

## Clinical Features and Magnetic Resonance Imaging Findings in 7 Dogs with Central Nervous System Aspergillosis

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**Background:** Systemic aspergillosis is a manifestation of *Aspergillus* sp. infection that can result in central nervous system (CNS) involvement with marked alterations in CNS function. Information regarding the clinical presentation and magnetic resonance imaging (MRI) findings in cases of aspergillosis with CNS involvement is lacking, resulting in a need for better understanding of this disease.

**Hypothesis/Objectives:** The primary objectives were to describe the clinical features and MRI findings in dogs with CNS aspergillosis. The secondary objectives were to describe clinicopathologic findings and case outcome.

**Animals:** Seven dogs with CNS aspergillosis.

**Methods:** Archived records from 6 institutions were reviewed to identify cases with MRI of CNS aspergillosis confirmed with serum galactomannan enzyme immunoassay (EIA) testing, culture, or supported by histopathology. Signalment, clinical, MRI, clinicopathologic, histopathologic, and microbiologic findings were recorded and evaluated.

**Results:** Aspergillosis of the CNS was identified in 7 dogs from 3 institutions. The median age was 3 years and six were German Shepherd dogs. Five dogs had signs of vestibular dysfunction as a component of multifocal neurological abnormalities. The MRI findings ranged from normal to abnormal, including hemorrhagic infarction and mass lesions.

**Conclusions and Clinical Importance:** Until now, all reported MRI findings in dogs with CNS aspergillosis have been abnormal. We document that CNS aspergillosis in dogs, particularly German Shepherd dogs, can be suspected based on neurologic signs, whether MRI findings are normal or abnormal. Confirmatory testing with galactomannan EIA, urine, cerebrospinal fluid (CSF) or tissue culture should be performed in cases where aspergillosis is a differential diagnosis.

**Key words:** *Aspergillus*; Galactomannan; German Shepherd dog; Fungal.

**A**spergillus infections in dogs most commonly result in sinonasal disease, but may affect the pulmonary system or cause disseminated aspergillosis.<sup>1–6</sup> *Aspergillus fumigatus* is the most common species involved in sinonasal and bronchopulmonary disease, whereas disseminated aspergillosis has been reported to be caused by *A. terreus*, *A. deflexus* or, less frequently, other species of *Aspergillus*.<sup>1,2,7</sup> Illness because of *Aspergillus* has been reported in multiple species including dogs, cats, birds, horses, cattle, and humans.<sup>1,8–12</sup> Humans infected

### Abbreviations:

CNS	central nervous system
CSF	cerebrospinal fluid
EIA	enzyme immunoassay
MRI	magnetic resonance imaging
OD	oculus dexter
OS	oculus sinister
RBC	red blood cell
TNCC	total nucleated cell count
WBC	white blood cell

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with *Aspergillus* species often are immunocompromised.<sup>11</sup>

Systemic aspergillosis in dogs may cause discospondylitis, osteomyelitis, lymphadenopathy, nephritis, splenitis, and meningoencephalitis.<sup>1,3–6</sup> Systemic infections most commonly are reported in the German Shepherd dog, possibly because of heritable abnormalities of the humoral and cellular immune system in this breed.<sup>1,3–7,13–16</sup> In a retrospective study of 30 dogs with systemic aspergillosis, the majority (57%) were euthanized within 1 week of diagnosis.<sup>1</sup> Dogs that were treated with antifungal medications, all of which lacked severe or neurological signs, had reported survival times of <25 months.<sup>1</sup>

Several publications describe the clinical signs and diagnostic imaging findings in dogs affected by sinonasal disease or discospondylitis, but there is little available literature regarding dogs with aspergillosis of the central nervous system (CNS). Over the past 2 decades, 13 dogs with aspergillosis involving the CNS have been reported.<sup>1,3,5</sup> Neurologic signs were identified in 8 dogs.<sup>1,3,5</sup> Magnetic resonance imaging (MRI) results were reported for 3 dogs.<sup>1</sup> All 3 had abnormal MRI

studies, characterized by multifocal T2W hyperintense lesions (3/3) and foci of contrast enhancement on T1-weighted images of the brain parenchyma (3/3) or meninges (2/3).<sup>1</sup> In general, reported cases of CNS aspergillosis have had poor outcomes.

Given the relative lack of information concerning CNS aspergillosis in dogs, the primary objectives of this study were to describe the clinical findings and MRI features in dogs with CNS aspergillosis. The secondary objectives were to describe clinicopathologic findings and case outcome. We utilized a multicenter approach to enhance case identification of a disease process that may become more widely recognized as a consequence of improved antigen-based diagnostic testing.<sup>17</sup>

## Materials and Methods

Medical records databases between 2007 and 2014 from 6 institutions (Texas A&M University, Virginia Tech University, Auburn University, Purdue University, Washington State University and University of Georgia) were reviewed to identify dogs with systemic aspergillosis using the following search terms: “canine”, “discospondylitis”, “diskospondylitis”, “MRI”, “platelasia *Aspergillus* EIA”, “*Aspergillus*”, and “aspergillosis”. Dogs were included in the study if clinical signs compatible with CNS disease were present, MRI of the CNS had been performed and a diagnosis of aspergillosis was confirmed by culture (sterile site or from tissue samples collected at necropsy), *Aspergillus* galactomannan enzyme immunoassay (EIA) or both.

