

## The first case of feline sinonasal aspergillosis due to *Aspergillus fischeri* in Japan

Rui KANO<sup>1)\*</sup>, Tomoko TAKAHASHI<sup>2)</sup>, Takeshi HAYAKAWA<sup>3)</sup>, Yoshiki YAMAYA<sup>3)</sup>, Atsuhiko HASEGAWA<sup>4)</sup> and Hiroshi KAMATA<sup>1)</sup>

<sup>1)</sup>Veterinary Pathobiology, Nihon University College of Bioresource Sciences, 1866 Kameino, Fujisawa, Kanagawa 252–0880, Japan

<sup>2)</sup>Veterinary Radiology, Nihon University College of Bioresource Sciences, 1866 Kameino, Fujisawa, Kanagawa 252–0880, Japan

<sup>3)</sup>Division of Veterinary Respiratory Medicine, Animal Medical Center, Nihon University College of Bioresource Sciences, Kameino, Fujisawa, Kanagawa 252–0880, Japan

<sup>4)</sup>Teikyo University Institute of Medical Mycology, 539 Otsuka, Hachioji, Tokyo 192–0395, Japan

(Received 4 September 2014/Accepted 6 April 2015/Published online in J-STAGE 3 May 2015)

**ABSTRACT.** Feline upper respiratory tract infection due to *Aspergillus* spp. is considered an emerging disease, with the number of reported cases continuing to rise. In this study, we report the first case of feline sinonasal aspergillosis caused by *Aspergillus fischeri* in Japan. The patient presented after 2 months of progressive facial deformity around the nose and nasal discharge. The isolate from this case was susceptible to itraconazole (ITZ), voriconazole and micafungin, but was resistant to amphotericin B. However, the infected cat died approximately 1 month after referral, despite treatment for 12 days ITZ administered orally at 10 mg/kg.

**KEY WORDS:** *Aspergillus fischeri*, feline, molecular identification, sinonasal aspergillosis

doi: 10.1292/jvms.14-0454; *J. Vet. Med. Sci.* 77(9): 1183–1185, 2015

Feline upper respiratory tract (URT) infection due to *Aspergillus* spp. is considered an emerging disease, with the number of reported cases continuing to rise [3–5, 7–9]. *Aspergillus fumigatus* has been the most frequently reported etiologic agent of sino-orbital aspergillosis in cats [4, 7]; two other *Aspergillus* species, *A. udagawae* and *A. fischeri*, have also been implicated. The minimum inhibitory concentrations (MICs) of amphotericin B (AMB) and azoles [4, 8, 9] are elevated for *A. udagawae* and *A. fischeri* compared with *A. fumigatus*, but distinguishing *A. udagawae* and *A. fischeri* from *A. fumigatus* requires molecular analyses. Some reports described that the feline infections due to *A. udagawae* and *A. fischeri* do not respond to treatment with AMB, itraconazole (ITZ) or micafungin (MCF) [4, 8, 9]. These results suggest that speciation and antifungal susceptibility testing of infecting agents are important to ensure effective treatment of feline URT aspergillosis. In this study, we report the first Japanese feline case of sinonasal aspergillosis caused by *A. fischeri*.

**Case:** A castrated Russian blue cat (11 years old; weight, 2.9 kg) was referred to the Nihon University Animal Medical Center, Kanagawa, Japan, in June 2014 after exhibited progressive facial deformity around the nose with nasal discharge over the previous 2 months. The cat suffered from diabetes mellitus and had been treated with insulin for 5 years. However, control by insulin injection was not suc-

cessful in the patient cat. Physical examination of the cat showed swelling on the nose (Fig. 1), depression and weight loss. The results of hematological examination, a serum biochemistry panel and urinalysis are shown in Table 1. The cat was positive for the serum *Aspergillus* galactomannan antigen (Health Sciences Research Institute, Inc., Yokohama, Japan). CT scan revealed a soft-tissue mass within the right nasal cavity (Fig. 2). A chest and abdominal CT scan did not detect typical *Aspergillus* infection signs. Histopathologic examination of biopsy samples from this mass revealed granulomatous inflammation containing many branching hyphal filaments (Fig. 3). A sample of nasal discharge was inoculated on Sabouraud's dextrose agar (SDA) and incubated at 28°C. Velvety-grayish white colonies developed within 2 weeks. Based on gross and microscopic characteristics, the isolate was identified as an *Aspergillus* species, and the case was diagnosed as sinonasal aspergillosis.

