NOTE Internal Medicine

The First Case of Feline Prototheca wickerhamii Infection in Japan

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ABSTRACT. A 3-year-old castrated male domestic short-haired cat was presented with nodules on the left nasal wing and the left ear flap. Prototheca cells were found after excision biopsy of one of the nodules located on the left ear flap. The patient cat was generally in good condition without skin problems. Prototheca wickerhamii was isolated from all 6 masses after they were surgically nucleated. The cat was recovered two months after intervention with no recurrence of skin nodules. This report deals with the first case of feline protothecosis in Japan.

KEY WORDS: cutaneous infection, excision biopsy, feline, Prototheca wickerhamii, protothecosis.


Prototheca species are unicellular algae usually without chlorophyll that are found in nature [4, 8, 11, 12]. They have been reported as pathogens responsible for refractory subcutaneous diseases and systemic infection in both humans and animals in many countries [5]. Although rare, protothecosis has mostly been reported mostly in immunosuppressed dogs and cats [7]. The major clinical manifestations reported are prolonged hemorrhagic enteritis in dogs and cutaneous nodules in cats [1–3, 5]. Feline cases are almost always considered to be fatal, and an effective countermeasure has yet to be established [1, 6, 9]. To our knowledge, the case reported herein may be the first case of feline protothecosis in Japan.

A 3-year-old castrated male domestic short-haired cat was presented with firm, smooth and discrete nodules on the left nasal wing and left ear flap without swelling of regional lymph nodes (Fig. 1a and 1b). The patient cat was confirmed to be free from Feline Immunodeficiency Virus and Feline Leukemia Virus infection based on a blood examination and it was in apparently in good condition. It was reared indoors-outdoors.

Fine needle biopsy specimens taken from skin nodules revealed many granular cells that were positive for Giemsa staining, suggesting a mast cell tumor. The cat was initially orally administered 1.1 mg/kg of prednisolone once/day, 2.2 mg/kg of orbifloxacin once/day, 1.1 mg/kg of diphenhydramine twice/day and famotidine 0.5 mg/kg of famotidine twice/day.

Within two weeks of starting treatment, the cat began to vomit. The size of the mass was unchanged. Accordingly, the treatment was discontinued, and a fine needle biopsy into the mass was again carried out. From the microscopic findings of the specimen, granuloma was strongly suggested due to the presence of yeast-like organisms. Therefore, antifungal chemotherapy was conducted with 2 mg/kg of terbinafine once/day. The masses, however, were further enlarged four weeks later, and we changed the drug administration strategy to 10 mg/kg/day of itraconazole given orally for two weeks. However, the treatment was not successful.

Then, some excision biopsy samples were taken from a nodule located on the left ear flap. Histopathological examination showed an extensive granulomatous inflammation from the superficial dermis to the subcutis. The epidermis was mildly acanthotic. The inflammatory cells mainly consisted of an amount of histiocytes and multinucleated giant cells. These cells were accompanied by a moderate amount of lymphocytes and neutrophils. Each multinucleated giant cell contained heavily colonized organisms. These organisms were spheroid, ovoid or irregular shaped. They were not well stained by hematoxylin-eosin staining but were

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Fig. 1. (a) The firm, smooth, apparently-circumscribed and florid mass on the left nasal wing (arrow). (b) The mass on the left ear flap (arrow) with the same appearance as the mass in (a).
apparently stained by periodic acid-Schiff (PAS). Some organisms having endospores seemed to have wheel-shaped nuclei (Fig. 2).

Excision biopsy samples cultured on Sabouraud’s dextrose agar at 27°C for 1 week developed yeast-like colonies and were subcultured on Prototheca isolation medium (PIM) [10]. The morphological characteristics and findings in assimilation tests of clinical isolates were identical to those of *P. wickerhamii* (Table 1). The clinical isolates were resistant against itraconazole, voriconazole, gentamicin and kanamycin.

