Disseminated aspergillosis in a German shepherd mixed breed dog with unusual initial localization to the iliac wing

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Keywords
Aspergillosis, *Aspergillus terreus*, Osteomyelitis, Pyelonephritis, Systemic mycosis, Ureteral obstruction.

Summary
A female, 1.5 years old, mixed-breed dog, was presented for left hind limb lameness. Radiographs revealed an irregular periosteal proliferation on the left iliac wing. The clinical condition worsened with generalised enlargement of the lymph nodes, azotaemia, and pyelonephritis. The magnetic resonance imaging of the pelvis and a surgical biopsy diagnosed a mycotic myositis and osteomyelitis of the iliac wing and gluteal muscles. *Aspergillus terreus* was isolated from culture of urine and lymph nodes aspirates. The antifungal susceptibility test showed moderate sensitivity to Itraconazole. After one month of therapy with itraconazole, the dog presented discospondylitis of L1-L2 and partial ureteral obstruction due to mycotic bezoar that was resolved with medical treatment and itraconazole dose elevation. After twelve months, itraconazole was suspended; a severe osteomyelitis of the left femur developed, and the dog was euthanised. The necropsy confirmed the presence of mycotic osteomyelitis of the iliac wing and femur, discospondylitis, lymphadenitis and severe granulomatous pyelonephritis. Systemic aspergillosis has rarely been reported in the literature, especially in Italy. The pelvic bone involvement is rare both in dogs and humans. Although itraconazole treatment allowed remission of the clinical signs for one year, it was not able to cure the dog.

Introduction

Disseminated aspergillosis is a rare disease in dogs, more frequently reported in USA (Corrigan *et al.* 2016, Starkey and McLoughlin 1996, Van Wie *et al.* 2013, Schultz *et al.* 2008), rather than in other countries (Bruchim *et al.* 2006). Systemic aspergillosis has been reported recently in Italy as a disease in three dogs (Morabito *et al.* 2020).

Aspergillus spp. are saprophytic and ubiquitous fungi, which usually colonise immunocompromised humans and animals (Bieganska *et al.* 2014). German shepherd dogs seem to be predisposed to the localised sino-nasal (Seyedmousavi *et al.* 2015) and generalised forms, possibly because of heritable abnormalities of the humoral and cellular immune responses (Schultz *et al.* 2008).

In dogs, the disseminated form has a haematogenous spread and clinical signs are initially mild and nonspecific as fever, anorexia, weight loss, vomiting, and lethargy. Specific signs are also reported according to the localisation of the infection. Common sites of embolic dissemination of fungal hyphae are the intervertebral discs, kidneys, and uveal tracts. Central nervous system, lungs and long bones represent other common localisations of the infection. Orthopaedic symptoms may be present as a consequence of osteomyelitis and discospondylitis, while signs of renal involvement can be related to pyelonephritis (Schultz *et al.* 2008).

Due to the variety of nonspecific clinical signs, the diagnosis of systemic aspergillosis is usually belated. Therefore, most of the dogs are severely ill at the time of diagnosis. The prognosis is usually poor

even when a specific therapy is instituted (Schultz *et al.* 2008, Morabito *et al.* 2020). The antifungal therapies available for animals are limited because of the costs of last generation antimycotic drugs, like voriconazole and posaconazole (Koehler *et al.* 2013, Corrigan *et al.* 2016).

In humans and dogs, obstructive uropathy related to aspergillosis is a very rare condition and, only few cases are reported in the literature (Yoon *et al.* 2010, Najafi *et al.* 2013, Starkley and McLoughlin 1996, Schultz *et al.* 2008).

This report describes a case of disseminated aspergillosis in a young female German shepherd mixed breed dog in Italy.

