Aspergillosis of the dog and cat

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Cases of aspergillosis are increasing in small animals in Japan

Because of increasing:
aging
chemotherapy,
inbreeding?
Molecular identification for *Aspergillus* species—Why?

Identification of clinical isolates in species level of *Aspergillus* is important, in relation with the susceptibilities of these organisms to various antifungal drugs.

*A. fumigatus* and *N. fischeri* produce the toxic metabolite, gliotoxin which has been implicated in infectivity.

Molecular identification has received wide recognition as a rapid and easy identification system.
Currently accepted classification of *Aspergillus* section *Fumigati*, including only isolates implicated in human disease

<table>
<thead>
<tr>
<th>Species</th>
<th>AMB</th>
<th>ITZ</th>
<th>VRZ</th>
<th>CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fumigatus</em></td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td><em>A. lentulus</em></td>
<td>1-2</td>
<td>0.5-1</td>
<td>1-2</td>
<td>2-16</td>
</tr>
<tr>
<td><em>A. viridinutans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. pseudofischeri</em></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.015</td>
</tr>
<tr>
<td><em>N. fischeri</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>N. udagawae</em></td>
<td>0.5-4</td>
<td>0.125-0.5</td>
<td>0.25-1</td>
<td>0.015-0.06</td>
</tr>
<tr>
<td><em>N. hiratsukae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Balajee et al., Eukaryot. Cell. 2006; 5, 1705-1712
Aspergillus species can be identified by morphological characteristics, however this is limited because morphology differences are small and largely dependent on growth conditions.

Molecular analysis is a rapid and easy identification method. However, it has not been proved what is the most useful system to identify the species.
Orbital aspergillosis has been reported in humans who received steroid treatment and chemotherapy, and were suffering from immunosuppressive diseases, including acquired immunodeficiency syndrome, but is extremely rare in cats. 

*A. fumigatus* is reported as the most frequent cause of sino-orbital aspergillosis in humans and small animals.
Case 1:
A 1 year and eleven months old spayed female American short Hair cat.

**Chief complaint:**
progressive protrusion of the left third eyelid and serous ocular and nasal discharge of 3 months duration.
Computerized tomography (CT) revealed a soft-tissue mass within the left orbit.

Histopathologic examination of this mass from the left eye orbit revealed granulomatous inflammation with many branching hyphal filaments in the granules.

Serum Aspergillus antigen titers measured by the latex galactomannan agglutination test (Pastorex Aspergillus; Sanofi Diagnostics Pasteur, Marnes-la-coquette, France) was positive.
Velvety, grayish-green colored colonies developed from this mass samples after 1-week incubation on Sabouraud's dextrose agar at 25°C; based on gross and microscopic characters, this isolate was identified as *A. fumigatus*.

The case was diagnosed as proven aspergillosis due to *A. fumigatus*. 
Schema for molecular identification procedure of the isolate

Cultured on SDA agar at 30 °C for 2-3 days

DNA extraction

PCR amplification ITS and β-tublin regions

Sequencing

Homology search by BLAST data base analysis
Results of comparative sequence analyses in GenBank

ITS region sequence of the clinical isolate was 100% identical to both *A. udagawae* and *N. fischeri*, and < 98% identical to *A. fumigatus*.

β-tubulin region sequence analyses of clinical isolate was 99% identical to *A. udagawae*. 
## Therapy for disseminated Aspergillosis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>Cat</td>
<td>0.25</td>
<td>IV</td>
<td>48 h</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>0.25-0.5</td>
<td>IV</td>
<td>48 h</td>
</tr>
<tr>
<td>AMB (lipid)</td>
<td>Cat</td>
<td>1</td>
<td>IV</td>
<td>3 d/week</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>2-3</td>
<td>IV</td>
<td>3 d/week</td>
</tr>
<tr>
<td>ITZ</td>
<td>Cat</td>
<td>10</td>
<td>PO</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>2.5-5.0</td>
<td>PO</td>
<td>12 h</td>
</tr>
</tbody>
</table>

AMB; Ampotericin B, ITZ; Itraconazole

Therapy for this case

ITZ 20 mg / kg orally once a day
AMB 0.2 mg / kg, 3 days per week

↓ 4 weeks

The mass continued to increase
ITZ was discontinued
AMB was continued
Micafungin
1 mg / kg, 3 days per week

