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Disseminated Aspergillosis Without Bone Lesions In A Dog

A García[1*],M Gil[2], E Varela[2], V Roncero[3], R Barrera[4], P Ruiz[4] ,M. Hermoso de Mendoza[5]

[1*]Departamento de Producción Animal, CICYTEX-La Orden, Guadajira, Badajoz.
[2]Servicio de Recepción y Diagnóstico de Muestras, Hospital Clínico Veterinario UEX, Cáceres.
[3]Cátedra de Histología y Anatomía Patológica, Facultad de Veterinaria UEX, Cáceres.
[4,] Servicio Clínico de Pequeños Animales, Hospital Clínico Veterinario UEX, Cáceres.
[5]Cátedra de Patología Infecciosa, Facultad de Veterinaria UEX, Cáceres.

***Corresponding author:** A García
*fredgarsa@gmail.com

Abstract
This case report describes the clinical and pathological findings in a German Shepherd dog with disseminated aspergillosis, a rare disease that usually has a grave prognosis**.** Disseminated infections tipically affects bone, causing spondylitis, diskospondylitis, osteitis or osteomyelitis. In this case there was neither significant changes in the vertebrae and intervertebral disks nor infection and inflammation of the bone or bone marrow although the dog showed dorsal and cervical pain.

Veterinary practitioners should be aware of the difficulty of its early diagnosis and the possible absence of skeletal pathology in x-rays taken to detect this infection.

Keywords:
Aspergillus terreus; dogs; neurological diseases; German Shepherd

Introduction
Disseminated aspergillosis is a relatively uncommon but potentially fatal disease in dogs, known since 1978 [13]. Many dog breeds are occasionally affected, but the German Shepherd dog seems to be predisposed, maybe because of a deficiency in mucosal immunity [5,14].

Extrinsic factors that may predispose dogs to this fungal infection include mucosal lesions, immunosuppression, catheterisation or concomitant diseases [10]. Albeit other penetration ways are possible, inhalation and ingestion are the most probable.

The vegetative hyphae invade tissues and spread hematogenously, maybe by aleurioconidiae formation and liberation [3,7], causing granulomatous inflammation in diverse organs as kidney, spleen, NCS and frequently bone. Clinical manifestations are less than specific and may include mixed respiratory, urinary, digestive, locomotor and neurologic symptoms [14,3]. Veterinary practitioners should be aware of its difficult early diagnosis and the importance of predisposing factors such as the dog's breed and immunosuppressive medications.

Clinical features
A four-year-old male German shepherd dog was referred from a private veterinary clinic to the Veterinary Clinical Hospital of the University of Extremadura.

The animal had shown progressive weight loss and lethargy over the last month and a 1-week history of head tilt to the left, mental dullness, disorientation, convulsions and moaning. It had been treated with doxycycline and prednisone (Dacortin®, Merck).

The patient exploration at the VCH showed a rectal temperature of 38.6ºC, mucous membranes pink and moist, capillary refill time less than 2 seconds, a pulse of 68 beats per minute and a respiratory rate of 60 breaths per minute with an auscultated arrhythmia. The dog showed weakness and a painful extension and flexion of neck and all along the spinal column.

The most remarkable findings after a complete neurologic examination were myosis, ventrolateral strabismus and protruded left eye third eyelid. Right eye was normal. Moderate bilateral atrophy was noted in the temporalis and masseter muscles, and also affected thoracic and pelvic limb muscles. The column radiographs taken showed normal appearance of the spinal vertebrae and intervertebral discs.