Case description

A spayed female, 1.5 years old, German shepherd mixed breed dog was referred for an orthopaedic consultation for lameness on the left hind limb of 2 week duration. The physical and orthopaedic examination revealed a painful swelling on the left iliac wing region. The radiographs of the pelvis showed an irregular margin of the crest of the left iliac wing (Figure 1) and through the ultrasonographic examination an enthesitis of the middle gluteal muscle was suspected. Treatment initially consisted of firocoxib (5 mg/kg q24h, Previcox, Merial, Toulouse, France) and omeprazole (0.7 mg/kg q24, Mepral, Bracco, Milano, Italy) for 10 days, without improvement. During treatment, the dog presented hyperthermia (39.2 °C), anorexia and peripheral lymph nodes enlargement. Full blood count (FBC), serum chemistry profile, fine needle aspirates of the prescapular and popliteal lymph nodes and magnetic resonance imaging (MRI) of the pelvis were performed. A mild leucocytosis consisting of mature neutrophilia was observed on the FBC. The serum chemistry profile revealed mild azotaemia, increased concentration of total protein and C-reactive protein (Table I, T_0). Cytological examination of the lymph nodes showed a granulomatous lymphadenitis associated with the presence of fungal hyphae (Figure 2). The MRI showed a wide lesion of the deep and middle gluteal, longissimus lombi, piriformis, and partially of the iliopsoas muscles. The iliac

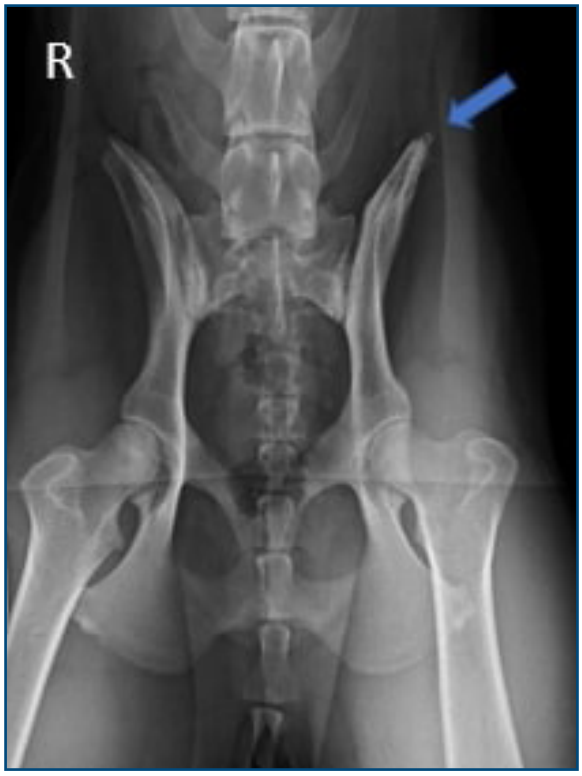

Figure 1. *Radiograph, ventro-dorsal projection of the pelvis of a dog with lameness, slight swelling of the gluteal region and pain on palpation.* Blue arrow indicates the periosteal reaction of the left iliac wing.

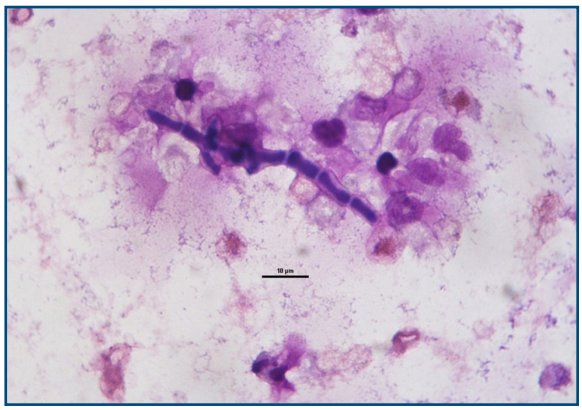

Figure 2. *Fine needle aspirate, prescapular lymph node.* Evidence of a fungal septate hypha of *Aspergillus terreus* embedded in necrotic material. May-Grünwald Giemsa stain. x100 objective.

