Histoplasmosis is caused by *Histoplasma capsulatum* (*H. capsulatum*), which is a dimorphic soil-borne fungus. *H. capsulatum* is widely distributed along major rivers in worldwide temperate and tropical climates. A major endemic area was known to center on the Mississippi River and its tributaries in the United States [10]. The organisms commonly infect hosts by inhalation of conidia from the mycelial phase, which then convert to the yeast phase at the host's body temperature. The lesion formed in the lungs is usually latent and clinically vague. The yeast cells proliferate within macrophages and are transported to systemic organs by a mononuclear phagocytic system in severe cases [3]. In disseminated histoplasmosis, in which necrotizing and granulomatous inflammation develop observed in various systemic organs with nonspecific clinical symptoms, most cases of the diseases are progressive and fatal.

In the United States, histoplasmosis has become the second most commonly reported fungal disease after cryptococcosis in feline deep mycotic infections [5]. In Japan, however, there have been no reports of feline histoplasmosis. Feline histoplasmosis has been reported primarily in the United States [1, 2, 4, 8, 11, 15, 21, 23], and all disseminated cases have shown pulmonary lesions by pathological examination, except for one primary gastrointestinal case [21]. In the case, however, histological examination of the lungs was not done, so it is unclear whether *H. capsulatum* primarily induces intestinal lesions in cats. In dogs, cases with lesions restricted to the abdominal organs have been reported [6]. No lesions in the respiratory tract have been confirmed by histologic examination in these cases, and infection via an oral route by ingestion of the mycelial phase or infected rodents has been postulated.

In Japan, histoplasmosis has generally been considered to be an imported mycosis. Recently, however, autochthonous cases have been reported in humans [13]. Seven cases of canine histoplasmosis have been reported in Japan [12, 17, 18, 20, 22]. In addition, Murata et al. have compared canine cases with worldwide human and equine cases with respect to partial sequences on the internal transcribed spacer (ITS1/2) regions of the ribosomal DNA genes [17]. This phylogenetic analysis performed by nested polymerase chain reaction (nested PCR) and sequence analysis have revealed that these canine cases in Japan were closely related to the Asian autochthonous human cases.

In this report, we describe cytological, histopathological, and immunohistochemical findings of intestinal histoplasmosis in a cat. This is the first report of feline histoplasmosis in Japan.
lated in the abdominal and thoracic cavities. The upper part of the colon, approximately 10 cm in length, was markedly dilated with a thickened wall (Fig. 2). The mucosal surface of this part was necrotic and ulcerated, and was partly perforated with outflow of the contents to the abdominal cavity. The pancreatic lymph node attached to the pancreas was markedly enlarged approximately 17 mm in diameter and colored dark red. In the lungs, no significant changes were observed, except for a few dispersed white fine nodules on the surface. The trancheobronchial lymph node was enlarged and firm. Skin lesions were not observed. The vaccination history was unknown, but vaccination against feline leukemia virus (FeLV) had not been done. Serum tests for FeLV antigen and feline immunodeficiency virus antibody were not performed.

Tissue samples including the heart, spleen, trancheobronchial lymph node, trachea, lung, diaphragm, small and large intestine, liver, pancreas, kidney, and pancreatic lymph node were fixed in neutral-buffered 10% formalin for histopathological examination. Tissues were routinely processed, embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin (HE). Grocott-Gomori methanamine-silver stain (GMS) was applied to selected tissue sections. For the immunohistochemistry, rabbit anti-histoplasma yeast antibody (Meridian Diagnostics, Inc., Cincinnati, OH, U.S.A.: 1:1,000) and peroxidase-conjugated anti-rabbit IgG antibody (Histofine Simple Stain MAX-PO(R); Nichirei, Tokyo, Japan) were used as primary and secondary antibodies, respectively. Color development by AEC (AEC SUBSTATE KIT FOR PEROXIDASE; Vector laboratories, Burlingame, CA, U.S.A.) and counterstaining with Mayer’s hematoxylin were performed after the immunoreaction.

To obtain a partial sequence of *H. capsulatum*, a nested PCR was performed using extracted DNA from paraffin-embedded tissues of the colon and the pancreatic lymph node. The method of DNA extraction, the primer sequences specific to *H. capsulatum*, and the method of nested PCR were selected according to previously published reports [14, 17]. The primer sequences for nested PCR were as follows: the primers for the first PCR were ITS-5 (5′-GGAAGTAAAGATTGGTAAACAGG-3′) and ITS-4 (5′-TCCTCAGCTTTATGGATATGC-3′), and those for the second PCR were HeAF (5′-CACGCCGTGGGCGCTGGAGGCCT-3′) and HeCR (5′-ATGGTGGGGCAGAGCCCGCC-3′) with a complementary sequence of 5′-GGCCGGCTCCYGCCCACCAT-3′.

