

Case Report

Transuterine transmission of *Aspergillus terreus* in a case of disseminated canine aspergillosis

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Aspergillus terreus was isolated from the organs of a German Shepherd pup removed from the bitch by cesarean intervention. In the following days, the bitch developed signs of canine disseminated aspergillosis and was euthanized. The fungus was isolated from a necrotic lesion in the uterus and other organs. To the best of our knowledge, this is the first report of the transuterine transmission of *A. terreus* during a case of canine disseminated aspergillosis.

Keywords canine disseminated aspergillosis, *Aspergillus terreus*, transuterine transmission, German Shepherd dog

Introduction

Canine disseminated aspergillosis (CDA) is characteristically caused by *Aspergillus terreus* in German Shepherd dogs, predominantly females [1]. The reasons for the predisposition of this breed to the infection are not clear [1]. The portal of entry is uncertain, but it has been suggested that hematogenous dissemination of the fungus may be facilitated by production of aleuriospores [2]. While various organs may be involved, the lungs appear least involved possibly due to their intrinsic capabilities to avert fungal infections [3]. Prognosis is generally poor because the dogs are presented in advanced stages of the infection, usually with central neurological signs, indicating cerebral involvement. In addition, the intrinsic resistance of *A. terreus* to antimycotic drugs [4], further reduces the prospects of successful therapy. In fact, only one successful treatment of CDA – with itraconazole – has been reported to date [5].

Case report

A 3-year-old German Shepherd (GS) bitch, kept in a breeding kennel, showed no contractions at 60 days of pregnancy and was treated with oxytocin to induce parturition. When this treatment was unsuccessful, a cesarean operation was performed. Five pups were extracted, 3 dead and 2 alive. No particular clinical symptoms were observed in the bitch prior, during or immediately after surgery, except for a light nystagmus during anesthesia.

The 3 dead pups were brought to the Kimron Veterinary Institute (KVI) but, at the owner's request, only one was examined. Portions of the pup's stomach contents, kidney, liver and lung, as well as the placenta were inoculated onto 5% sheep blood agar, McConkey agar and nutrient agar plates (fungal infection was not suspected at the time) and incubated at 37°C. Two days later, rich confluent fungal growth was found on all media inoculated with material from the placenta and the pup's stomach contents and a few colonies on those inoculated with kidney, liver and lung samples. Since a preliminary examination of the mycelium indicated that the fungus was probably an *Aspergillus* spp., the isolates were transferred to Sabouraud dextrose and Czapek agar plates and incubated at 30°C for identification. The same day the remaining two pups died as well, but since the owners were not yet informed as to the suspected diagnosis, they were not submitted for further examinations. In addition, the bitch started to

Received 3 June 2007; Accepted 1 September 2007

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show neurological signs, i.e., tilted head and lopsided walk. The clinical symptoms, the presence of the fungi in the placenta and the pup's organs and the bitch's breed led to a tentative diagnosis of CDA. The bitch was brought to the KVI where whole blood samples were collected and, at the request of the owners, the animal was euthanized. A full postmortem examination was performed, with portions of the kidney, uterus, spleen, lung, udder, liver, heart, brain and mediastinal, mesenteric, popliteal and retropharyngeal lymph nodes submitted for histopathological, bacteriological and mycological examinations. Urine was submitted for the latter two types of studies. Whole blood and serum were submitted for biochemical analysis (albumin, alkaline phosphatase, alanine aminotransferase, amylase, aspartate aminotransferase, chloride, cholesterol, creatinine phosphokinase, creatinine, total calcium, gamma-glutamyl transpeptidase, lactate dehydrogenase, potassium, protein, inorganic phosphate, sodium, total bilirubin, urea) (Thermo Clinical Lab System, Konelab 30I, Finland) and differential blood count (red

blood cells, mean corpuscular volume, hematocrit, hemoglobin, white blood cells and platelets) (Technikon H1E System, Miles Diagnostics Division, USA).

A large number of cotton-like corpuscles were seen in the urine (Fig. 3) and a microscopic examination of the urine showed the corpuscles to consist of fungal hyphae. The most prominent lesions noted as part of the pathologic examinations of the bitch were two necrotic areas in the uterine mucosa, one of which was perforated (Fig. 1). In addition, splenomegaly, necrotic areas in the renal papillae and several white masses on the parietal meninges were observed. Observation of Grocott-Gomori methenamine silver stained sections of the uterine lesions revealed severe diffuse necrotizing placentitis with massive fungal invasion (Fig. 2a). Amorphous necrotic eosinophilic material containing degenerated cells and fungal hyphae with a large number of aleuriospores were seen in the allantochorion (Fig. 2b). The underlying chorionic villi were necrotic, congested and edematous with an infiltration of polymorphonuclear cells and histiocytes. Hyphae

