged survival following antifungal drug therapy, and so treatment should be considered in such cases. The longest survival time occurred in the dog treated with ABLC, novel azoles, and IV echinocandins, which currently is cost prohibitive to most owners. Daily therapy with caspofungin was not maintained in this case for financial reasons. A previous report described survival of more than 3 years in a dog treated for systemic aspergillosis with itraconazole alone.[6]

Limitations of this study are caused by its retrospective nature, and therefore diagnostic tests were performed at the discretion of the clinician at the time. The variability in the diagnostic tests performed most likely introduced bias into the actual laboratory and imaging findings. Necropsy or histology follow-up was not available in all dogs with imaging findings. The low number of cases in each group of causative *Aspergillus* spp. limited our ability to conclude the presence of small differences between groups with confidence. Treatment regimes varied and there was no way to determine if one treatment regime was better than another. Although the survival times of treated and untreated dogs differed, treated dogs had a less severe clinical presentation, and the majority of the untreated dogs were euthanized shortly after presentation because of disease severity. Prospective treatment studies considering different treatment regimes with different clinical cases need to be conducted to determine a potential optimal therapy. Lastly, the fact that this was a referral population might have influenced the severity of disease in these patients as well as which diagnostic and treatment options were available, and both of these issues may have influenced survival.

In conclusion, systemic aspergillosis should be considered in young to middle-aged primarily female German Shepherd dogs that present with nonspecific clinical signs or those related to the musculoskeletal, neurologic, respiratory, or gastrointestinal systems. The most common radiographic abnormality is diskospondylitis, but polyostotic bone lesions and enlarged tracheobronchial lymph nodes may also be seen. The diagnosis should be particularly suspected in dogs with abdominal ultrasonographic evidence of mottled and irregular kidneys with or without nodules or masses, pyelectasia, and an aggregate of echogenic material in the renal pelvis. *A. deflectus* in this case series was nearly as common as *A. terreus*, and the clinical presentation did not appear to differ with infecting species. Treatment with antifungal drugs should be considered in dogs with less severe presentations because long-term remission may be possible.

Footnotes

[a] GraphPad Prism, version 4.00, GraphPad Software Inc, San Diego, CA
[b] Cardinal Health, Dublin, OH
[c] Abelcet, Enzon Pharmaceuticals Inc, Indianapolis, IN
[d] Lamasil, Novartis Pharmaceuticals Corporation, East Hanover, NJ
[e] Vfend, Pfizer Incorporated, New York, NY
[f] Noxafil, Schering-Plough, Kenilworth, NJ
[g] Cancidas, Merck & Co Inc, Whitehouse Station, NJ
[h] Eraxis, Pfizer Incorporated
[i] Mycamine, Astellas Pharma US Inc, Deerfield, IL

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