ITZ (Janssen Pharmaceutical K.K., Tokyo, Japan) was administered orally at 10 mg/kg, once daily for 12 days. In addition, antifungal susceptibility tests were initiated for the infecting organism, and plans were made to flush the nasal airway with appropriate antifungal solutions based on the results of these tests. However, the owner declined the use of topical antifungal therapy pending the results of susceptibility testing because of the nasal airway needs to be performed in an anesthetized condition that is a high risk for the patient. The owner desired an effective and safe treatment.

In the meantime, the case continued to deteriorate, and the cat died on day 19, i.e., one week after completion of the ITZ regimen (and 28 days after the initial referral). Necropsy was not performed.

**Molecular identification of fungal species:** Isolation of genomic DNA of the isolate was reported previously [9].

The internal transcribed spacer (ITS) region of the isolated *Aspergillus* was amplified using the universal fungal

\*CORRESPONDENCE TO: KANO, R., Veterinary Pathobiology, Nihon University College of Bioresource Sciences, 1866 Kameino, Fujisawa, Kanagawa 252–0880, Japan.  
e-mail: kanou.rui@nihon-u.ac.jp

©2015 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.



Fig. 1. Pus discharge and an ulcer were observed on the mass on the left side of the bridge of the nose.

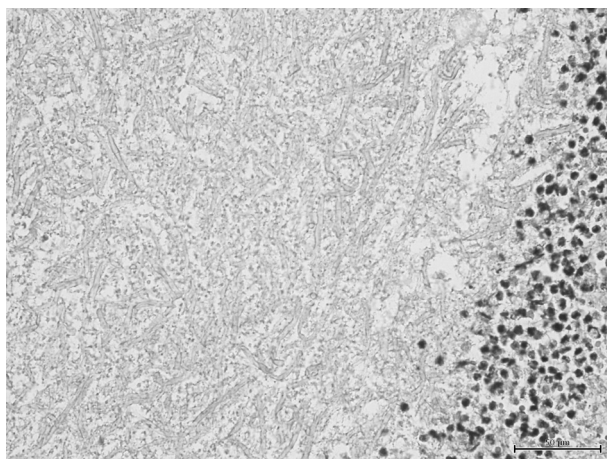


Fig. 3. Histopathologic examination of the mass from the right nasal cavity of the case revealed chronic purulent inflammation with many branching hyphal filaments (HE stain).

primers ITS5 (5' GGAAGTAAAAGTCGTAACAAGC) and ITS4 (5' TCCTCCGCTTATTGATAGC) [2]. PCR amplification and sequence analyses were performed as described previously [2].

Comparative sequence analyses by nucleotide BLAST analysis on the National Center for Biotechnology Information (NCBI) website showed that the sequence of the ITS region amplified from the isolate from the case was 100% identical to that of *A. fischeri* (teleomorph of *Neosartorya fischeri*; GenBank accession no. FJ624264).

The sequences determined in this study have been deposited in GenBank (*Aspergillus fischeri* genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial sequences, strain: NUBS14001 clinical isolate from feline sinonasal aspergillosis; DDBJ accession number, LC011422).

*Testing of in vitro susceptibility to antifungal drugs:* The

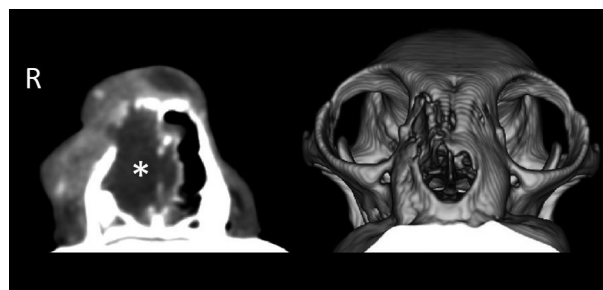


Fig. 2. The left panel shows a computed tomographic (CT) image after administration of a contrast agent at the level of the canine teeth. The animal's right nasal cavity (R) is occupied by a large mass (\*) that lacks contrast enhancement. The right panel shows a reconstructed image from multiple CT images of the head; the right side of the nasal bone is largely destroyed.