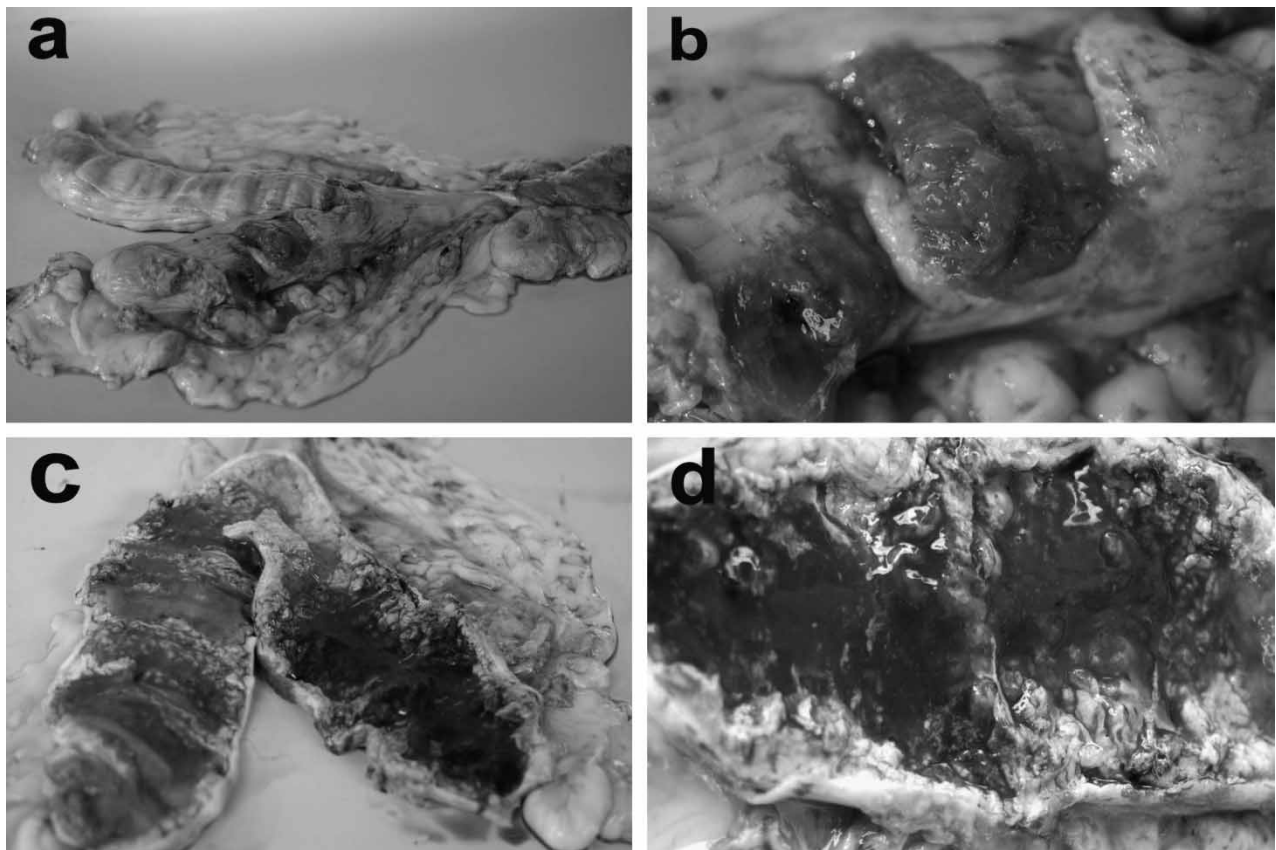


Fig. 1 Uterine lesions. (a) External aspect, (b) External aspect – detailed view – note perforation of left lesion. (c) Internal aspect, (d) Internal aspect – detailed view of lesion.