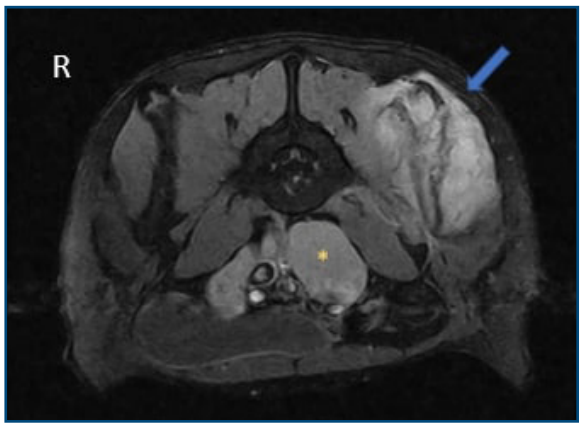

Figure 3. *Magnetic resonance imaging (MRI) of the pelvic region, post-contrast T-1 weighted sequence.* The blue arrow indicates the increased contrast uptake of the deep and middle gluteal, longissimus lombi, piriformis and partially of the iliopsoas muscles muscles. Note the irregular appearance of the iliac bone with periosteal reaction and bone lysis. The asterisk indicates severe lymphadenomegaly of the left sacral lymph node.

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wing presented periosteal proliferation and bone lysis, and an enlargement of the sacral lymph node was observed (Figure 3). A surgical biopsy of the left middle and deep gluteal muscles and of the iliac wing was performed and a severe chronic granulomatous, necrotising myositis, and severe chronic panosteitis and bone lysis were diagnosed. The presence of fungal hyphae in the biopsy samples was documented on histopathology. After the MRI, the clinical condition of the dog worsened. The dog became progressively depressed and presented vomit, and polyuria and polydipsia. Abdominal ultrasonography and urinalysis were performed. A bilateral dilatation of the renal pelvis (right renal pelvis diameter 26 mm, left renal pelvis diameter 12 mm) and the ureters (diameter 6 mm) with scattered hyperechoic content associated with enlargement of the mesenteric lymph nodes were noticed. Urinalysis revealed isosthenuria and microscopic examination of a wet-mount of urine sediment revealed pyuria

Table I. *Clinicopathological findings of the described Aspergillosis case evaluated upon admission and during the follow up period.*

Variable	Units	T0	T1	T2	T3	Reference Interval
Haematology						
- Haematocrit value	%	49.6	47.7	36.6	52	37.0-55.0
- RBC	$\times 10^{12}$/L	7.5	7.2	5.7	7.6	5.5-8.5
- WBC	$\times 10^{9}$/L	18.5	17.5	20.6	13	6.0-17.0
- Neutrophils	$\times 10^{9}$/L	13.1	12.8	14.4	5.9	3.0-12.0
- Lymphocytes	$\times 10^{9}$/L	3.1	3.4	4.9	4.8	1.0-4.8
- Monocytes	$\times 10^{9}$/L	1.3	0.8	1.1	0.5	0.1-1.4
Eosinophils	$\times 10^{9}$/L	0.6	0.4	0.3	1.5	0-0.7
- PLT	$\times 10^{9}$/L	34.6	25.5	21.7	26.4	16.0-50.0
Chemistry						
CK	U/L	200				50-290
- ALT	U/L	20	25	67	38	20-55
- AST	U/L	35	91	46	31	20-42
- ALP	U/L	66		52	123	42-180
- GGT	U/L	1.8		1.4	2.0	0–5.8
Glucose	mg/dL	86	142	81	98	65-115
Total Bilirubin	U/L	0.21		0.2	0.32	0.07-0.33
- Creatinine	mg/dL	2.08	1.29	4.3	1.64	0.65-1.35
- Urea	mg/dL	67	32.23	86.48	38.91	18-55
- Total Proteins	g/dL	8.21	8.16		6.88	5.60-7.90
- Albumin	g/dL	2.91	2.82		3.13	2.80-3.70
- A:G		0.55	0.53		0.83	0.60-1.30
- CRP	mg/dL	2.98			0.01	0-0.8
- Calcium	mg/dL	10.5		9.9	9.8	9.0-11.8
- Phosphate	mg/dL	4.6		6.3	4.3	2.6-4.90
- Sodium	mEq/L	141	146	146	144	143-154
- Chloride	mEq/L	104		111	113	108.0-118.0
- Potassium	mEq/L	3.9	4.2	4.3	4.7	3.9-5.3
Urinalysis						
- USG		1,012	1,018	1,016	1,020	> 1,030
- pH		5.5	5.5	6	5.5	
- Protein (dipstick)	mg/dL	0	0	0	0	Absent
- Urine Sediment		Mycotic hyphae, leucocytes	Erythrocytes and rare leucocytes	Mycotic hyphae, erythrocytes and leucocytes	Mycotic hyphae	
UPC		0.3			0.1	0.0-0.5
Culture						
Urine		*Aspergillus terreus*				
Lymph nodes		*Aspergillus terreus*				