Table 1. Hematological examination, serum biochemistry panel and urinalysis of the patient cat

Ht	23%	ALB	42 U/l	Na	152 mEq/l
Hb	7.3 g/dl	ALP	31 U/l	K	2.7 mEq/l
PLT	$772 \times 10^3/\mu\text{l}$	ALT	29 U/l	Cl	110 mEq/l
WBC	$17,900/\mu\text{l}$	AST	10 U/l		
Stab	$0/\mu\text{l}$	BUN	42 mg/dl	Urine gravity 1.034	
Seg	$12,440/\mu\text{l}$	CRE	0.8 mg/dl	Glycosuria 2,000 mg/l	
Lym	$1,969/\mu\text{l}$	TCHOL	380 mg/dl		
Mon	$1,790/\mu\text{l}$	GLU	316 mg/dl		
Eos	$1,700/\mu\text{l}$	Ca	10.8 mg/dl		
Baso	$0/\mu\text{l}$	P	2.3 mg/dl		

*in vitro* susceptibilities of the isolate to the antifungal drugs AMB, ITZ, voriconazole (VRZ) and micafungin (MCF) were assessed by the E-test method [11]. The drug susceptibility tests for this isolate revealed that the MICs of AMB, ITZ, VRZ and MCF by E-test were more than 32 mg/l, 0.25 mg/l, 0.064 mg/l and 0.012 mg/l, respectively.

This work represents the first reported Japanese case of feline sinonasal aspergillosis due to *A. fischeri*. In summarizing 22 cases of feline sinonasal aspergillosis in Australia, Barrs *et al.* reported that the fungal pathogens were *A. fumigatus* (n=4), *Neosartorya fischeri* (n=1), *A. lentulus* (n=1) or other *Neosartorya* spp. (n=16) [4]. Therefore, non-*fumigatus* aspergilli, including *A. fischeri*, should be considered potential fungal pathogens in feline URT aspergillosis.

*A. fischeri* is known as a causative agent of human respiratory aspergillosis [1], but it has not been well investigated with regard to susceptibility to AMB and azoles. The isolate from this case was susceptible to ITZ, VRZ and MCF, but was resistant to AMB. Cantón *et al.* suggested that the *in vitro* breakpoints (resistance) for AMB and azoles of *Aspergillus* spp. were MICs  $\geq 4$  mg/l [6].

AMB is frequently selected for treatment of canine and feline cases of aspergillosis, including feline URT aspergillosis [10]. Therefore, the decision-making process for determining the therapy for therapy of feline URT aspergillosis

should include determination of the MICs of other antifungal drugs.

We administered oral ITZ to the patient cat, but could not confirm the efficacy of this treatment due to subsequent mortality. The poor response in this case may have reflected progression of the infection. In addition, the pathogenesis of diabetes mellitus might interfere with ITZ therapy and has been recognized as a risk factor in feline URT aspergillosis [5]. More aggressive management using intravenous administration or nasal air way flushing with ITZ solution may have been needed.

This case was diagnosed as aspergillosis by a serum *Aspergillus* galactomannan antigen test as well as histopathologic examination and mycological identification. The antigen test has been reported to have poor specificity, but moderate sensitivity as a noninvasive screening test to rule out infection in feline patients with suspected URT aspergillosis [12]. Therefore, the test should be evaluated for rapid, noninvasive screening of feline URT aspergillosis, including *A. fischeri* infection.

The low susceptibility to antifungals seen in isolates of non-*fumigatus* aspergilli indicates that molecular identification of *Aspergillus* species and *in vitro* susceptibility testing are needed in the selection of effective antifungal drugs and prediction of the prognosis of feline URT aspergillosis. Further studies are required to determine the distinct resistance profiles of infecting non-*fumigatus* aspergilli, since antifungal susceptibility may be a major determinant of treatment outcome. Aspergillosis is generally an opportunistic infection, and host immunocompetence is thought to be an important determinant in the development of the infection. The frequency of use of chemotherapy and immunosuppressive drugs is increasing in the veterinary field. Due to the risk of opportunistic fungal infections should exercise care when they use these drugs.

In conclusion, feline URT infection due to *Aspergillus* spp. is considered an emerging disease, with the number of reported cases continuing to rise. To our knowledge, this was the first feline case of sinonasal aspergillosis in Japan caused by *A. fischeri*. The isolate from this case was susceptible to ITZ, VRZ and MCF, but was resistant to AMB. Therefore, the cat was administered ITZ orally for 12 days, and it died on day 19. The poor response in this case may have reflected progression of the infection. A rapid diagnosis method and effective treatment are needed based on speciation and antifungal susceptibility testing of infecting agents.