Preoperative examinations indicated no significant findings in X-ray and ultrasonography analyses, and no microbes were isolated from peripheral blood samples. The result of blood examination showed leucopenia (WBC, 3,000/μl; bandcells, 120/μl; segmentedcells, 1320/μl; lymphoid corpuscles, 1680/μl; monocytes, 120/μl; eosinophilic leukocytes, 120/μl).

After one-week of treatment with 10 mg/kg of G-CSF, the total WBC count was increased to 10,200/μl. Then, five of the six nodules were removed from the nasal bridge, sole, hock-joint of the left hind limb, earflap and tail along with some normal skin margin. Concerning the remaining nodule located on the nose wing, it was incised with as wide a margin of grossly normal cartilage as possible because it was difficult to resect with an adequate margin. *P. wickerhamii* was isolated in the cultures of specimens taken from all of the nodules, and the organism was also microscopically found in the cartilage of the grossly normal nose wing. It has been suggested that infection with Prototheca in the case of cutaneous protothecosis occurs via trauma to the skin [5]. The present case was reared indoors-outdoors and

![Fig. 2. Histopathologic findings of the mass on the left ear flap (PAS stain) revealed granulomatous dermatitis with inflammatory cells around organisms. Many phagocytosed Prototheca cells were detected in macrophages (arrow).](image)

<table>
<thead>
<tr>
<th>Species</th>
<th>Daure cell</th>
<th>Sporangiospores</th>
<th>Spherical</th>
<th>Capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical isolate</td>
<td>&lt;8.5 μm</td>
<td>8.5 μm</td>
<td>4.3 μm</td>
<td>5.7 μm</td>
</tr>
<tr>
<td><em>P. moriformis</em></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>P. stagnora</em></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>P. ulmea</em></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td><em>P. wickerhammi</em></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><em>P. zopfii</em></td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

a) +, all cell stages obviously nearly spherical; V, Variety of cell stages spherical or ellipsoidal.

b) +, the primary cells cultured on PIM supplemented with 1% glucose at 25°C have a capsule; –, have no capsule.

Table 1b. Alcohol and sugar assimilation test of the clinical isolate and recognized species of Prototheca

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N-Acetyl-D-glucosamine</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Glycerol</th>
<th>Inositol</th>
<th>Lactose</th>
<th>D-Mannitol</th>
<th>Melibiose</th>
<th>Melibiose</th>
<th>α-Methyl-D-glucoside</th>
<th>Salicin</th>
<th>Sucrose</th>
<th>Starchose</th>
<th>Trehalose</th>
<th>D-Xylose</th>
<th>Ethanol</th>
<th>1-Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical isolate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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</tr>
<tr>
<td><em>P. moriformis</em></td>
<td>NT</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>NT</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>+/W</td>
</tr>
<tr>
<td><em>P. stagnora</em></td>
<td>NT</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>NT</td>
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<td>–</td>
<td>–</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td><em>P. ulmea</em></td>
<td>NT</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>NT</td>
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<td>NT</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>NT</td>
</tr>
<tr>
<td><em>P. wickerhammi</em></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td><em>P. zopfii</em></td>
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<td>–</td>
<td>+</td>
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<td>V</td>
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<td>–</td>
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<td>+</td>
</tr>
</tbody>
</table>

+, positive; –, negative; W, weakly positive; +/W, positive or weakly positive; V, variable; NT, not tested.
had no wounds before. However, the microbe might have been inoculated through a small wound.

In our case, the cutaneous nodules located on the nasal bridge, sole, hock-joint of the left hind limb, ear flap, frontal region of the head and tail were successfully excised. However, it was difficult to remove the lesions, especially on the left nose wing, with an adequate margin. Pathological examination of the nodule from the nose revealed prototheca cells in the cartilage of the marginal area, suggesting the surgery was not successful for complete recovery. Therefore, it was anticipated that another mass would form. In such cases of protothecosis, it may be important to make a decision concerning surgical intervention as soon as possible in order to completely remove the lesions based on accurate diagnosis in an early stage.

REFERENCES