NOTE *Pathology*

Gastrointestinal basidiobolomycosis in a dog

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ABSTRACT. An 8-year-old, spayed, female Shiba dog was presented to a referring veterinarian with a complaint of chronic diarrhea and anorexia. Ultrasound and radiographs revealed an irregular mass in the pelvic cavity. The mass and the affected section of colon were surgically removed. Histopathological examination revealed multifocal coalescing granulomas and effaced intestinal structures. Central necrotic debris surrounded by multinucleated giant cells, lymphocytes, plasma cells and neutrophils was observed. Numerous, irregularly branched hyphae with pale basophilic, thin walls and occasional bulbous enlargements at the tips were present. Polymerase chain reaction identified *Basidiobolus ranarum*, successfully confirming a definitive diagnosis of basidiobolomycosis. To the best of our knowledge, this is the first report of intestinal basidiobolomycosis in a dog.

KEY WORDS: *Basidiobolus ranarum*, canine, gastrointestinal basidiobolomycosis

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Basidiobolomycosis is a rare disease caused by *Basidiobolus ranarum*, an environmental saprophyte found worldwide in soil, decaying organic matter and the gastrointestinal tracts of amphibians, fish and reptiles [1, 4]. *B. ranarum* is a fungus belonging to the Entomophthoraceae family of the class Zygomycetes and is primarily associated with subcutaneous infections that are presumably acquired after minor trauma to the skin or insect bites [1, 4]. Most cases of basidiobolomycosis have been reported from tropical and subtropical regions of Africa, South America and recently, the United States. The majority of these reports have emerged in the last decade [7, 11]. Gastrointestinal manifestations have been described rarely in human medicine, with the colon showing the most frequent involvement [1, 4, 6, 7,10–12]. In the field of veterinary medicine, two reports on cutaneous and systemic infection with *B. ranarum* have been reported in the southern part of the United States [2, 9].

Here, we describe, to the best of our knowledge, the first case of intestinal basidiobolomycosis in an 8-year-old, spayed, female Shiba dog in Japan.

The dog was brought to a referring veterinarian in a severely emaciated condition with a history of chronic diarrhea and anorexia. The rectal temperature was high (39.7C [103.5F]). A blood test revealed (values with reference ranges) albumin (ALB), 2.0 g/d*l* (2.3–4.0 g/d*l*); blood urea nitrogen (BUN), 38 mg/d*l* (7–27 mg/d*l*); and C- reactive protein (CRP), >7.0 mg/d*l* (<1.00 mg/d*l*). Ultrasound and radiographs (Fig.1) revealed an irregular mass measuring 12 × 2 × 3 cm in the pelvic cavity. These findings were suggestive of a malignant neoplasm. The dog's general condition rapidly deteriorated, and the dog underwent emergency surgery on the ninth day after the initial consultation. The mass involved the majority of the colon, and it was radically excised along with the colon and ileum (Fig. 2). The dog died after the surgery, and necropsy was not performed.

The surgical specimen was fixed in 10% formalin, embedded in paraffin, cut into 4 μm-thick sections and stained with hematoxylin and eosin. Some sections were also prepared using Gomori methenamine silver stain and periodic acid–Schiff (PAS) reaction. Histopathological examination revealed transmural inflammation involving the entire colonic wall (Fig. 3) and extending to the pericolic fat, with effacement of the normal intestinal structures. The iliac lymph nodes were also involved. The colonic mass contained multifocal and coalescing granulomas. Numerous cross and longitudinal sections of hyphae, which were irregularly branched with pale basophilic, thin walls and occasional septae (diameter, 5–20 μm), were observed in central necrotic areas of granulomas. The hyphae were surrounded by multinucleated giant cells, lymphocytes, plasma cells and neutrophils (Figs. 4 and 5). However, Splendore-Hoeppli material ("eosinophilic cuff""), reported in humans [5], was not observed. In addition to hyphae, zygospores were present as spherical bodies that measured up to 30 μm in diameter (Fig. 5), with thin outer walls, foamy cytoplasm and a nucleus containing a large nucleolus. Occasional 30 μm diameter spore-like bulbous enlargements were observed at the tips of the hyphae. The presence of hyphae was confirmed using Gomori methenamine silver stain (Fig. 6) and PAS reaction. Forms of these fungi were consistent with those of *B. ranarum* previously reported [2, 3, 5, 10, 11].