The signalment, neurologic examination findings, clinical pathology test results, diagnostic imaging findings, culture results, histopathologic results from biopsy and necropsy, and case outcome were recorded when available.

Magnetic resonance images were acquired on multiple MRI systems including both low and high field strength magnets (0.2T–3.0T). One radiologist (BY), blinded to signalment, clinical findings and neurolocalization, but not diagnosis, retrospectively reviewed and described the MRI findings for each case. The evaluation of MRI included CNS and all surrounding structures. All studies (with the exception of the 3T system) included both pre- and postcontrast spin echo or turbo spin echo T1-weighted (T1W: repetition time [TR] 461–992 ms; echo time [TE] 15–20 ms; slice thickness 2.5–5.0 mm), spin echo or turbo spin echo T2-weighted (T2W: TR 2,500–9,000 ms; TE 80–119 ms; slice thickness 2.5–5.0 mm), and T2-weighted fluid attenuation inversion recovery (T2-FLAIR: TR 5,600–35,000 ms; TE 90–119 ms; slice thickness 2.5–5.5 mm) images. A subpopulation of these studies included T2\* gradient recall echo (T2\*: TR 840–1,021 ms; TE 80–119 ms; slice thickness 4.0–5.0 mm) images. The studies performed at 3T included pre- and postcontrast T1-weighted FLAIR (T1-FLAIR: TR 2,200–4,500 ms; TE 2.4–11 ms; slice thickness 2.5 mm), T2W (TR 4,000–4,500 ms; TE 94 ms; slice thickness 2.5 mm), T2-FLAIR (TR 9,000–9,030 ms; TE 94 ms; slice thickness 2.5 mm) and T2\* (TR 1,030–1,200 ms; TE 19.9 ms; slice thickness 2.5 mm) images.

## Results

### Clinical Features

Seven dogs ranging in age from 2 to 13 years old, admitted at 3 institutions (Texas A&M University, Virginia Tech University, and Auburn University), met study inclusion criteria. The duration of clinical signs before evaluation ranged from 10 days to 2 months.

Dog 1, a 3-year-old male intact German Shepherd dog, presented with a 10-day history of an acute onset of circling and lethargy. On physical examination, the dog had a body condition score (BCS) of 2/5 with no other abnormalities identified. Neurologic examination identified circling to the left, decreased postural reactions in all limbs (worse on the right), absent menace response and vision oculus dexter (OD), decreased facial sensation on the right, positional ventral strabismus OD, and generalized proprioceptive ataxia. Neuroanatomical localization was consistent with a multifocal disease process involving the forebrain and caudal brainstem.

Dog 2, a 3-year-old female spayed German Shepherd dog, presented with a 2-week history of head tilt and 2-day history of a change of iris color in the left eye to red (rubeosis). On physical examination, the dog had a body temperature of 102.8°F and rubeosis of the left eye. Neurologic examination identified a head tilt to the left, absent menace response and direct pupillary light response (PLR) oculus sinister (OS), and paraspinal hyperesthesia from the cervical to lumbosacral regions of the vertebral column. Neuroanatomical localization was multifocal with involvement of the left retina or optic nerve, vestibular system and meninges or vertebral column.

Dog 3, a 2-year-old male castrated German Shepherd dog, presented for an 18-day history of ataxia and head tilt to the left with a 3-month history of right thoracic limb lameness. On physical examination, no significant abnormalities were detected. Neurologic examination identified a head tilt to the left, generalized vestibular ataxia, and decreased postural reactions on the left thoracic and both pelvic limbs. The neuroanatomic localization was central vestibular system.

Dog 4, a 4-year-old male castrated German Shepherd dog, presented with a 2-month history of progressive paraparesis and a 1-month history of head tilt to the right. On physical examination no abnormalities were identified. Neurologic examination identified a head tilt to the right, and nonambulatory tetraparesis. Segmental reflexes were decreased in all limbs, with the exception of a hyper-reflexive left patellar reflex. Paraspinal hyperesthesia was present in the cervical region of the vertebral column. The neuroanatomic localization was multifocal with involvement of the vestibular system and spinal cord or lower motor neuron system.

Dog 5, a 10-year-old male castrated German Shepherd dog, presented with a 3-week history of decreased appetite, lethargy, nasal discharge and reluctance to walk. The dog was referred after cluster seizures occurred, in addition to epistaxis. At the time of admission, the dog was in status epilepticus. Bilateral serosanguinous nasal discharge and normal nasal airflow was present on examination. Seizures ceased after two boluses of diazepam<sup>a</sup> (1 mg/kg IV each) and phenobarbital<sup>b</sup> (13 mg/kg IV once). After cessation of seizures, the patient had stuporous mentation, intermittent decerebrate posturing and extensor rigidity. The neuroanatomic localization was multifocal involvement of the forebrain and brainstem.