**ACKNOWLEDGMENTS.** This study was supported by a grant (“International joint research and training of young researchers for zoonosis control in the globalized world”) from the Ministry of Education, Culture, Sports, Science

and Technology (MEXT) Japan, and by a Nihon University Multidisciplinary Research Grant (2013–2014). We thank to Chieko Ishikawa<sup>2)</sup> who took CT scan.

## REFERENCES

- Balajee, S. A. and Marr, K. A. 2006. Phenotypic and genotypic identification of human pathogenic aspergilli. *Future Microbiol.* **1**: 435–445. [Medline] [CrossRef]
- Balajee, S. A., Tay, S. T., Lasker, B. A., Hurst, S. F. and Rooney, A. P. 2007. Characterization of a novel gene for strain typing reveals substructuring of *Aspergillus fumigatus* across North America. *Eukaryot. Cell* **6**: 1392–1399. [Medline] [CrossRef]
- Barachetti, L., Mortellaro, C. M., Di Giancamillo, M., Giudice, C., Martino, P., Travetti, O. and Miller, P. E. 2009. Bilateral orbital and nasal aspergillosis in a cat. *Vet. Ophthalmol.* **12**: 176–182. [Medline] [CrossRef]
- Barrs, V. R., Halliday, C., Martin, P., Wilson, B., Krockenberger, M., Gunew, M., Bennett, S., Koehlmeyer, E., Thompson, A., Fliegner, R., Hocking, A., Sleiman, S., O'Brien, C. and Beatty, J. A. 2012. Sinonasal and sino-orbital aspergillosis in 23 cats: aetiology, clinicopathological features and treatment outcomes. *Vet. J.* **191**: 58–64. [Medline] [CrossRef]
- Barrs, V. R. and Talbot, J. J. 2014. Feline aspergillosis. *Vet. Clin. North Am. Small Anim. Pract.* **44**: 51–73. [Medline] [CrossRef]
- Cantón, E., Espinel-Ingroff, A. and Pemán, J. 2009. Trends in antifungal susceptibility testing using CLSI reference and commercial methods. *Expert Rev. Anti Infect. Ther.* **7**: 107–119. [Medline] [CrossRef]
- Giordano, C., Gianella, P., Bo, S., Vercelli, A., Giudice, C., Della Santa, D., Tortorano, A. M., Peruccio, C. and Peano, A. 2010. Invasive mould infections of the naso-orbital region of cats: a case involving *Aspergillus fumigatus* and an aetiological review. *J. Feline Med. Surg.* **12**: 714–723. [Medline] [CrossRef]
- Kano, R., Itamoto, K., Okuda, M., Inokuma, H., Hasegawa, A. and Balajee, S. A. 2008. Isolation of *Aspergillus udagawae* from a fatal case of feline orbital aspergillosis. *Mycoses* **51**: 360–361. [Medline] [CrossRef]
- Kano, R., Shibahashi, A., Fujino, Y., Sakai, H., Mori, T., Tsujimoto, H., Yanai, T. and Hasegawa, A. 2013. Two cases of feline orbital aspergillosis due to (*Aspergillus udagawae*) and (*A. viridinutans*). *J. Vet. Med. Sci.* **75**: 7–10. [Medline] [CrossRef]
- Michael, J. D. and Vanessa, R. D. B. 2012. Feline sinonasal and sinoorbital *Aspergillus fumigatus* complex and Penicillium infections. pp. 659–662. In: Infectious Diseases of Dog and Cat, 4th ed. (Green, C. E. ed.), Saunders Elsevier, St. Louis.
- Pfaller, M. A., Messer, S. A., Boyken, L., Hollis, R. J. and Diekema, D. J. 2003. *In vitro* susceptibility testing of filamentous fungi: comparison of Etest and reference M38-A microdilution methods for determining posaconazole MICs. *Diagn. Microbiol. Infect. Dis.* **45**: 241–244. [Medline] [CrossRef]
- Whitney, J., Beatty, J. A., Martin, P., Dhand, N. K., Briscoe, K. and Barrs, V. R. 2013. Evaluation of serum galactomannan detection for diagnosis of feline upper respiratory tract aspergillosis. *Vet. Microbiol.* **162**: 180–185. [Medline] [CrossRef]