↓ 5 weeks

No response to therapy was seen and the cat continued to deteriorate and expired.
The *in vitro* susceptibilities of the isolate against the antifungal drugs

<table>
<thead>
<tr>
<th>Minimal Inhibitory Concentration</th>
<th>AMB</th>
<th>ITZ</th>
<th>VRZ</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate</td>
<td>&gt;32 μg/ml</td>
<td>0.38 μg/ml</td>
<td>0.19 μg/ml</td>
<td>0.19 μg/ml</td>
</tr>
</tbody>
</table>

AMB; Ampotericin B, ITZ; Itraconazole, VRZ; Voriconazole, POS; Posaconazole
Conclusion

To our knowledge, this is the first case of the recovery of *A. udagawae* from orbital aspergillosis in a cat.

The cat failed therapy with AMB and this correlated with *in vitro* low susceptibility of *A. udagawae* to AMB.

It is important to identify the isolate correctly as to guide therapeutic decisions canine and feline aspergillosis.

A. terreus infection

A. terreus has been recognized to be an etiological argent of disseminated infection due to Aspergillus species in dogs and cats. Human and animal aspergillosis due to A. terreus is poor to response against AMB therapy.

We isolated Aspergillus sp. from a canine case with renal disease and identified it as A. terreus by the molecular analysis.
Case 2: A 6 year old male Shibainu dog

Chief complaint: anorexia and weakness of 4 days duration.

Clinical findings:
On physical examination, clinical abnormality sing was not find.
Hematologic and serum biochemistry analysis showed
leukocytosis, high levels of CRP, BUN, creatinin and liver enzymes.

<table>
<thead>
<tr>
<th>Hematology and serum biochemistry in patient dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
</tr>
<tr>
<td>WBC</td>
</tr>
<tr>
<td>CRP</td>
</tr>
<tr>
<td>TP</td>
</tr>
<tr>
<td>BUN</td>
</tr>
<tr>
<td>Creatinin</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>ALT</td>
</tr>
<tr>
<td>ALP</td>
</tr>
</tbody>
</table>
Bilateral renomegalies were on the lateral view of radiograph.

Radiographic evaluation could not find skeletal and respiratory system abnormality.

Dilation of renal pelvis and mass were on the view of ultrasound.
Microscopic examination of the urine specimen from left renal pelvis showed many white blood cells, red blood cells, and many branching hyphae.

The latex galactomannan agglutination test of patient serum was positive with Pastorex Aspergillus (Sanofi Diagnostics Pasteur, Marnes-la-coquette, France).
Isolation and identification

From this urine samples, velvety, brown-cinnamon–colored colonies grew after 5-day incubation on potato dextrose agar at 25°C; based on gross and microscopic characters, this isolate was identified as *A. terreus*.

ITS and β-tubulin sequence of the clinical isolate was 100 % identical to *A. terreus*.

The case was diagnosed as proven aspergillosis due to *A. terreus*.
The *in vitro* susceptibilities of the isolate against the antifungal drugs

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<th>VRZ</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate</td>
<td>0.19 μg/ml</td>
<td>0.047 μg/ml</td>
<td>0.19 μg/ml</td>
</tr>
</tbody>
</table>
Therapy for this case

ITZ 5 mg / kg orally twice a day
- 4 weeks
VRZ 5 mg / kg orally twice a day
- 4 weeks
The dog was in poor appetite.
Serum ALT and ALP were increasing.
The concentration of VRZ in sera was 20.32 \( \mu \text{g/ml} \).
VRZ was decreased to 1 mg / kg orally twice a day.
- 1 week
The concentration of VRZ in sera was 0.28 \( \mu \text{g/ml} \)
- 5 weeks
A. terreus has been cultured in urine sample.
VRZ was increased to 2.5 mg / kg orally twice a day.
Conclusion

The case had been treated with ITZ (5 mg/kg BID for 4 weeks), however, *A. terreus* was still isolated from urine sample.

VRZ was administered to the case, since antifungal susceptibility testing revealed that this isolate had higher *in vitro* MIC to ITZ rather than VRZ.

However, the primary focus of *A. terreus* infection was not determined in this patient.
General conclusion

For appropriate therapy on animal aspergillosis, the isolates should be identified molecularly and their susceptibility to antifungal drugs should be determined by MIC testing.