Dog 6, a 13-year-old male castrated Weimaraner dog, presented with a 2-month history of sneezing, bilateral,

muroid nasal discharge, lethargy, and abnormal behavior. Four months before admission, the dog had been diagnosed with stage IIIb lymphosarcoma and was undergoing chemotherapy and in clinical remission. At the time of admission, the dog had normal mentation, exudate on the external aspect of the nasal planum, ulceration of the left nasal planum, and absent airflow through the left nostril on physical examination. Overnight, the dog had a generalized seizure that ceased with the administration of diazepam (3 mg/kg per rectum). The next morning, a neurologic examination was normal. The neuroanatomic localization was forebrain.

Dog 7, a 3-year-old male castrated German Shepherd dog, presented with a history of head tilt and ataxia of 2-month duration. Similar clinical signs were reported by the owner to have occurred at 6 months of age and then resolved spontaneously. Physical examination identified a body temperature of 103.8°F and BCS of 1/5. Neurologic examination identified a right head tilt, positional ventral strabismus OD, absent menace response (vision not recorded) OS, vestibular ataxia and falling to the right, decreased postural reaction in the left pelvic limb and paraspinal hyperesthesia in the lumbar region of the vertebral column. The neuroanatomical localization was multifocal involvement of the vestibular system and spinal cord.

### *Clinicopathologic Findings*

Abnormal CBC, biochemistry and urinalysis results are included in supporting information. Urinalysis with sediment cytology identified fungal hyphae in the urine of dogs 5 and 7 (2/5 dogs). Special stains were not performed on urinalysis sediment.

A cisternal CSF analysis was performed in 6 dogs. The total nucleated cell count (TNCC) was increased in 4/6 samples evaluated (dogs 1, 4, 6, and 7), with a median of 248 cells/ $\mu$ L and range 20–1,450/ $\mu$ L (reference interval, 0–5 cells/ $\mu$ L). Cytologic examination identified neutrophilic pleocytosis in all cases with increased TNCC. The red blood cell count (RBC) was increased in 4/6 samples (dogs 1, 3, 4, and 7), with a median of 1,270 RBC/ $\mu$ L and a range of 976–15,450/ $\mu$ L. The microprotein concentration was increased in 4/6 samples (dogs 1, 4, 6, and 7), with a median of 78 mg/dL and a range of 38–1,682 mg/dL (reference interval, 0–30 mg/dL). Dog 2 had a normal TNCC, but percentages of neutrophils and eosinophils were increased on cytologic evaluation. Dog 3 had a normal TNCC but an increased percentage of neutrophils on cytology. The increase in TNCC in dog 4 (20/ $\mu$ L) was paralleled by an increase in RBC (8,980/ $\mu$ L), without evidence of erythrophagia or xanthochromia. These findings suggest that RBC contamination may have led to the increase in TNCC.

