

Journal of the Hellenic Veterinary Medical Society

Vol 68, No 4 (2017)



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doi: [10.12681/jhvms.16074](https://doi.org/10.12681/jhvms.16074)

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To cite this article:

KALOGIANNI, L., SOUBASIS, N., SARIDOMICHELAKIS, M., VLEMMAS, I., VELEGRAKI, A., PAPAZOGLU, L., KRITSEPI-KONSTANTINOU, M., & RALLIS, T. (2018). Intra-abdominal Aspergillosis due to *Aspergillus fumigatus* in a Dog. *Journal of the Hellenic Veterinary Medical Society*, 68(4), 681–686. <https://doi.org/10.12681/jhvms.16074>

Intra-abdominal Aspergillosis due to *Aspergillus fumigatus* in a Dog

Kalogianni L.¹, Soubasis N.¹, Saridomichelakis M.², Vlemmas I.³, Velegraki A.⁴,
Papazoglou L.¹, Kritsepi-Konstantinou M.⁵, Rallis T.¹

¹*Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences,
Aristotle University of Thessaloniki, Thessaloniki, Greece*

²*School of Veterinary Medicine, Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece*

³*Laboratory of Pathology, School of Veterinary Medicine, Faculty of Health Sciences,
Aristotle University of Thessaloniki, Thessaloniki, Greece*

⁴*Mycology Research Laboratory, Department of Microbiology, Medical School,
National and Kapodistrian University of Athens, Athens, Greece*

⁵*Diagnostic Laboratory, Faculty of Health Sciences,
Aristotle University of Thessaloniki, Thessaloniki, Greece*

ABSTRACT. A 3 year-old, spayed female, mixed-breed dog presented with abdominal distension due to a large mass, detected during abdominal palpation and confirmed by abdominal imaging. Cytological examination of the mass was suggestive of pyogranulomatous inflammation. During exploratory laparotomy, extensive peritoneal adhesions and multifocal nodular lesions on the liver, spleen and omentum were revealed. One week later, the dog deteriorated and was euthanized. Numerous firm masses were observed in the liver, spleen, left kidney, stomach, small and large intestine during necropsy. The lungs, heart, and ocular structures were macroscopically normal. Histopathology results (surgery and necropsy) revealed fungal hyphae enclosed in the pyogranulomatous lesions. Polymerase chain reaction (PCR) products showed 100% homology with *Aspergillus fumigatus* and agar gel double diffusion was positive for IgG antibodies against the same fungus.

Keywords: *Aspergillus fumigatus*, dog, granuloma, peritonitis

Corresponding Author:

Kalogianni Lamprini
Companion Animal Clinic, Department of Veterinary Medicine, Faculty of Health
Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.
PO Box 50208, GR-54013, Thessaloniki, Greece
Αλληλογραφία: Καλογιάννη Λαμπρινή
Κλινική Ζώων Συντροφιάς, Τμήμα Κτηνιατρικής, Σχολή Επιστημών Υγείας,
Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη.
ΤΘ 50208, ΤΚ 54013, Θεσσαλονίκη
E-mail: altamyra@msn.com

*Date of submission: 10-10-2016
Date of acceptance: 17-1-2017*

ΠΕΡΙΛΗΨΗ. Σκύλος τριών ετών, θηλυκός στερημένος, ακαθόριστης φυλής προσκομίστηκε με διάταση της κοιλιακής κοιλότητας εξαιτίας ευμεγέθους μάζας, η οποία ανευρέθηκε κατά την ψηλάφηση της κοιλίας και επιβεβαιώθηκε με απεικονιστικές εξετάσεις. Η κυτταρολογική εξέταση της μάζας ανέδειξε πυοκοκκιωματώδη φλεγμονή. Κατά την ερευνητική λαπαροτομή αποκαλύφθηκαν εκτεταμένες συμφύσεις μεταξύ των οργάνων της περιτοναϊκής κοιλότητας και πολυεστιακές αλλοιώσεις στο ήπαρ, το σπλήνα και το περιτόναιο. Μια εβδομάδα αργότερα, η κατάσταση του ζώου επιδεινώθηκε και διενεργήθηκε ευθανασία. Κατά τη νεκροτομική εξέταση παρατηρήθηκαν πολλαπλές σκληρές μάζες στο ήπαρ, το σπλήνα, τον αριστερό νεφρό, τον ορογόνο του στομάχου, το λεπτό και το παχύ έντερο. Οι πνεύμονες, η καρδιά και οι οφθαλμοί βρέθηκαν μακροσκοπικά κατά φύση. Η ιστοπαθολογική εξέταση από τα ιστοτεμάχια που λήφθηκαν κατά τη λαπαροτομή όπως και κατά τη νεκροτομή αποκάλυψαν μυκητιακές υφές που περιβαλλόταν από τον κοκκιωματώδη ιστό των αλλοιώσεων. Το προϊόν της αλυσιδωτής αντίδρασης της πολυμεράσης ήταν κατά 100% ομόλογο με τον *Aspergillus fumigatus* και η διπλή ανοσοδιάχυση σε άγαρ βρέθηκε θετική για IgG αντισώματα ενάντια στον ίδιο μύκητα.