A:G = Albumin-to-globulin ratio; ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; AT, antithrombin; CK, creatinine kinase; CRP = C-reactive protein; GGT = Gamma-glutamyltransferase; PLT = Platelets; UPC = Urinary protein to creatinine ratio; RBC = Red blood cells; T_0 = Presentation; T_1 = 7 days later, preoperative; T_2 = 7 days postoperative; T_3 = 12 days postoperative; USG = Urine specific gravity; WBC = White blood cells.

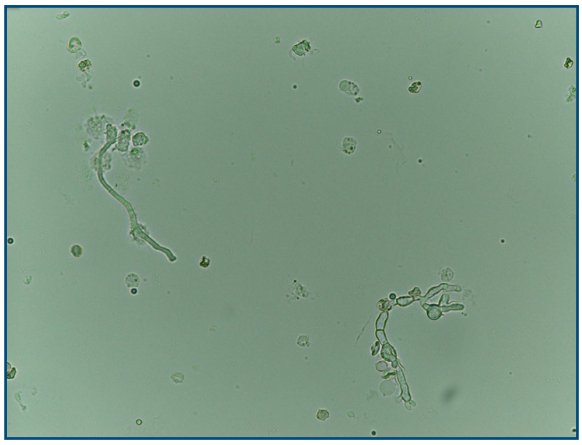

Figure 4. *Wet-mount urine sediment preparation.* Two septate hyphae of *Aspergillus terreus* surrounded by scattered leucocytes and red blood cells. x40 objective.

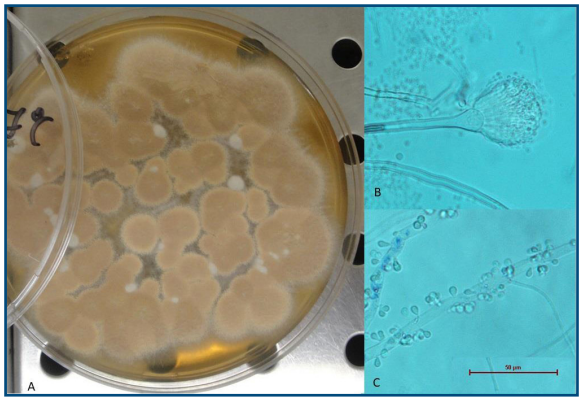

Figure 5. A. terreus *from urine:* **A.** *Culture on sabouraud dextrose agar;* **B.** *Compact, biseriate conidial heads;* **C.** *Aleurioconidia produced directly on the submerged hyphae - Lactophenol cotton blue stain.*

and the presence of fungal hyphae (Figure 4) (Table I, T_0). The final diagnosis was systemic mycosis with osteomyelitis localised to the left iliac wing and fungal pyonephrosis/pyelonephritis.

The dog was hospitalised and antifungal therapy with itraconazole (10 mg/kg q24h OS, Itraconazolo Teva, Milano, Italy) was started along with intravenous (IV) fluid therapy. The culture from the urine and the lymph nodes resulted positive for moulds belonging to *Aspergillus terreus* group, based on phenotypic characteristics, according to Raper and Fennell (Raper and Fennell 1965) (Figure 5). The antifungal susceptibility test showed moderate sensitivity to Itraconazole, resistance to fluconazole and a good sensitivity to voriconazole. The dog progressively improved clinically, as the dilation of the renal pelvis diminished (right renal pelvis diameter 14 mm, left renal pelvis diameter 3.9 mm) and azotaemia resolved (Table I, T_1). The patient was discharged with antimycotic therapy (itraconazole 10 mg/kg q24h PO).