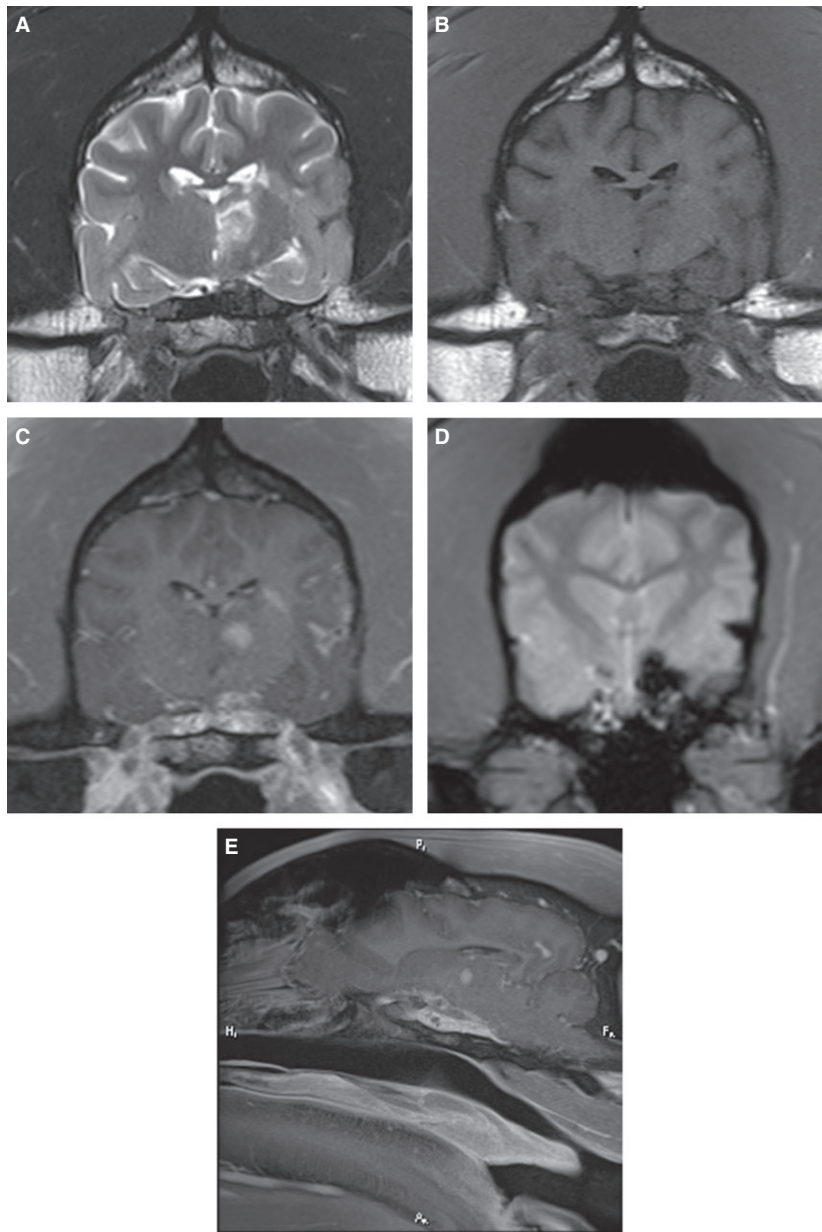
### *Magnetic Resonance Imaging*

Magnetic resonance imaging findings were diverse. All dogs had MRI of the head performed, whereas dog 4 also had MRI of the vertebral column from C1-S1 performed. Two dogs were scanned with 0.2 T (dogs 5 and 6), 4 dogs

were scanned with 3.0 T (dogs 1–4) and 2 dogs were scanned with a 1.0 T magnet (dogs 4 and 7). Dog 4 had C1-S1 scanned with 3.0 T and the head scanned with 1.0 T. In 3 of the 7 patients (dogs 2, 3 and 7), no MRI abnormalities of the brain, cranial cervical spinal cord, or meninges were found. These 3 dogs were scanned with a 3.0 T (dogs 2 and 3) and 1.0 T magnet (dog 7). Of the 3 patients with identifiable brain lesions (dogs 1, 5 and 6), all had lesions demonstrating T1W isointensity or mild hypointensity as compared to normal brain parenchyma. In two of these cases, lesions were T2W and T2-FLAIR hyperintense and the third was mixed in intensity on T2W and T2-FLAIR. Two of 3 cases (dogs 1 and 6) with brain lesions had mass effect (displacement and distortion of normal structures) whereas the third did not. In one case (dog 5) brain lesions were exclusively intra-axial, involving both the forebrain (frontal and olfactory lobes) and mesencephalon. In 1 case (dog 1) intra-axial lesions involved the thalamus and internal capsule and a plaque-like extra-axial lesion was identified near the optic chiasm (Fig 1). The third case (dog 6) had a large contiguous mass extending from the nasal region, through the cribriform plate, to involve the frontal lobe and MRI findings consistent with extensive white matter edema. In each of these 3 cases, moderate or strong contrast enhancement of the lesions was observed. In dog 1, diffuse unilateral contrast enhancement of the pachymeninges and leptomeninges was identified, including strong contrast enhancement of the plaque. In this case, there was T2\* GRE signal void of the plaque and enhancing meninges, indicating hemorrhage (Fig 1). No other cases demonstrated evidence of hemorrhagic lesions.

In 1 patient (dog 2), lesions of the brain were not seen, but the left cochlea had decreased T2W signal intensity, slightly increased T1W signal intensity and mild contrast enhancement compared to the contralateral cochlea. This dog also had ipsilateral ocular changes, including T2W hypointensity in the vitreous and iris with strong contrast enhancement of the iris, ciliary body, and optic disc.

In 1 case (dog 4) without identifiable brain lesions, multiple lesions of the spinal cord were present. The dog previously had been examined (1 month before presentation) and MRI of the vertebral column and brain was performed. Both the initial MRI of the brain and vertebral column and repeated MRI of the entire vertebral column were reviewed. At the C5-C6, T11-T12, T12-T13, L5-L6, L6-L7, and L7-S1 vertebral articulations lysis of the vertebral endplates and increased T2W signal of the disc spaces and surrounding paravertebral musculature were observed. Variable contrast enhancement of the intervertebral disc spaces, bony endplates, and paravertebral tissues were observed at all affected articulations. At the C5-C6 articulation, dorsal displacement of the nucleus of the intervertebral disc was identified, with marked ventral extradural spinal cord compression. The herniated nuclear material was T2W hyperintense, T1W isointense, and had strong contrast enhancement. The spinal cord at this level contained a focal, ill-defined T2W hyperintense lesion that was isointense on T1-weighted images but strongly enhanced