Λέξεις ευρετηρίασης: *Aspergillus fumigatus*, κοκκίωμα, περιτονίτιδα, σκύλος

CASE HISTORY

A 3 year-old, mixed breed, spayed female dog was referred with a 3 month history of progressive anorexia, weight loss and abdominal distension.

Nine weeks prior to referral, the dog had been diagnosed with acute monocytic ehrlichiosis and leishmaniosis and had been treated, initially with doxycycline hydrochloride (Ronaxan, Merial) for 3 weeks, and then with miltefosin (Milteforan, Virbac) and amoxicillin-clavulanic acid (Synulox, Pfizer) for 4 weeks and with prednisolone (Prezolon, Takeda Hellas) for 6 weeks. There was no clinical improvement and the dog was referred to the first author's Clinic.

On admission pallor and poor body condition were observed and a large, firm abdominal mass with an irregular surface was palpated. Complete blood count (ADVIA 120, Siemens Healthcare Diagnostics, United States) revealed severe non-regenerative anaemia (hematocrit 16,9 %, reference range 38-55%) neutrophilic leucocytosis (96,500/ μ l, reference range 6,000-17,000/ μ l), with a regenerative left shift (band neutrophils 24,100/ μ l), and mild thrombocytopenia (190,000/ μ l, reference range 200,000-500,000/ μ l). Thoracic radiographs were normal whereas the abdominal radiograph revealed splenomegaly, caudal displacement of the small intestine and diffuse radiopacity. Abdominal

ultrasonography showed a small amount of anechoic fluid, splenomegaly, hepatomegaly with normal echodensity, enlarged mesenteric lymph nodes and a mixed-echogenicity tumour that was subsequently aspirated. Cytological examination of the fine needle aspiration smear showed pyogranulomatous inflammation (many non-degenerative neutrophils and macrophages), whereas the abdominal fluid was haemorrhagic and contained non-degenerative neutrophils, reactive macrophages and no organisms. Blood, urine and abdominal fluid cultures were negative for aerobic and anaerobic bacteria.

Exploratory laparotomy confirmed the imaging findings and revealed discrete nodular lesions (1-3 mm) on the liver, spleen and omentum; complete abdominal exploration was impossible due to extensive adhesions. Samples were collected from accessible nodules on the omentum for bacterial culture, histopathology and imprint cytology. The latter showed reactive macrophages and erythrophagocytoses, and when cultured, there was no growth of aerobic/anaerobic bacteria. Aerobic and anaerobic cultures were negative.

The dog was hospitalized for 1 week and treated with Lactated Ringer's solution, cefoxitin (Mefoxil, Vianex) and butorphanol (Butador, Chanelle Veterinary) for the first 24 hours that was later replaced by

fentanyl transdermal patch (Durogesic, Janssen-Cilag). Two days after discharge, the dog was re-admitted due to lethargy, anorexia and tachypnoea. Phagocytosed bacteria were observed on peritoneal fluid cytology. Due to the guarded prognosis, extent of intra-abdominal lesions and financial concerns, the dog was euthanized.

During post-mortem examination, approximately one litre of serosanguineous, turbid abdominal fluid was noted. In addition to the findings of surgical exploration of the abdominal cavity, multifocal discrete nodules, 1-3 mm in diameter, were present on the peritoneal surface of the diaphragm, the omentum was diffusely thickened, and numerous firm masses (1-5 cm in diameter) were observed on the liver, spleen, left kidney, stomach and the serosal surface of the small and large intestine. There was a rupture of small intestinal wall in a segment unrelated to the surgical biopsy sites surgical biopsy, resulting in septic peritonitis. Macroscopic lesions were not observed in the pulmonary, cardiac or ocular tissues.