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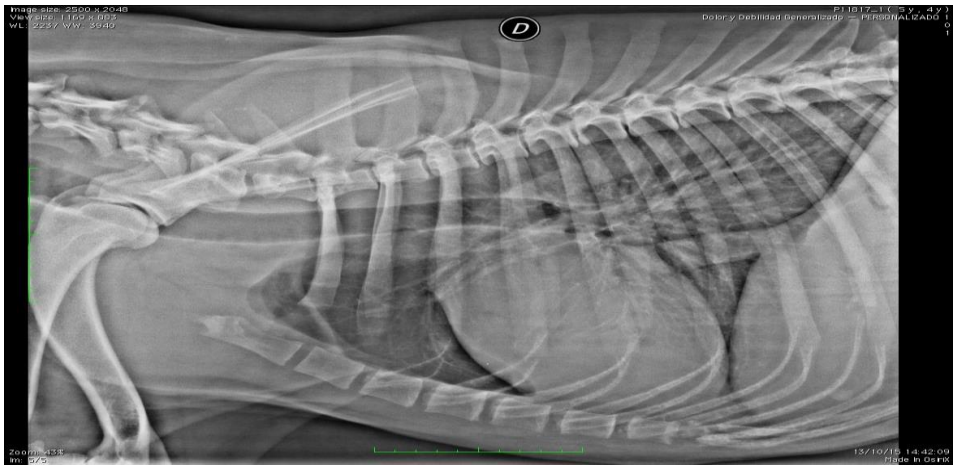

Figure 1 left lateral thoracic radiograph where no apparent abnormalities in the vertebrae and intervertebral discs were found.

Laboratory findings included mild normochromic, normocytic nonregenerative anemia, and leukocytosis with marked regenerative neutrophilia. The blood chemistry showed azotemia with elevated urea and creatinine levels, hyperphosphatemia, hyperproteinemia due to hypergammaglobulinemia, hypernatremia, hyperchloremia, and increased liver enzymes levels (Table 1). Urinalysis revealed a slight alkaline pH value with proteinuria and the microscopic assessment showed presence of erythrocytes and leukocytes in the urinary sediment. An arterial blood gas measure using a Radiometer NPT-7 Series Blood Gas Analyzer showed normal oxygenation with hypercapnia, with values of pH 7.44, pCO_2 17 mmHg, and pO_2 101 mmHg,

Table 1 (Hematologic and blood biochemical parameters).

Parameter	**Dog's values**	**Reference ranges**
Red blood cell (RBC) count (x10^6/µl)	5.37	5.9 – 7.6
White blood cell (WBC) count (/µL)	40.01 x10^3	6.80 – 14.00 x10^3
Mean corpuscular volume (fL)	70.50	62.0 – 71.0
Mean corpuscular hemoglobin concentration (g/dL).	34.03	33.0- 36,5
Absolute reticulocyte count (x10^3/µl).	18,236	> 60
Segmented neutrophils (x10^3/µl).	34.44	4.12 – 10.35
Band neutrophils (x10^3/µl).	2.56	0.00 – 0.10
Monocytes (x10^3/µl).	0.53	0.00 – 0.21
Analyte	**Dog's values**	**Reference ranges**
Urea (mg/dL).	163	21.4 – 51.3
Creatinine (mg/dL)	3.4	0.7 – 1.2
Phosphorus (mg/dL)	8.3	3.3 – 5.7
Sodium (mEq/L).	181	140-161
Chloride (mEq/L).	145	113-123
Plasma proteins (g/dL)	8.6	5.50 – 7.30
Gamma glutamyl transferase (GGT) (U/L)	12	1.0-6.0
Glutamic-pyruvic transaminase (GPT) (U/L)	201	16.00-49.00
Alkaline phosphatase (ALP) (U/L)	356	18.00-100.00

The animal was hospitalized and received intravenous fluid therapy of lactate Ringer's solution with methadone hydrochloride 0.5 mg/kg body weight IV (Semfortan®, Esteve) ranitidine hydrochloride 2 mg/kg IV (Zantac®, GlaxoSmithKline), and enrofloxacin antibiotic treatment (Alsir® 5%, Esteve).

Specific Enzyme-linked immunosorbent assays (ELISA) were performed with the commercially available kits IDEXX SNAP* Test 4Dx® (filariosis, ehrlichiosis, Lyme disease and anaplasmosis), and SNAP* Test Leishmania. In both tests negative results were obtained.