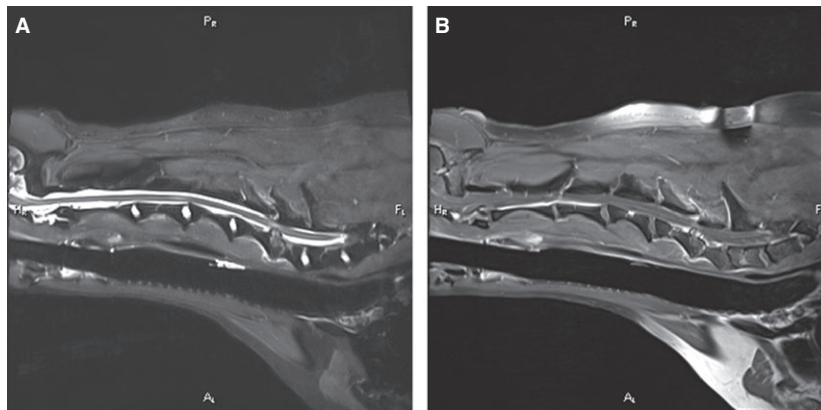
**Fig 1.** (A–E) MRI images from dog 1. T2-weighted (A), precontrast T1-FLAIR (B), fat-saturated postcontrast T1-FLAIR (C), T2\*-weighted (D) transverse images made at the level of the caudate nuclei, and fat-saturated postcontrast T1-FLAIR sagittal image made to the left of midline (E). Note the intra-axial lesion involving the left thalamus and internal capsule, the extra-axial lesion ventral to the thalamus, and the T2\* signal loss between pituitary stalk and parietal lobe of the plaque and meninges.

on T1-fat suppressed images after administration of gadolinium.<sup>c</sup> The vertebral lesions were consistent with multifocal discospondylitis with concurrent disc herniation causing a focal spinal cord lesion that could have represented contusion, gliosis, or regional myelitis (Fig 2).

### Microbiology Results

*Aspergillus* spp. was cultured from 6/6 dogs (dog 1 from kidney tissue, dog 2 from urine, dog 4 from urine and surgical site, dog 5 from the urine, meninges and lung, dog 6 from nasal biopsy specimen, and dog 7

from urine and CSF). Cerebrospinal fluid was only submitted for culture from dog 7. The species of *Aspergillus* was fully investigated in dogs 1, 4, 5, 6, and 7. *Aspergillus terreus* was identified in dogs 1, 4, 5, and 7; *A. fumigatus* was identified in dog 6. The *Aspergillus* galactomannan EIA test was performed on blood and was positive in all 3 cases tested (dog 1 at 6.17 index, dog 3 at 8.22 index, dog 4 at 7.11 index; reference,  $\geq 0.5$  index is positive). Additional infectious disease testing was performed in dogs 1 and 3 and was negative (EIA antigen for histoplasmosis and blastomycosis; serum titers for neosporosis, cryptococcosis, and toxoplasmosis).



**Fig 2.** (A, B) MRI images from Dog 4. T2-weighted (A) and fat-saturated, postcontrast T1-FLAIR (B) sagittal images of the cervical vertebral column. Note the disc herniation causing ventral compression of the spinal cord, the signal changes within the spinal cord, and the irregularity of the vertebral endplates at the level of the C5-6 intervertebral disc.

### Outcome

The owner of dog 1 declined antifungal treatment and the patient was discharged with an anti-inflammatory course of prednisone<sup>d</sup> (0.54 mg/kg PO q24 h). The dog continued to deteriorate, and 3 weeks later was euthanized. On external examination of the brain nodules and plaques within the leptomeninges were observed overlying the left half of the cerebral hemispheres, the cerebellum and the ventral surface of the brain (Fig 3). Microscopically, severe, chronic granulomatous, and necrotizing meningoencephalitis and vasculitis were identified within the diencephalon and telencephalon. Intralesional hyphae were present in the granulomatous lesions (Fig 4). Granulomatous and necrotizing inflammation with associated intralesional hyphae also was found in the kidneys, diaphragm, liver, and a perihepatic lymph node.

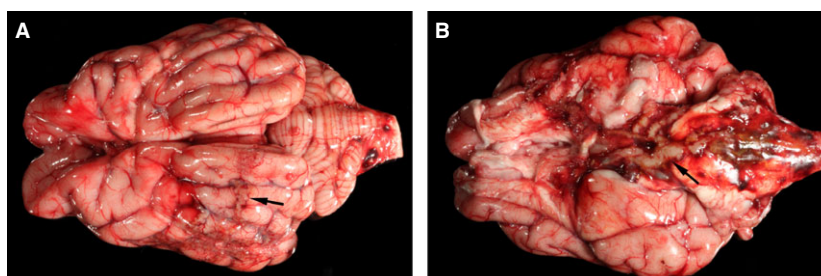
Because of the diagnosis of aspergillosis, the owner of dog 2 elected to have euthanasia performed by the referring veterinarian the day after discharge, and no necropsy was performed.