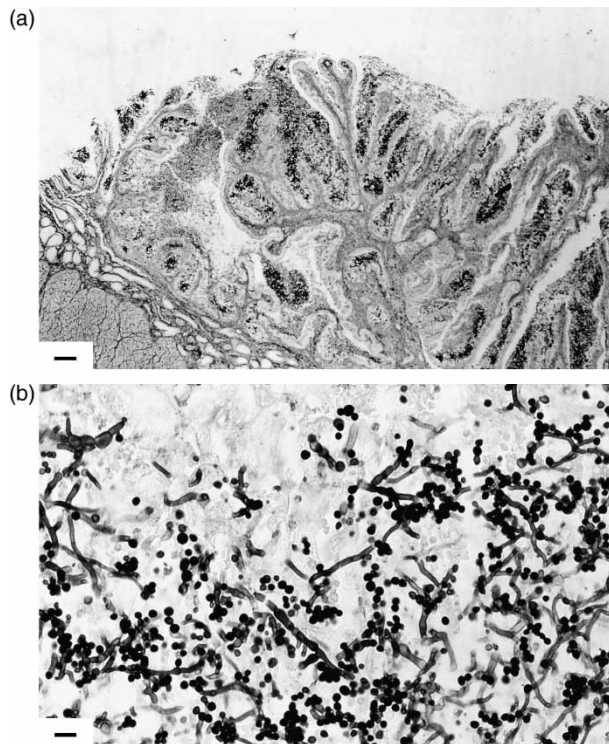


Fig. 2 Grocott's modification of Gomori's methenamine silver stain of uterine lesions. (a) Massive fungal (dark brown elements) invasion (bar = 200 µm). (b) Hyphae and large number of globose aleuriospores (bar = 10 µm).

were seen in the kidney and lungs as well. A few foci of perivascular cuffing with small number of lymphocyte and histiocytes, but no hyphae, were seen in the brain.

Bacteriological cultures resulted in the isolation of a few colonies of plasma coagulase negative staphylococci from the uterus. The bacteriological cultures of the other organs were negative. All biochemical parameters and blood cell were within normal ranges,

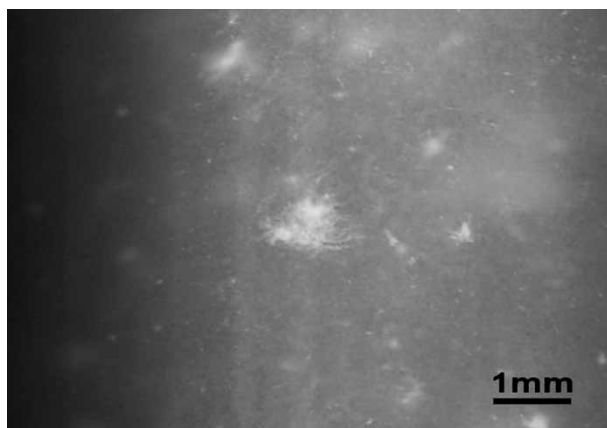


Fig. 3 Cotton-like corpuscles in urine – hyphal filaments evident.

except for a low platelet count of 20×10^3 (normal 143.3×10^3 – 400×10^3).

The mycological examination of the bitch's organs yielded confluent growth of *A. terreus* (see identification below) from the urine, kidney, uterus, spleen and the mediastinal and mesenteric lymphnodes. In addition, a few colonies of the fungus were recovered from the lung, udder, liver, heart (valves), brain (cerebellum) and popliteal and retropharyngeal lymphnodes. Blood cultures were negative.

The fungal colonies on SDA and Czapek agar were quick-growing (diameter >5 cm after 7 days), velvety, cinnamon colored with a dark brown reverse. A yellow-brown pigment was produced. Microscopic morphology was consistent with *Aspergillus* spp., with compactly columnar, biserial heads, smooth walled, hyaline stipes (length 100–150 µm), subspherical vesicles (diameter 12–18 µm), metuleae and phialides of similar length (2–2.5 µm) and spherical, hyaline, smooth, small conidia (diameter <3.5 µm). Cleistothecia were not observed. Consequently [6], the isolate was identified as *A. terreus*, as expected on the basis of other cases of CDA.

Discussion

Among the two common canine infections caused by *Aspergillus* spp., i.e., nasal and disseminated aspergillosis, the latter is the less frequently encountered [7]. In Israel, one or two cases are diagnosed yearly (unpublished data), mostly in dogs examined at the Koret School of Veterinary Medicine Teaching Hospital. Quite possibly, other cases may go undiagnosed by private practitioners.

A. terreus is a fungus common in the environment and consequently it is important to ascertain that it was the cause of the observed clinical symptoms. Since the pups were removed by cesarean surgery, peri- or post-natal infection may be excluded. The necrotic focus observed in the uterus of the bitch was the most prominent fungal lesion found during the pathological examination. While this does not necessarily make it the portal of the fungus' entry, it is the most likely source of dissemination. Moreover, it further substantiates the transuterine mode of transmission.

To the best of our knowledge, this is the first report of the transuterine transmission of *A. terreus* during a case of disseminated canine aspergillosis.

Acknowledgements

The authors wish to thank Ms. Dorith Natan from the Histology Laboratory of the Weizmann Institute of

Science in Rehovot for the preparation of the GMS stained slides.

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