Microscopic agglutination tests for Leptospira-antibody detection included the serovars: *Australis*, *Autumnalis*, *Bratislava*, *Canicola*, *Grippotyphosa*, *Copenhageni* (*Icterohaemorrhagiae*), *Pomona* and *Saxkoebing*, and were also negatives.

Although the aerobic bacterial culture of spinal fluid obtained by lumbar puncture did not show bacterial growth after 24 hours of incubation, on the third day the animal developed a disseminated intravascular coagulation (DIC) and finally died four days later; a full necropsy could be conducted.

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The dog carcass was in poor condition and emaciated. Gross pathologic lesions included multiple miliary, white-yellow foci in kidneys, spleen, heart, and pulmonary pleura, which also showed congestion and edema.

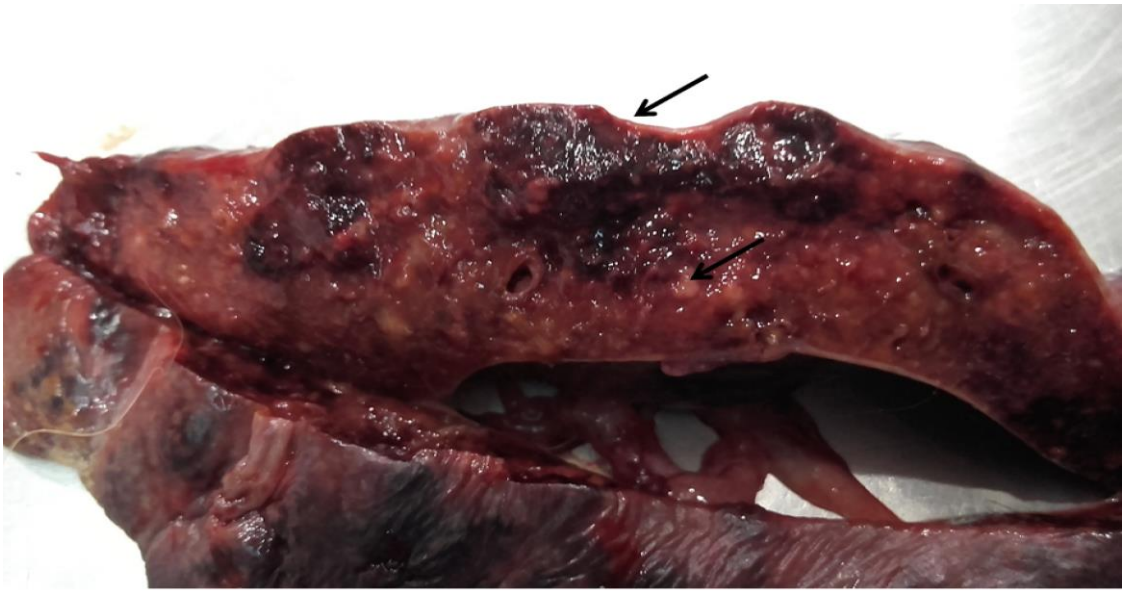

Figure 2 Spleen: Multifocal infarction and multiple whitish nodules in parenchyma.

There were multifocal and coalescing solid white nodules 1 to 3 mm in diameter within myocardium, epicardial and endocardial surfaces.