Radiographs of the right ulna of dog 3 identified an osteolytic lesion of the proximal ulna. A fine needle aspirate of this lesion identified the mass as a sarcoma. The owner of dog 3 declined treatment for aspergillosis

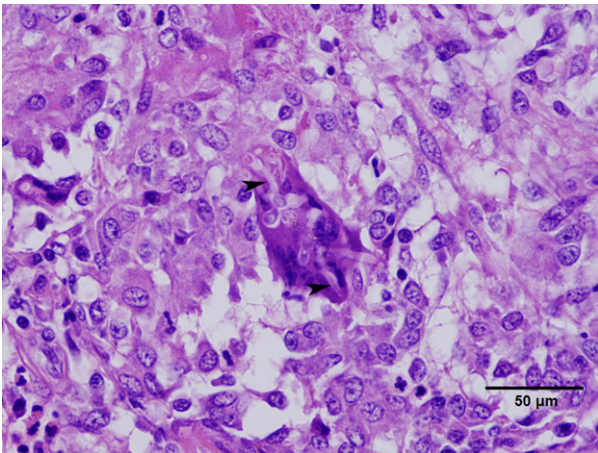
and the sarcoma in the right thoracic limb, and this dog was lost to follow-up after 1 month.

Dog 4 was diagnosed with a cervical disc extrusion secondary to discospondylitis (see MRI findings above). Ventral slot decompression was performed between the fifth and sixth cervical vertebrae. Biopsy of disc material disclosed marked necrosis, minimal neutrophilic inflammation and fungal hyphae. Culture of an aerobic swab from the ventral slot surgical site identified *A. terreus*.

Dog 4 was treated with voriconazole<sup>e</sup> (5 mg/kg PO q12 h) and terbinafine<sup>f</sup> (6.25 mg/kg PO q12 h), and amphotericin B liposomal formulation<sup>g</sup> (25 mg/kg IV q3d for 8 treatments). In an attempt to monitor response to treatment, the *Aspergillus* galactomannan EIA test was serially measured during treatment. This test result was 8.88 index after 1 week of voriconazole and terbinafine treatment, 6.91 index after 2 weeks of voriconazole and terbinafine treatment and 3 amphotericin B treatments, and 9.31 index after 8 amphotericin B treatments and 2 months of treatment with voriconazole and terbinafine (reference  $\geq 0.5$  index is positive). The dog was ambulatory tetraparetic with a head tilt to the right, after dedicated, rigorous physical rehabilitation and 11 months of antifungal treatment.



**Fig 3.** (A) Dorsal aspect of brain of Dog 1 with irregular raised plaques within the leptomeninges overlying the left temporal and parietal lobes (arrow). (B) Ventral aspect of brain of Dog 1 with irregular raised plaques overlying the ventral brainstem (arrow).



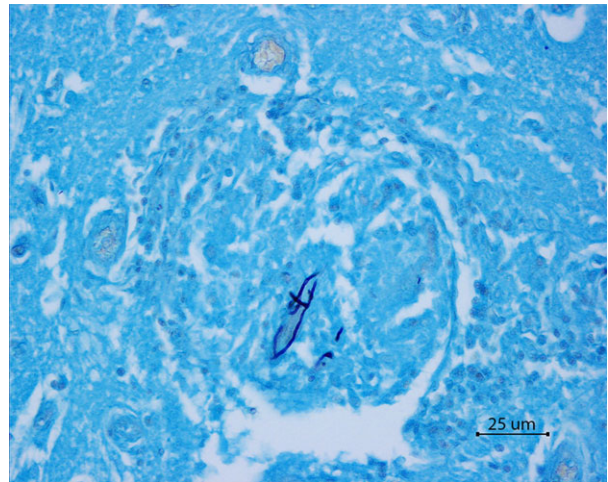
**Fig 4.** Hematoxylin and Eosin (H&E) 100× magnification stained intralésional hyphae (arrowhead) in the granulomatous lesions of the neuropil.

After anesthesia for MRI, dog 5 developed deficits of cranial nerves IX, X, and XII noted as absent gag reflex and tongue movement and never resumed adequate spontaneous respiration. Because of continued decline in status the dog was humanely euthanized.

On external examination of the brain of dog 5, severe bilateral softening of the olfactory and frontal lobes was observed. The cerebellar vermis was coned, and beneath the dura dark red material consistent with hemorrhage from an acute foramen magnum herniation was found. Mild herniation of the caudal occipital lobes was observed beneath the cerebellar tentorium. Multifocally within the leptomeninges were nodular to plaque-like lesions. Throughout the cerebrum, predominantly within the grey matter of the olfactory, frontal and parietal lobes, were multifocal macroscopic and microscopic nodules. Microscopically, the lesions contained macrophages, neutrophils and fungal hyphae. The nodules were more marked in the periventricular regions. Fungal hyphae stained positive for Grocott's methenamine silver stain (GMS) (Fig 5). Similar nodules also were present in the pancreas, liver, lungs, and kidneys. Fungal hyphae were present in the L6-7 intervertebral disc.

Rhinotomy was performed after MRI in dog 6, and identified a friable, light tan mass within the left caudal nasal cavity, extending into the left frontal sinus. Cytology and histopathology of endoscopic nasal biopsies were consistent with fungal rhinitis.