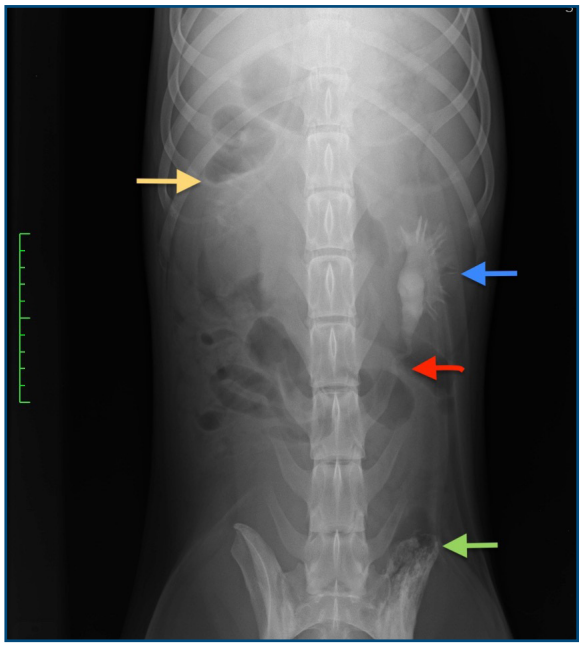

Figure 6. *Descending urography, ventro-dorsal projection of the abdomen.* The blue arrow indicates the dilated left renal pelvis and proximal portion of the left ureter due to obstruction by suspected fungal bezoars (red arrow). Yellow arrow underlines very reduced contrast uptake of the right kidney. Green arrow indicates the severe progression of bone lysis of the left iliac wing in comparison to the first X-ray.

After one month, the dog was presented for acute vomiting. Moreover, the owner noted a reduced urine production in the previous 24 hours. Physical examination detected pain at palpation of the abdomen, renal area, and lumbar region. A discitis at the L1-L2 intervertebral disc was evident on the radiographs. Acute kidney injury was suspected based on increased serum creatinine concentration, on the persistence of hyphae in the urine sediment (Table I, T_2) and on the results of abdominal ultrasonography. In particular, the latter revealed worsening of the bilateral pyelonephritis/pyonephrosis (right renal pelvis diameter 24 mm, left renal pelvis diameter 9.3 mm) and proximal hydroureters (proximal right ureter diameter 14 mm, proximal left ureter diameter 10 mm), floating hyperecoic intraluminal debris and retroperitoneal effusion. A descending intravenous urography was performed and evidenced a partial obstruction of the left ureter associated with dilatation of the ipsilateral pelvis. Based on the excretory urography the right kidney appeared very poorly perfused. Mycotic bezoars were suspected to be the cause of the ureteral obstruction (Figure 6). A urine culture and susceptibility test were repeated and the sensitivity to itraconazole appeared decreased. Voriconazole was proposed as a more efficient therapy, but the owner refused due to financial restraints. The dosage of itraconzole was then increased at 7 mg/kg q12h and conservative treatment for ureteral obstruction was started with

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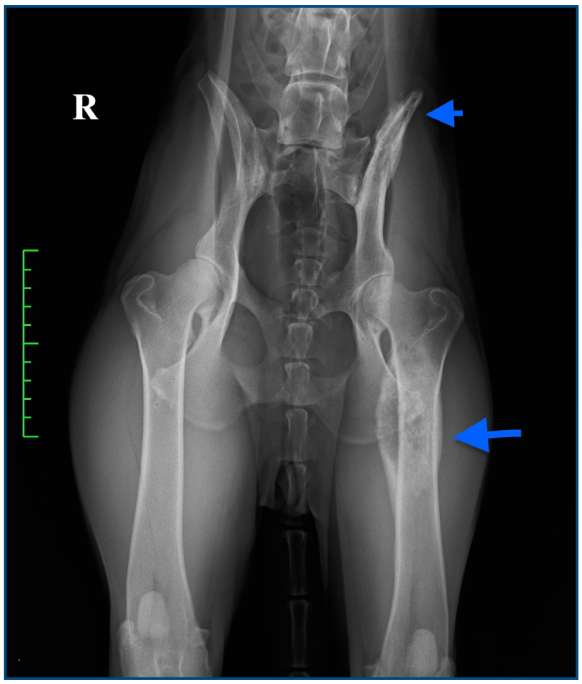

Figure 7. *Radiograph, ventro-dorsal projection of the pelvis and femurs.* The blue arrow indicates the severe focal periosteal reaction and mild bone lysis in the proximal third of the left femoral diaphysis due to mycotic osteomyelitis. The arrow-head underlines the bone lysis and periosteal reaction of the left iliac wing due to mycotic osteomyelitis. Note the decreased muscle mass of the left tight in comparison to the right one.