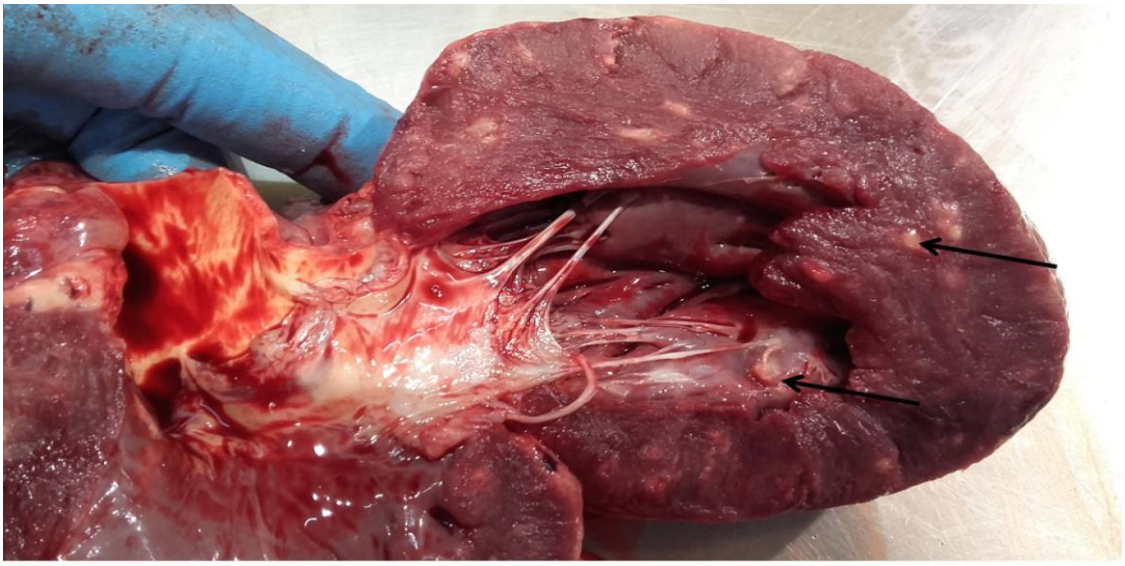

Figure 3 Heart: Whitish multifocal nodules in myocardium and endocardial surfaces.

Both kidneys showed those disseminated foci along the renal surface, and also throughout the medulla and cortex. The spleen was enlarged and multifocal infarcts were noticeable with multifocal white masses similar to those seen on hearth and kidneys. The lymph nodes that drained affected organs were enlarged and the brain revealed generalized congestion. However, post mortem examination of the skeletal system did not reveal any abnormality.

Histologic analysis revealed infiltration of multiple organ systems with fungal hyphae and granulomatous inflammatory cells. Kidneys and spleen revealed granulomatous interstitial nephritis and granulomatous splenitis with abundant giant cells and fungal hyphae forms inside. The heart showed necrotizing myocarditis with intralesional fungal hyphae and meningoencephalitis with necrotic areas was observed in the brain.

Blood agar and Sabouraud dextrose agar plates inoculated with spinal fluid and affected organ homogenates revealed the presence of pure cultures of mycelium fungal colonies after 5-6 days of incubation at 30ºC, which were identified as *Aspergillus terreus* by its morphometric characteristics.

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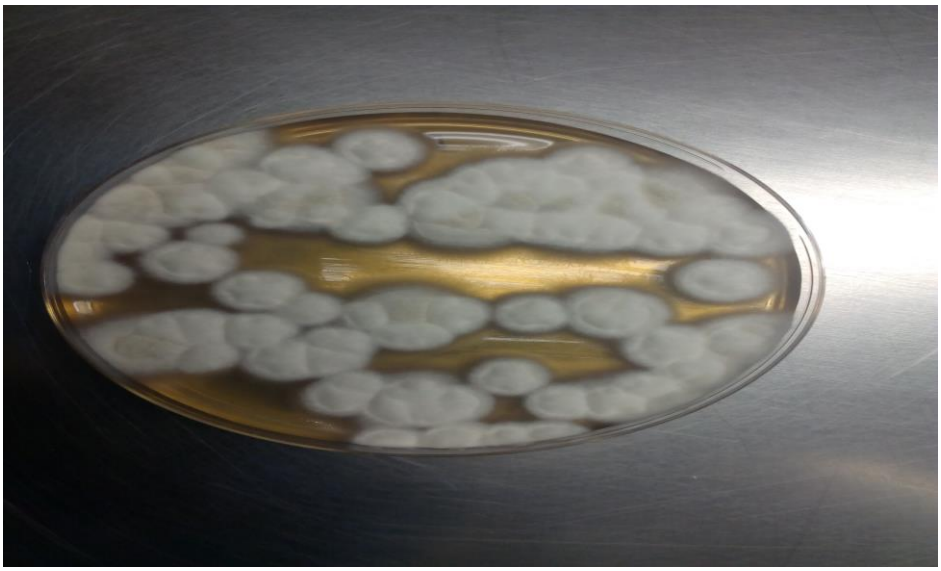

Figure 4 Colonies growth of *A. terreus* on Sabouraud's dextrose agar from spinal fluid.