Dog 6 was discharged 24 hours after anesthetic recovery. The dog was treated with fluconazole<sup>h</sup> (3.3 mg/kg PO q12 h), terbinafine (4.3 mg/kg PO q12 h) and levetiracetam<sup>i</sup> (33 mg/kg PO q12 h). Chemotherapy was discontinued and the prednisone dosage was decreased (0.5 mg/kg PO q24 h). The dog was examined 9 days after discharge, with no changes in physical or neurologic examination. One month after diagnosis the dog was admitted to an emergency clinic because of status epilepticus. At the time of admission, the superficial cervical lymph nodes were enlarged. Fine



**Fig 5.** Grocott's Methenamine Silver (GMS) 40× magnification stained intralésional hyphae in granulomatous lesion of the forebrain.

needle aspirates of the lymph nodes were cytologically consistent with lymphoma. The dog was humanely euthanized but a necropsy was not performed.

Dog 7 was treated with fluconazole<sup>l</sup> (5 mg/kg IV q12 h) for 9 days, and discharged with voriconazole<sup>e</sup> (4.9 mg/kg PO q12 h) and terbinafine (28.5 mg/kg PO q24 h). The dog was lost to follow-up after discharge.

## Discussion

Aspergillosis occurs most commonly in German Shepherd dogs and has the ability to spread systemically, including to the CNS.<sup>1,3-7,15,18</sup> The dogs in this study were mainly young adult German Shepherds. In most cases, neurologic examinations identified multifocal involvement of the nervous system, including vestibular dysfunction. The dogs reported here had a range of MRI abnormalities and in some cases had normal MRI. Most dogs from which CSF was acquired had neutrophilic pleocytosis and increased protein concentration. The diagnosis of systemic aspergillosis initially was made with the *Aspergillus* galactomannan EIA test in 3/3 tested cases. Diagnosis was confirmed by culture of urine, CSF, or tissue in 6 cases in which culture was performed. Postdiagnosis survival data from this report should be viewed cautiously because the number of included animals was small, treatment protocols varied among dogs, and owner willingness to treat was variable.

In this cohort of cases, 6 of 7 dogs were German Shepherd dogs, five of which were young adults, consistent with previous reports.<sup>1,3,4,6,7,13-15,18</sup> Previous investigations of German Shepherd dogs affected by *Aspergillus* have indicated a possible hereditary immunodeficiency.<sup>16,18</sup> In contrast with the largest published series of dogs with CNS aspergillosis,<sup>1</sup> the majority of dogs in this report were male. The only breed in our study that was not a German Shepherd dog was a Weimaraner in which sinonasal *Aspergillus* had crossed the

cribriform plate. The mass was biopsied in the nasal region and confirmed to be *Aspergillus fumigatus*. This dog was considered immunocompromised because of previous chemotherapy for lymphosarcoma.

The majority of dogs in this study had a history of clinical neurologic dysfunction of <2 months. Neurologic signs were multifocal in most cases (6/7), and there was evidence of vestibular dysfunction in five of those cases. Vestibular dysfunction has been reported in 6 dogs with aspergillosis previously.<sup>1,3</sup> It is unknown whether *Aspergillus* has a predilection for affecting the vestibular system. In one case (dog 7), it is unknown whether vestibular signs were caused by CNS aspergillosis, because they were described 2 years before diagnosis.

In this case series, the MRI appearance of CNS aspergillosis was highly variable. Included dogs had normal studies (3/7), hemorrhagic infarction (1/7), and mass lesions (3/7). These features are in contrast with the MRI findings recognized in humans with CNS aspergillosis. In humans, lesion patterns have been identified on MRI including hemorrhagic and infarctive lesions in the distribution of perforating arteries, possible ring enhancement, and a lack of meningeal (0/13 patients in 1 report) or ventricular changes despite histopathologic evidence of their involvement.<sup>19–21</sup> Cerebral aspergillosis recognized on MRI in humans often affects the corpus callosum secondary to striate and perforate artery occlusion, an MRI finding which is unlike those of other fungal CNS diseases.<sup>19</sup> Neither callosal involvement nor ring enhancement was noted in any of the dogs in this report or a previous study of dogs with CNS aspergillosis.<sup>1</sup> Dogs with CNS aspergillosis seem to have meningeal lesions identified by MRI more commonly than affected humans. One of 4 dogs in this cohort (dog 1) had evidence of leptomeningeal and pachymeningeal involvement on MRI and histopathology. Two of the three previously reported studies of brain MRI of dogs with CNS aspergillosis described meningeal change.<sup>1</sup>

Our MRI data may have been impacted by certain limitations. We suspect the variability in MRI findings is the result of differences in disease progression and the relative insensitivity of MRI in detecting inflammatory brain lesions at standard field strengths.<sup>22,23</sup> The normal MRI studies were performed on 3.0T (2/3) and 1.0T systems (1/3). Other cases in our report may have had meningitis or ventriculitis that was not detected by MRI, but not all cases had necropsy or biopsy performed to confirm this hypothesis. Although evidence was not present on the MRI of CNS aspergillosis in all dogs, dogs with normal MRI had strong evidence to support the diagnosis of aspergillosis, with a positive CSF culture in dog 7, a strongly positive galactomannan EIA *Aspergillus* test in dog 3, and urine culture in dog 2. Dogs 2 and 7 had systemic evidence of infection. Both dogs had increased body temperatures at admission and dog 7 was in poor body condition. We identified hyperglobulinemia in both cases, an inflammatory leukogram in dog 7, and hyphal fungal elements in the urine sediment of dog 7.

