

Pathological and Immunohistochemical Investigation of Naturally Occurring Systemic *Candida albicans* Infection in Dogs

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Abstract: Systemic candidiasis is a rare disease of animals. In this study, the clinical course, and cultural and pathological findings of systemic candidiasis are described and presumed predisposing factors are investigated in 3 dogs. Pathologically, pyogranulomatous lesions in various organs were present in all dogs. Blastospores, pseudohyphae, and true hyphae of *Candida albicans* were observed with periodic acid Schiff, Gomori's methenamine silver, and immunohistochemistry. Administrations of broad-spectrum antibiotics and corticosteroids and, in one dog, parvoviral infection were thought to be predisposing factors leading to opportunistic infection. The combined effect of immunosuppressants and antibiotics might have led to *Candida* colonization and dissemination in these dogs.

Key Words: *Candida albicans*, dog, immunohistochemistry, pathology, systemic candidiasis

Köpeklerde Doğal Sistemik *Candida albicans* Enfeksiyonunun Patolojik ve İmmunohistokimyasal Yönden Değerlendirilmesi

Özet: Hayvanlarda sistemik kandidiazis tanısı güç ve az rastlanılan bir hastalıktır. Çalışmada üç köpekte rastlanılan sistemik kandidiazisin klinik seyri, patolojik bulguları ve kültür sonuçları değerlendirilerek hastalığa yatkınlığı arttıran olası faktörler tartışıldı. Patolojik olarak tüm köpeklerde çeşitli organlarda pyogranüloamatöz lezyonlar saptandı. Periyodik asit Schiff, Gomori metenamin silver ve immunohistokimyasal boyamalarda *Candida albicans*'in blastospor, yalancı ve gerçek hifalarından oluşan gelişim formları görüldü. Geniş spektrumlu antibiyotik kullanımı ve bir olguda rastlanılan parvoviral enfeksiyonun fırsatçı kandida enfeksiyonu için hazırlayıcı olduğu düşünüldü. Muhtemelen bu köpeklerde immun baskılanma ve antibiyotik tedavisi *Candida* kolonizasyonu ve yayılmasına öncülük etmişti.

Anahtar Sözcükler: *Candida albicans*, köpek, immunohistokimya, patoloji, sistemik kandidiazis

Introduction

Candida species are natural inhabitants of the alimentary, genital and upper respiratory tracts of mammals (1). These yeast-like fungi can cause opportunistic infections in animals treated with antibiotics, corticosteroids, cytotoxic agents and immunosuppressive drugs (1-3). In the veterinary literature, occasional skin and intestinal infections caused by *Candida* species have been reported (4,5). Of the

Candida species, *C. albicans* and *C. tropicalis* are the most commonly isolated in animals (6). Although focal infections with *Candida* species are common, there are only a limited number of reports describing disseminated candidiasis (1,5,7,8).

In this study, pathological and microbiological aspects of systemic candidiasis were examined in 3 dogs. In addition, predisposing factors for *Candida* infection were determined and discussed in detail.

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Materials and Methods

Animals and History: Dog 1 was a male, 10-year-old fox terrier. The dog was previously diagnosed with atopic dermatitis and was treated with dexamethosone (0.6 mg/kg) and cefazolin (30 mg/kg) for 1 week followed by concurrent administrations of prednisolone (2 mg/kg) and enrofloxacin (5 mg/kg) for another week. The dog died 15 days after the diagnosis without any sign of improvement. Dog 2 was a male, 12-year-old mixed breed, which was examined due to chronic diarrhea. The dog was treated with prednisolone (2 mg/kg) and oxytetracycline (7 mg/kg) for 1 week. Trimethoprim-sulphamethoxazole (5 mg/kg) was used in place of oxytetracycline for another 12 days. The dog did not respond to therapy and died in 19 days. Dog 3 was a male, 7-month-old mixed breed. The dog showed signs of anorexia, depression, lethargy, vomiting, and fever (40.5 °C) for 3 days followed by hemorrhagic enteritis. The dog was treated as a case of parvoviral enteritis, and given ampicillin (10 mg/kg). However, the dog died 8 days after the onset of the clinical signs.

Pathology: Necropsy of the animals was performed and tissue samples were taken for histopathological examination. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5-7 µm, and stained with hematoxylin and eosin. Selected sections were also stained with periodic acid – Schiff (PAS) and Gomori's methenamine silver (GMS).