This causative agent was subsequently confirmed by direct sequencing of the PCR products obtained by amplification with the universal fungal primers ITS1 5'-TCCGTAGGTGAACCTGCGG-3 and ITS4 5'-TCCTCCGCTTATTGATATGC-3' [12].

A BLAST search against the GenBank database showed 99,9% identity with the rRNA ITS region of *A. terreus*. Reliable identification of *Aspergillus* spp. requires molecular analysis in addition to phenotypic methods [1]. Therefore, according to these results, disseminated aspergillosis by *A. terreus* was diagnosed.

Discussion

Disseminated *A. terreus* infection in a dog was first reported in 1978 [13]. The infection is described as rare and generally has a poor prognosis. The two most frequent etiologic agents are *Aspergillus terreus* and *A. deflectus* [9,8], followed by *A. fumigatus*. However, other species, such as *Aspergillus niger*, *Aspergillus flavipes*, *Aspergillus versicolor*, *Aspergillus alabamensis* and *Aspergillus felis* have also been identified [5,2].

The German Shepherd breed appears to be predisposed to disseminated *A. terreus* infection; however, other breeds, including the Dalmatian, English setter, pug, Rhodesian ridgeback, springer spaniel, most retrievers, Weimaraner and whippet, have occasionally been affected [14,3]. Although the reason is unknown, some authors believe that this predisposition may be related to a breed-specific IgA deficiency or dysfunction leading to a deficiency in mucosal immunity [5]. Comparative studies of serum immunoglobulin concentrations in healthy dogs indicate that the IgA level in the German shepherd dog is significantly lower than that in other breeds [14].

Extrinsic factors that may predispose dogs to infection include injury to any of the mucous membranes, the use of catheters, administration of antibiotics, and immunosuppressive drugs like corticosteroids or the presence of other diseases [10]; in our case the animal was treated with corticosteroids.

The fungus port of entry in this case rests unknown. Obvious primary gastrointestinal or pulmonary lesions have not been observed, and the registered ones can be justified as resulting from hematogenous dissemination. Common pathological findings in this disease include diskospondylitis, osteomyelitis, pyelonephritis, and parenchimatous organs granulomatous infiltrate and infarction [4]. In our case, although the dog showed dramatic dorsal and cervical pain, diskospondylitis and osteomyelitis evidence were conspicuously absent in the radiographs taken.

Symptoms of disseminated aspergillosis in dogs usually takes several months to develop and include weight loss, pyrexia, inflammatory ocular disease, neurological deficits, lethargy, muscle wasting, weakness, vomit, spinal column pain and lameness [11,7]. Laboratory abnormalities include neutrophilia, azotemia, increased total serum protein concentrations, and isosthenuria [9,7]. The clinical symptoms and biochemical alterations registered in this case coincide in most the points with this scheme. Death in this case occurred before an etiological diagnosis and adequate treatment were possible, but systemic antifungal long-term treatment with itraconazole has been described as effective in disseminated aspergillosis in dogs. However successful treatment is not common and most patients are euthanized due to central nervous system involvement and intractable pain when treatment is interrupted [6].

Conclusions

In the case described above it was no possible to have a definitive diagnosis of disseminated fungal infection before the patient's death. Veterinary practitioners must seriously consider this uncommon sickness as a possible differential

diagnosis of neurological diseases in dogs, without forgetting that it could affect multiple organs and systems. *Aspergillus* should be considered as a causative agent of disseminated disease particularly in German shepherd dogs and other dogs with compromised immunity [5].

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