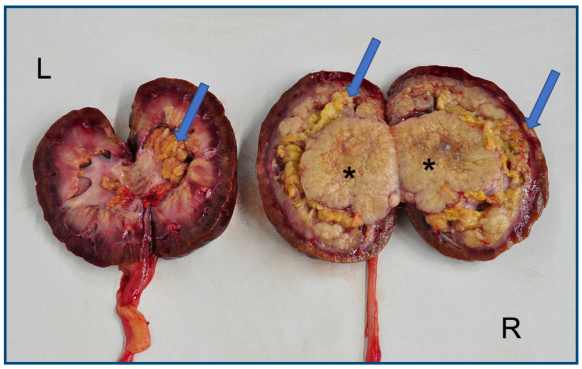

Figure 8. *Post-mortem sagittal section of the kidneys.* Note a large mass invading the right renal pelvis consistent with mycotic granuloma (asterisks). Note in both pelvis the presence of yellow material compatible with mycotic bezoars (blue arrows).

IV fluids (ringer lactate 3 ml/kg/h Braun, Melsungen, Germany) and analgesia (buprenorphine 15 mcg/kg every 6 h, Temgesic, Indivior Italia Srl, Milano, Italy). The dog developed post-obstructive polyuria 24 h after the beginning of the conservative treatment and the bilateral dilatation of the renal pelvis and ureters partially resolved (right renal pelvis diameter 7 mm, left renal pelvis diameter 3.5 mm). Renal function improved (Table I, T_3) and the dog was discharged after one week of hospitalisation.

The dog was re-examined periodically for approximately one year. The physical examination was unremarkable, except for the palpation of an irregular margin on the left iliac wing. The dog was staged, based on the IRIS staging of chronic kidney disease, as stage 2, non-proteinuric (Table I), normotensive. A mild bilateral dilatation of the renal pelvis and of the proximal ureters associated with hyperechoic content and enlargement of the mesenteric lymph nodes persisted at every abdominal ultrasound re-examination. After 12 months from the beginning of treatment, Itraconazole was temporarily discontinued because of severe vomiting. The clinical condition of the dog progressively deteriorated and, after two weeks, the dog was presented for acute and non-weight bearing left hind limb lameness. Radiographic examination revealed a lytic and proliferative lesion of the shaft of the left femur, along with a severe periosteal reaction. A mycotic osteomyelitis was strongly suspected (Figure 7). The dog was humanely euthanised and the necropsy identified a severe mycotic bilateral pyelonephritis with granulomatous lesions in the right renal parenchyma (Figure 8), renal, lombo-aortic and sacral lymphadenitis, severe osteomyelitis to the left iliac wing and left femur (Figure 9) and discospondylitis of L1-L2. From all these tissues, *A. terreus* was isolated.