Haematoxylin-eosin staining of biopsy samples collected during exploratory laparotomy (omentum nodules) and post-mortem examination (liver, spleen, left kidney, intestine) revealed pyogranulomatous inflammation surrounding central areas of oedema and necrosis that contained aggregates of fungal hyphae, whereas at the periphery of these lesions there were moderate numbers of eosinophils, lymphocytes and plasma cells (Figure 1). In Gomori's methenamine silver-stained sections (liver, kidney) hyphae were uniform, thin-walled, septate, branching at acute-angles and with a globose terminal segment (Figure 2).

PCR was performed in paraffin-embedded kidney samples, by automated, high-sensitivity closed system method (FFPE Plus LEV, Maxwell®, Promega, Madison, WI 53711, United States). Sequencing of the internal transcribed spacer (Velegraki et al., 1999), confirmatory beta-tubulin (Arabatzis et al., 2011) and 28S amplification products (Khot et al., 2009) showed 100% homology with *A. fumig-*

atus, whereas agar gel double diffusion was positive for IgG against the same fungus. Finally, conventional PCR of kidney samples for *Leishmania donovani* complex was negative. Due to sudden deterioration in the dog's clinical condition and disseminated neoplasia at the top of the differential diagno-

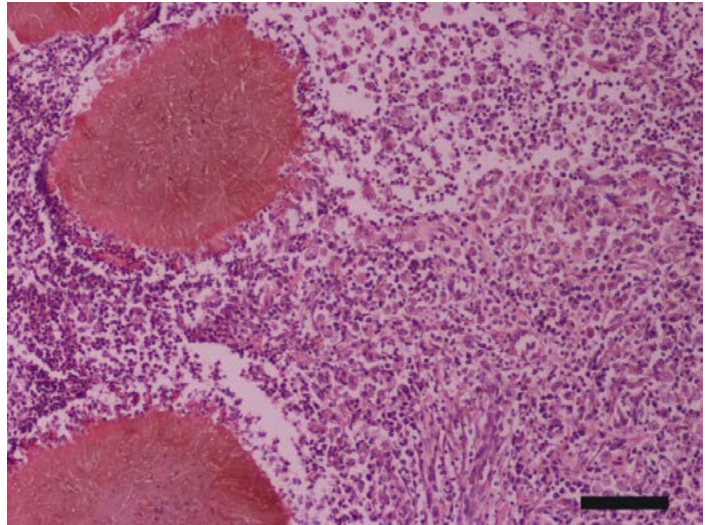


Figure 1. Histopathology sample, liver.

Pyogranulomatous inflammation, with neutrophils and macrophages surrounding areas of oedema and necrosis, that contain aggregates of fungal hyphae. Haematoxylin-Eosin, 10x magnification, bar=100 µm.

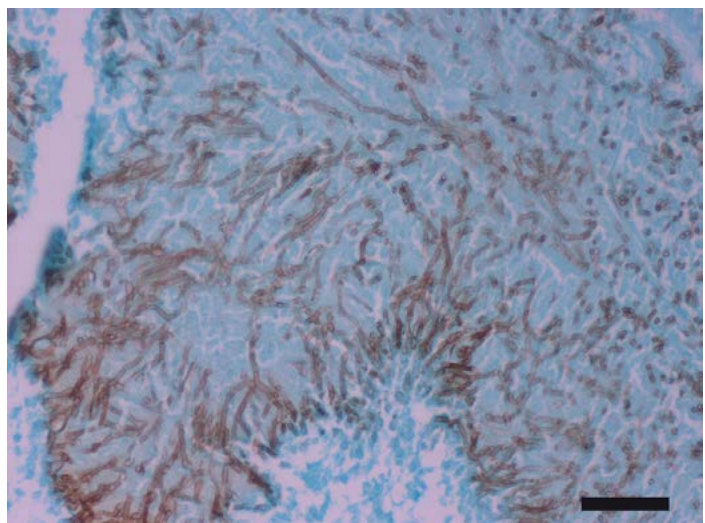


Figure 2. Histopathology sample, kidney.

Higher power view of the fungal aggregates showing dark-stained fungal hyphae. Hyphae are typically uniform, thin-walled, septate, acute-angle branching structures with a globose terminal segment. Gomori methenamine silver stain, 20x magnification, Bar=50 µm.

sis ante mortem, conventional diagnostics for leishmaniosis (bone marrow, lymph node and serology results) were not performed, and corresponding tissues were not preserved in paraffin blocks.