Fungal genomic DNA was extracted from the formalin-fixed, paraffin-embedded sample using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany). The taxon specific primers used for fungal amplification were Ba1/Ba2 (Ba1: 5′-AAAATCTGTAAGGTTCAACCTTG-3′ and

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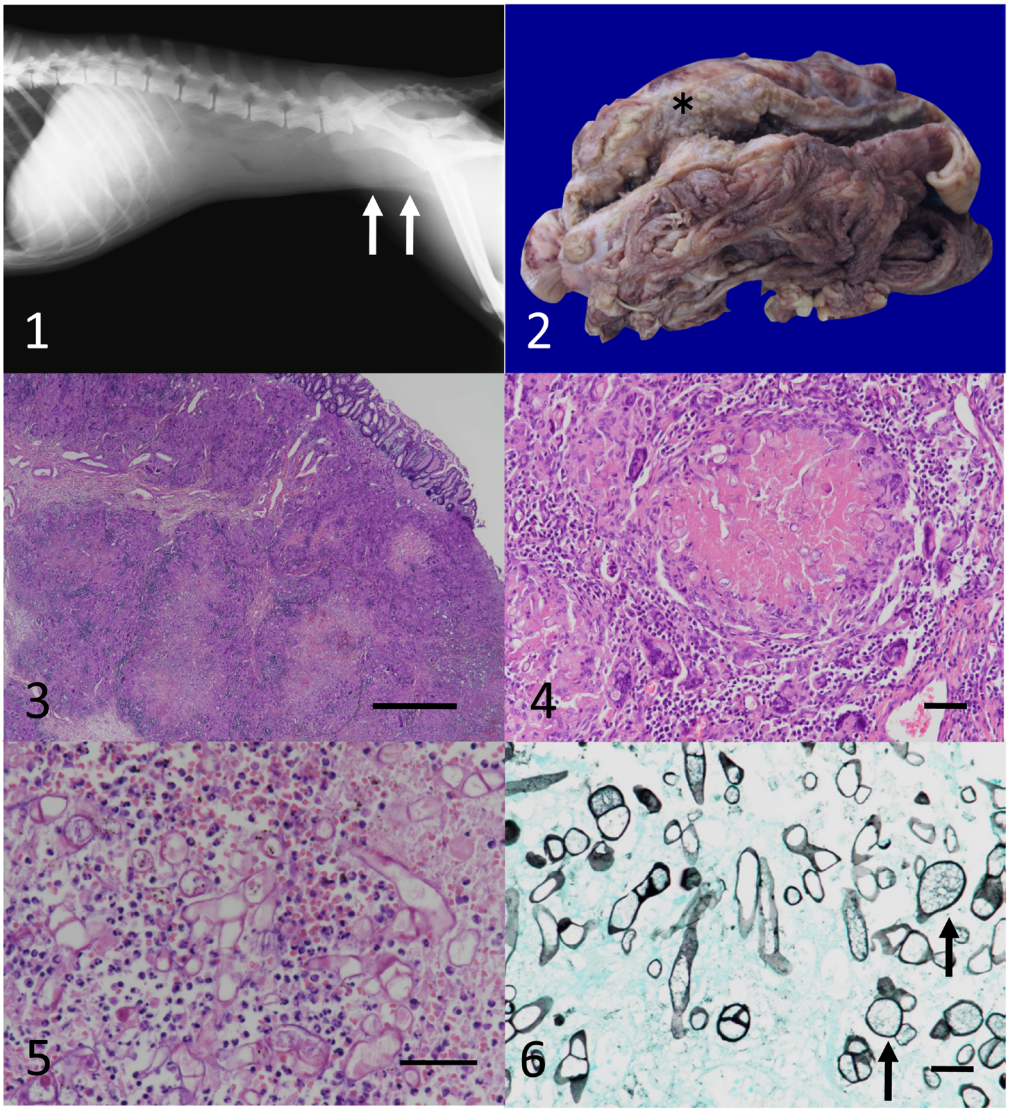