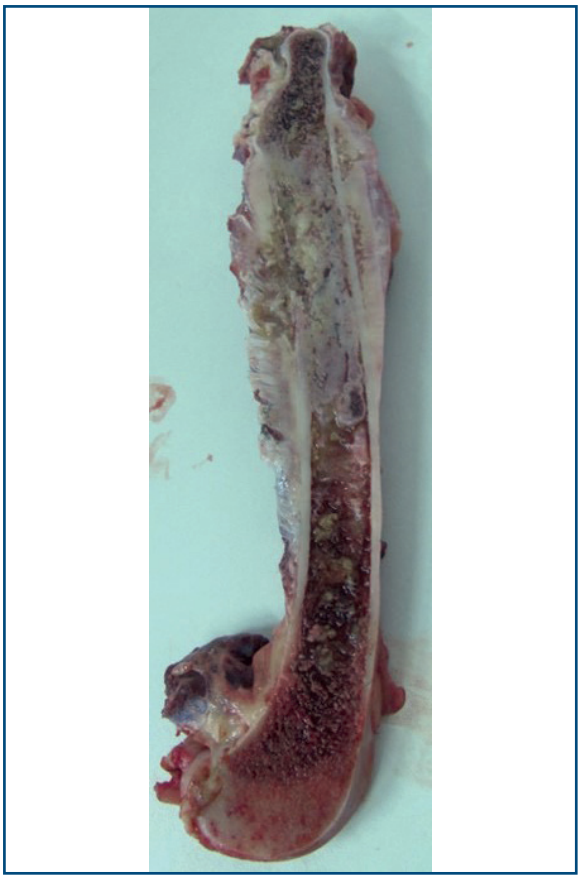

Figure 9. *Post-mortem longitudinal section of the left femur with mycotic osteomyelitis of the proximal third of the shaft of the femur.*

Discussion

Disseminated aspergillosis is considered an uncommon disease in dogs, and it usually carries a poor prognosis. The fungal species involved in this disease is more frequently *A. terreus*, unlike the more frequent sino-nasal aspergillosis that is primarily caused by *A. fumigatus* (Schultz *et al.* 2008).

Conversely, *A. terreus* species complex appears to be a rare cause of invasive Aspergillosis in human beings (where the most frequent agent is *A. fumigatus*), and often the isolates display a polyene resistance (Risslegger *et al.* 2017). Its incidence as cause of invasive aspergillosis in humans is higher in Innsbruck (Austria) and Houston (Texas-USA) hospitals than worldwide. Studies performed in Tyrol concluded that in this region patients develop *A. terreus* infections more often, because this fungus is more prevalent in the environment (Lackner *et al.* 2016). The dog of the present report was born in Emilia-Romagna region and lived in an apartment in Bologna.

In Italy, canine generalised aspergillosis appears to be a very rare disease, with three reported cases with a short follow-up before euthanasia, to the best of the authors' knowledge (Morabito *et al.* 2020). However, it cannot be excluded that the real prevalence of the disease is currently underestimated.

Female German shepherd dogs seem to be overrepresented in the literature about systemic Aspergillosis (Schultz *et al.* 2008, Van Wie *et al.* 2012, Perry *et al.* 2012, Morabito *et al.* 2020). The dog of the current case report was a young female mix-breed dog, linked to German shepherd dog. She did not have any other evident predisposing factors to fungal infection, like evident causes of immunosuppression or previous surgery (Bieganska *et al.* 2014). These data are in accordance with the previous veterinary literature, where most of the reported cases had no historical evidence of immunosuppression (Schultz *et al.* 2008). Canine transplacental aspergillosis transmission from an infected mother appears possible (Elad *et al.* 2008), however no information about the littermates was available for this dog.

At the time of first presentation, the dog was in good general condition and the initial clinical signs were just related to the iliac bone involvement causing lameness; only after generalisation of the disease, the dog showed nonspecific and systemic signs of generalised illness.

The abnormalities detected in the initial blood work were only neutrophilia and hyperglobulinaemia, two common but also nonspecific findings in dogs with systemic aspergillosis (Schultz *et al.* 2008). Raised creatinine and urea concentrations reflected kidney involvement, another common event in dogs with the generalised infection (Schultz *et al.* 2008).

Urinalysis and cytological examination of the fine needle aspirates of peripheral lymph nodes were helpful for the final diagnosis in the present case. Indeed, since urinary excretion of mycotic hyphae can occur in renal involvement, their observation at sediment examination is possible, as already reported by Morabito and colleagues (Morabito *et al.* 2020). Moreover, lymph node involvement is frequently reported (Morabito *et al.* 2020) and the cytological observation of fungal hyphae in their aspirates can be a useful and quick way to obtain the diagnosis of systemic mycosis. The sterile sampling of urine and lymph nodes could represent easily collectable specimens to perform fungal culture and even susceptibility test that can guide the treatment, as in our case. The sensibility test is an important step for the treatment because of the intrinsic amphotericin B resistance of *A. terreus* and the report of isolates with reduced azole-susceptibility (Zoran et al 2018).