Evaluation of CSF identified a neutrophilic pleocytosis with parallel increase in microprotein concentration

in 4/6 cases. Neutrophilic CSF pleocytosis can be seen in a variety of disease processes including neurotrauma and neoplasia (especially meningioma), although it is commonly seen in fungal and bacterial CNS infections.<sup>24</sup> Additionally, recent work suggests that CSF data alone does not accurately differentiate different CNS etiologies.<sup>25</sup>

Of the 3 dogs treated with antifungal treatment, 1 was still alive 11 months after treatment with no evidence of disease progression (dog 4), 1 was euthanized a month after treatment because of recurrence of lymphoma, and 1 was lost to follow-up. The successful treatment of dog 4 may be the result of a multimodal medical treatment plan that included voriconazole and terbinafine. In previous studies, dogs with systemic aspergillosis without CNS involvement that were treated with voriconazole had long survival times.<sup>1,13</sup> Together, these findings suggest a role for voriconazole in the treatment of systemic aspergillosis in dogs.

The diagnosis of CNS aspergillosis must be interpreted with caution in some of these cases. Although dogs 2, 3, 4, 6, and 7 had confirmation of aspergillosis by culture, serum testing, or histopathology of non-CNS organ systems, we did not have CNS tissue culture or histopathology. Thus, it is possible that neurologic signs resulted from a different underlying etiology. For example, an animal with systemic *Aspergillus* may have immunosuppression and be susceptible to opportunistic infections of the CNS or neoplasia. Additionally, in dog 3, galactomannan EIA was the sole methodology used to diagnose *Aspergillus*. Because the spinal cord of dog 3 was not evaluated by MRI, it is possible that an unidentified myelopathy could have contributed to the dog's clinical signs. Although there are factors known to cause false positive galactomannan EIA, such as administration of Plasmalyte<sup>k</sup> or beta-lactam antibiotics, dog 3 did not receive these treatments. The serum titer in dog 3 was 8.22; titers >1.5 have a sensitivity of 93% and a specificity of 92% for diagnosing *Aspergillus*.<sup>17</sup> Dog 2 had vestibular signs that were likely peripheral in nature based on examination findings and MRI changes of the cochlea on the affected side. The diagnosis of CNS aspergillosis in this case is supported by the changes in the optic disc OS, ipsilateral to the cochlear lesion. However, because of the fact that this case did not have histopathologic confirmation of the changes on MRI, it cannot be definitively proven to have had CNS involvement. The diagnosis of CNS aspergillosis in dog 7 also is supported by a menace change in an eye that was normal on MRI (ruling out chorioretinitis) and postural reaction deficits in the left pelvic limb and lumbar paraspinal hyperesthesia without evidence of discospondylitis on lumbar vertebral column radiographs.

In this cohort of cases, dogs with CNS aspergillosis had variable MRI changes. A diagnosis was established with galactomannan EIA testing or culture, with supportive findings on histopathology. Multiple methods of diagnostic investigation should be utilized when CNS aspergillosis is a possible etiology, because normal MRI cannot rule out CNS involvement. Although specific recommendations concerning treatment of CNS

aspergillosis cannot be made based on our data, the combination of voriconazole with other antifungal agents may improve survival.

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### Footnotes

- <sup>a</sup> Diazepam 5 mg/mL, Hospira, Inc., Lake Forest, IL  
<sup>b</sup> Phenobarbital 65 mg/mL, West-Ward Pharmaceutical Corp., Eatontown, NJ  
<sup>c</sup> Magnevist, Bayer Healthcare Pharmaceuticals Inc, Whippany, NJ  
<sup>d</sup> Prednisone 20 mg, Roxane Laboratories Inc., Columbus, OH  
<sup>e</sup> Voriconazole 200 mg, Mylan Pharmaceuticals Inc., Canonsburg, PA  
<sup>f</sup> Terbinafine 250 mg, Aurobindo Pharmaceuticals USA Inc., Dayton, NJ  
<sup>g</sup> Amphotericin B LC 5 mg/mL, Sigma-TAU Pharma Source, Gaithersburg, MD  
<sup>h</sup> Fluconazole 100 mg, Glenmark Pharmaceuticals Limited, Andheri, Mumbai, India  
<sup>i</sup> Levetiracetam 1,000 mg, Sandoz Inc., Princeton, NJ  
<sup>j</sup> Fluconazole 2 mg/mL, Hospira, Inc., Lake Forest, IL  
<sup>k</sup> Plasmalyte 148, Baxter Health, Deerfield, IL
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*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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### Supporting Information

Additional Supporting Information may be found online in Supporting Information:

**Table S1.** Clinicopathologic lab results.