Microscopically, severe necrotizing and granulomatous inflammation was observed in the wall of the dilated colon (Fig. 3), in the pancreatic lymph node, and in the trancheobronchial lymph node. In these tissues, normal structures had been destroyed. In the dilated wall of the colon, there were many macrophages containing yeast-like bodies as irregularly shaped eosinophilic round structures throughout the necrotic lesions (Fig. 4). The cell wall of the organisms was stained black by GMS (Fig. 5). Immunohistochemically, these organisms were positive for rabbit anti-histoplasma yeast antibody (Fig. 6). There were no yeast-like bodies in the mass attached to the pancreas and the trancheobronchial lymph node. Necrotizing and granulomatous tissues adhered to the serous surface of the abdominal organ were also found. In the lung, white fine nodules grossly observed were diagnosed as focal lipid pneumonia and a bronchiolalveolar adenoma. No yeast-like organisms were observed in the lung. The lesions accompanying the yeast-like organisms were not seen in any other organs.

Nested PCR did not detect the specific partial sequence of *H. capsulatum*.

In the present case, cytological, histopathological, and immunohistochemical findings suggested that the cause of the intestinal lesion may have been *H. capsulatum*. *H. capsulatum* has been grouped into three types: *H. capsulatum* var. *farcinomosum*, *H. capsulatum* var. *capsulatum*, and *H. capsulatum* var. *duboisii*. The size of the organisms observed in the present case was comparable to that of *H. capsulatum* var. *farcinomosum* or *H. capsulatum* var. *capsulatum*. We could not detect the partial sequence of the causative agent by nested PCR in this study, which may have been due to the severe necrosis of the lesions.

As for the organisms we observed in this case, it is necessary to differentiate them from other fungi or protozoans in the morphologic characterization and in considering the distribution of the lesions. *Leishmania donovani* is similar to *H. capsulatum* in morphology, but its lesions are mainly formed in the spleen and liver in visceral leishmaniasis in cats [16], and kinetoplasts were not seen in our case. *Sporothrix schenckii* induces ulcerative skin lesions [7], and *Blastomyces dermatitidis* causes suppurative inflammation in systemic organs but not the intestine [9].

The lesions in feline disseminated histoplasmosis often extend to the lymph nodes, spleen, liver, intestine, bone, bone marrow, eyes, skin, and muscle [3, 4, 19, 23]. In the present case, lesions were limited to the intestinal tract and lymph nodes, although bone, bone marrow, and eyes were not examined histologically. Histoplasmosis involving the intestinal tract from the terminal ileum to the colon with accessory lymph nodes has been reported in both dogs [6] and a cat [21]. Our case is similar to these cases; as such, the colon and gastrointestinal lymph nodes might be predilection sites in intestinal histoplasmosis.

There have been no cases without pulmonary lesions on histological examination in feline histoplasmosis. Although we obtained sections of the lung from multiple sites, no significant lesions associated with yeast-like organisms were observed. It has been postulated that the cases of canine abdominal histoplasmosis does not rule out the possibility that there were initial respiratory infections that resolved after dissemination occurred [6]. In the present case, the lung might not have been initially involved, as neither fibrosis nor cicatrization was observed.

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Fig. 1. Fine needle aspiration of the mass in the upper abdominal cavity. Many yeast-like organisms can be observed in the cytoplasm of a macrophage. They appear as basophilic dots with a clear halo. Wright-Giemsa. Bar=10 \( \mu m \).

Fig. 2. The upper part of the colon is markedly dilated with a thickened wall and the mucosal surface is necrotic and ulcerated.

Fig. 3. A wide range of necrotizing tissue in the colon with a marginal lymphatic structure (Lym). HE. Bar=1 mm.

Fig. 4. In the colonic lesion, the cytoplasm of the macrophages contains eosinophilic round structures (arrows) in necrotizing tissues. HE. Bar=10 \( \mu m \).

Fig. 5. The yeast-like organisms in macrophages are stained black. Grocott-Gomori methenamine-silver stain. Bar=10 \( \mu m \).

Fig. 6. The organisms show a positive reaction for anti-histoplasma yeast antibody. Immunoperoxidase-AEC with a hematoxylin counterstain. Bar=10 \( \mu m \).
REFERENCES