A bony prominence, the iliac crest, appeared to be the first affected site in the present case. No previous trauma or surgery were reported by the owners. Consequently, a possible direct inoculation of *A. terreus* after an undetected penetrating puncture or lesion can be suspected. Other entry routes appeared less probable because of the absence of respiratory and enteric signs of the disease. Very likely, a secondary haematogenous dissemination of the infection took place and involved the kidneys and lymph nodes; while discospondylitis developed after the beginning of the antifungal therapy with Itraconazole, indicating a lack of control of the infection. Long-term remission was partially achieved after antifungal therapy and drug dosage review, which carried no side effects for several months. Femoral osteomyelitis developed after 12 months from the initial presentation, during a period of withdrawal of Itraconazole. This last localisation again confirmed the inability of Itraconazole to control the dissemination of Aspergillosis, which within a few days from discontinuation of the antimycotic drug gained another haematogenous spread to the bone. The trend of initial improvement or apparent remission and subsequent relapse has already been reported for different antimycotic drugs like itraconazole, amphotericin B, ketoconazole and posaconazole (Schutz *et al.* 2008, Corrigan *et al.* 2016).

This unsatisfactory response to antifungal therapy is not completely understood; however, an inadequate concentration of these drugs in the urine and affected tissues, along with a lack of response of the immune system of affected dogs can explain the poor outcome with medical therapy (Corrigan *et al.* 2016). Moreover, the difficulties encountered in the diagnosis of the subtle and nonspecific signs, can prolong the time of diagnosis and treatment, although this do not seem to be the case for the

dog here reported. The costs for last generation azoles limit their use in veterinary medicine, since prohibitive for many owners, as in this case.

In humans, even in cases of disseminated infection, surgical debridement is suggested as an important part of the multimodal treatment strategy together with systemic antimycotic drug administration (Koehler *et al.* 2013). In our case, a wide biopsy was performed in the iliac wing; unfortunately, dissemination already occurred, and a surgical debridement was not performed. Probably a more aggressive surgical treatment could have improved the penetration of itraconazole in the primary bone affected site and potentially decreased the haematogenous spread.

To the best of the Authors' knowledge, the iliac crest has never been reported as site of first localisation of disseminated aspergillosis in dogs and pelvic bone involvement is rarely described in cases of mycotic infection both in dogs and humans (Koehler *et al.* 2013, Schultz *et al.* 2008). On the contrary, discospondylitis seems to be the most frequent skeletal lesion reported in dogs, followed by osteomyelitis of the long bones like humerus, tibia, femur and scapula (Schultz *et al.* 2008). In humans, the vertebrae are also the most affected site, but they are followed by the cranium, long bones, and joints (Reischies and Hoenigl 2014, Koehler *et al.* 2013).

Aspergillosis of the urinary tract may occur in 3 patterns: haematogenous spread with renal parenchymal involvement, *Aspergillus* casts of the renal pelvis leading to the formation of fungal bezoar, and ascending infection (Najafi *et al.* 2013). In the case here described, both a parenchymal involvement of the kidney and involvement of the renal pelvis and ureter were documented. Moreover, fungal hyphae were observed at urine sediment examination. The main hypothesis remains the haematological spread of the infection, however an ascending form cannot be excluded. The ureteral obstruction observed in the dog of the present report was successfully managed by conservative treatment; however in case of no response, surgical treatment is advised. Although the use of a nephrostomy tube to temporarily decompress the renal parenchyma can be necessary, secondary bacterial infections have been reported (Starkley *et al.* 1996). In humans, the endoscopic removal of the ureteral obstruction is typically performed; however, to the best of the Authors' knowledge, this treatment has never been reported in dogs (Najafi *et al.* 2013). The placement of a ureteral stent can also be considered, even if it could maintain the infection and it could be obstructed by the hyphal casts as already reported in humans (Kuntz *et al.* 2015, Najafi *et al.* 2013).

In conclusion, this report describes an uncommon presentation of disseminated aspergillosis sustained by *A. terreus* in a dog with an initial localisation of the mycotic lesions to the wing of the ilium. A further systemic involvement was recognised with severe renal lesions and ureteral obstruction. Despite a prompt diagnosis and treatment with high doses of itraconazole, that gave a long-term clinical remission, the recurrence appeared after one year, soon after temporary discontinuation of antifungal drug. Disseminated aspergillosis remains a very difficult disease to treat with often a negative prognosis in the long term. An aggressive approach, when possible, including both medical and surgical management could lead to a better control of the infection.

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