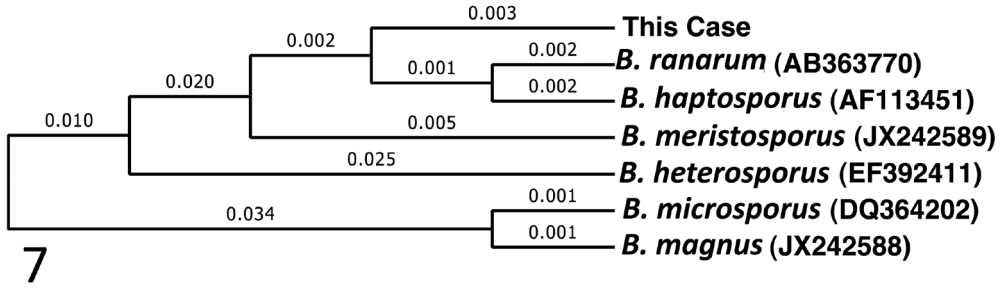

Fig. 1. Lateral abdominal radiograph of the affected dog. Note the irregular mass in the pelvic cavity (arrows).
Fig. 2. Gross features of the formalin-fixed mass in the colon. Multiple nodules scattered throughout the parenchyma can be seen on the cut surface (asterisk).
Fig. 3. Transmural granulomatous inflammation involving the entire colonic wall can be seen. Bar: 1 mm.
Fig. 4. Numerous fungal hyphae are observed within the granuloma. Bar: 50 μm.
Fig. 5. The fungal hyphae varied in size up to a diameter of 30 μm are observed. Neutrophils, a few macrophages and lymphocytes surround the hyphae. Bar: 50 μm.
Fig. 6. Gomori methenamine silver stain enhances the visibility of the fungal hyphae and spore-like structures (arrows). Bar: 25 μm.
Fig. 7. Phylogenetic tree generated from the sequence alignments of the D1/D2 domain of the nuclear large subunit (28s) regions of *basidiobolus* spp.

Ba2: 5′-TGCAGGAGAAGTACATCCGC- 3′), as described previously [1]. The resultant polymerase chain reaction (PCR) product was approximately 650 base pairs in length, and its nucleotide sequence was analyzed in a commercial laboratory (Eurofins Genomics, Tokyo, Japan). The obtained sequence of the PCR product showed 99% homology with that of 28s rDNA of *B. ranarum* on BLAST serch (GenBank accession number, AB363770) (Fig. 7).

B. haptosporus and *B. meristosporus*, which were previously classified as different organisms, are considered synonymous with *B. ranarum* at present [1, 8]. Therefore, it is conceivable that the organism detected in this study is *B. ranarum*.

In the present case, the histopathological and PCR findings suggested *B. ranarum* as the cause of the intestinal lesion. *B. ranarum* infection may present as subcutaneous, gastrointestinal or systemic lesions. Gastrointestinal basidiobolomycosis is rarely reported in the field of human medicine [6, 7,10–12]. *B. ranarum* infection is classified as an entomophthoromycosis, which is a rare form of zygomycosis [4, 12]. It was necessary to differentiate the organisms observed in this case from other fungi with similar morphological characteristics and to consider the distribution of the lesions. Pythiosis and zygomycosis share similar clinical and histological characteristics (all are represented by lesions characterized by pyogranulomatous inflammation associated with broad, irregularly branched, sparsely septated hyphae), making them difficult to distinguish from one another [3]. Gastrointestinal pythiosis in dogs is typically characterized by severe segmental, transmural thickening of the stomach, small intestine, colon and rectum. The histological findings of pythiosis are generally characterized by eosinophilic granulomatous inflammation. The organisms are usually found within areas of necrosis or at the center of discrete granulomas. Although *P. insidiosum* hyphae are difficult to visualize on hematoxylin and eosin-stained sections, they may be identified as clear spaces surrounded by a narrow band of eosinophilic material [3]. In humans, the characteristic histological findings of entomophoraceous infections include the presence of an eosinophilic cuff surrounding the hyphae [5]. This cuff is thought to represent an antigen– antibody complex [5]. Eosinophils are assumed to play a major role in the composition of this cuff in humans; however, the present case and previous cases in dogs did not show this cuff [2, 9]. In dogs, the inflammatory cells are primarily neutrophils, indicating an immune reaction different from that in humans.

Gastrointestinal basidiobolomycosis is considered to be an uncommon disease among humans and animals worldwide, although several cases may have been misdiagnosed as nonspecific inflammatory intestinal diseases or colon cancer in humans [4], because the clinical findings mimic malignancy and inflammatory bowel disease. Basidiobolomycosis should be included in the differential diagnoses of inflammatory bowel disease [6, 10].

Definitive diagnosis requires microbiological culture of the fungus obtained from tissues samples or PCR-based assays to identify *B. ranarum*. Molecular testing for basidiobolomycosis may prove to be the most accurate diagnostic method [1]. For the present case, the authors successfully confirmed the presence of *B. ranarum* using only paraffin-embedded tissue samples.

The incidence of gastrointestinal basidiobolomycosis may be increasing as a result of various environmental and demographic factors [7, 11], with the worldwide distribution attributed to global warming and increased travel within different geographical areas. In conclusion, we report the first case, to the best of our knowledge, of intestinal basidiobolomycosis in a dog.

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