DISCUSSION

Nasal aspergillosis is the most common clinical presentation of *Aspergillus* infection in dogs (Clercx et al., 1996, Day, 2012), but systemic aspergillosis (SA) has also been described in this animal species, in addition to humans and cats (Kabay et al., 1985, Barnes and Marr, 2006, Day MJ, 2012) and a rare bronchopulmonary form of the disease has also been reported (Clercx et al., 1996, Adamama-Moraitou et al., 2011). The responsible fungal species in canine SA are usually *A. terreus*, *A. deflexus* and *A. niger*, whereas *A. fumigatus* has been isolated in few cases (Day et al., 1986, Clercx et al., 1996, Bruchim et al., 2006, Schultz et al., 2008). The portal of entry is usually the respiratory tract with subsequent dissemination to organs with terminal capillary loops which is facilitated by the predisposition of this fungus to infiltrate the vascular wall (Kabay et al., 1985, Lamps, 2010).

Based on previously reported cases (Kabay et al., 1985, Schultz et al., 2008), it is unclear whether intra-abdominal aspergillosis can be the only manifestation of canine SA. The aim of this report is to present a rare case of canine SA due to *A. fumigatus* where lesions were apparently localized exclusively in the abdominal cavity.

In previously reported cases of canine SA with intra-abdominal granuloma formation, it is unclear whether lesions in other organs were simultaneously present (Clercx et al., 1996, Bruchim et al., 2006, Schultz et al., 2008). In the present case there was no clinical indication of ocular, central nervous system, vertebral or long bone involvement. Furthermore, careful retrospective re-examination of the radiographs that included the entire spine and many long bones, revealed no lesions that could be associated with SA. Therefore extra-abdominal lesions are unlikely, although detailed histologic examination of all the above organs would be necessary to definitively exclude this possibility. Considering the extent of intestinal involvement seen at necrop-

sy and the apparent lack of respiratory lesions, it is likely that the portal of infection was the intestinal wall and was followed by spreading to mesenteric lymph nodes and subsequently to adjacent abdominal organs (Kabay et al., 1985, Bruchim et al., 2006, Lamps, 2010).

The appearance of SA in a previously healthy young dog may suggest a compromised immune response against the fungal pathogen (Day et al., 1985, Barnes and Marr, 2006, Day, 2012). Prednisolone administration for 6 weeks along with canine monocytic ehrlichiosis and leishmaniosis may have resulted in a suppression of cellular immunity (De Luna et al., 1999, Harrus et al., 2003, Saridomichelakis, 2009) that permitted uninhibited fungal invasion of abdominal organs. Negative PCR for *Leishmania* may be attributed to previous treatment with miltefosine that reduces tissue parasitic load (Manna et al., 2008, Mateo et al., 2009, Andrade et al., 2011) and to PCR examination of kidney tissue which is not optimal for the detection of the parasite (Costa et al., 2003, Plevraki et al., 2006).

Anemia of chronic inflammation may, be one cause for hematocrit reduction in this dog, though it is often less severe. Blood loss within the larger necrotic lesions and the abdominal cavity may have contributed. The leukemoid response in this case (56,900/ μ l mature neutrophils with 24,100/ μ l band cells) can be attributed to the severe inflammation and tissue necrosis by the large, space-occupying granulomas, and prolonged corticosteroid use.

In SA, cytology has a low sensitivity because of low number of organisms within some lesions (Dial, 2007). In this dog, pyogranulomatous inflammation, a typical response to fungal agents, was repeatedly found in cytological examinations (mass lesions, peritoneal fluid) without evidence of an infectious agent. Histopathology showed a thick area of necrosis and a dense inflammatory infiltrate surrounding fungal components which may have made them inaccessible to fine needle aspiration. Routine haematoxylin-eosin staining may not always demonstrate the organisms or suffice for their morphological identification. For this reason, special stains, like Gomori's methenamine silver, are mandatory when fungal infections are suspected

(Kabay et al., 1985, Dial, 2007, Day, 2012).

Fungal culture from aseptically collected peritoneal fluid and tissue samples, the gold standard for species identification, was not possible because the results of histopathology became available post-mortem and suitable samples had not been stored. For this reason, molecular techniques (PCR) were employed and permitted definitive diagnosis and species identification. Serology was used as a complimentary examination because of the frequent false-positive and false-negative results (Dial, 2007, Schultz et al., 2008, Adamama-Moraitou et al., 2011).

In conclusion, taking into consideration that systemic aspergillosis can progress over several months, when confronted with a long-running histo-

ry and multiple abdominal masses with pyo-granulomatous findings on cytology, systemic aspergillosis should be included in the differential diagnosis.

ACKNOWLEDGMENTS

Dr Michael Arabatzis of the National and Kapodistrian University of Athens and Aristotle University of Thessaloniki, Greece, Curator of the UOA/HCPF Culture Collection sequences.

CONFLICT OF INTEREST

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. ■

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