C. albicans strain ATCC 26555 was used for polyclonal antibody production as previously described (9). Briefly, New Zealand White rabbits were inoculated 5 times at 4-week intervals with 10^9 CFU of killed *C. albicans* with incomplete Freund's adjuvant. Blood was collected from the rabbit and serum was prepared by standard methods. An immunoperoxidase method was performed as follows: formalin-fixed paraffin-embedded tissue samples were deparaffinized and hydrated through xylene and ethanol series from 100% to 70%. Hydrated samples were rinsed in distilled water and reacted with 3% hydrogen peroxide in methanol for 5 min at room temperature to quench endogenous peroxidase activity. The samples were washed with phosphate-buffered saline (PBS, pH 7.3) and incubated with normal goat serum for 30 min to block non-specific antibody binding. Then the slides were incubated with polyclonal rabbit anti-*C. albicans* antibody diluted 1:100 in PBS for 30 min in a humidified chamber. A commercial streptavidin-biotin peroxidase kit (LSAB2, DAKO A/S) was

used as directed by the manufacturer's manual and the antigen antibody binding was visualized by DAB substrate. Negative control tissue sections were incubated with normal rabbit serum. Previously known (3) *C. albicans* positive tissue sections were used as positive controls.

To detect parvoviral antigen, selected sections of small intestines, mesenteric lymph nodes and spleen were placed on poly-L-lysine coated microscope slides. After 2 h of incubation at 37 °C, the sections were deparaffinized in xylene and washed briefly in PBS. The tissues were digested with 0.1% proteinase K for 10 min at 37 °C. The sections were then washed in PBS for 15 min and then the sections were incubated with rabbit anti-parvovirus serum diluted 1:64 for 1 h at 37 °C, and then washed for 15 min in PBS. The sections were covered with goat anti-rabbit gamma globulin serum with fluorescein isothiocyanate (Sigma) for 45 min at 37 °C. The sections were then washed in PBS for 20 min and mounted with phosphate-buffered glycerol (pH 9.0). The fluorescein antibody reacted tissue sections were examined with a fluorescein microscope (Leica DMLB). As negative controls, tissue sections were incubated with normal rabbit serum, and as positive controls known tissue sections with parvoviral infection were used.

Microbiological examination: Pieces of the tissue samples with lesions were collected in sterile containers and cultured on sheep blood agar, MacConkey's agar and Sabouraud dextrose agar (SDA). Initial plates were inoculated and incubated at 37 °C for 18 to 24 h, and inoculated SDA plates were incubated both at room temperature and 37 °C for 3-7 days and then examined microbiologically. Yeast was identified with a commercial test kit, API 20 C AUX (BioMerieux).

Results

Necropsy findings: Dog 1—There was approximately 100-150 ml of a yellowish brown exudate in the abdominal cavity. The thoracic cavity contained approximately 200 ml of clear yellow fluid that clotted after the thorax was opened. Severe pericarditis and pericardial effusion were present. There were multiple pale yellow to tan, 1-5 mm diameter foci in the epicardium and the adjacent myocardium. Adhesions were present between the myocardium and epicardium. The liver was swollen and friable in consistency, and its capsule was focally thickened.

Dog 2—There was approximately 200-250 ml of yellowish brown exudate in the abdominal cavity. The surfaces of the liver and spleen were covered with a white-grayish pseudomembranous material. The liver and spleen contained 2-6 mm diameter granular lesions irregularly scattered throughout the parenchyma. There was white-reddish discoloration of the renal crest and greenish-white radial streaks in the medulla and cortex of both kidneys. The lung lobes were mottled with numerous 1-3 mm diameter, yellowish-white lesions.

Dog 3—The most striking gross lesions were observed in the duodenum, jejunum and ileum. In the small intestine, the mucosa was congested and there were multifocal petechial hemorrhages in the subserosa. There were grayish-white friable pseudomembranes on the mucosal surfaces of the jejunum and ileum. Brownish-red content covered with mucus was present in the lumen of the intestines. Peyer's patches were round to ovoid and reddish-brown throughout. The liver, lungs, spleen and

kidneys were congested. There were numerous multifocal, 1-7 mm diameter, white-grayish lesions on the peritoneum.

Histopathological findings: Dog 1—Numerous pyogranulomas were observed in the pericardium, epicardium, myocardium, liver, and peritoneum. Inflammatory and necrotic foci were similar in all affected tissues but were more prominent in the pericardium and myocardium. Necrosis was present in the pericardium and adjacent myocardium (Figure 1). There were extensive, irregular, coalescing foci of necrotic cellular debris diffusely infiltrated by macrophages and neutrophils, with fewer giant cells and lymphocytes. Many thrombi were also present in these areas. Microscopically, no inflammatory lesions were observed in the spleen, or thoracic or abdominal lymph nodes.

PAS and GMS stains revealed numerous blastospores (ovoid cells, 3 to 5 mm diameter), pseudohyphae and septate hyphae in the pericardium, myocardium, liver and

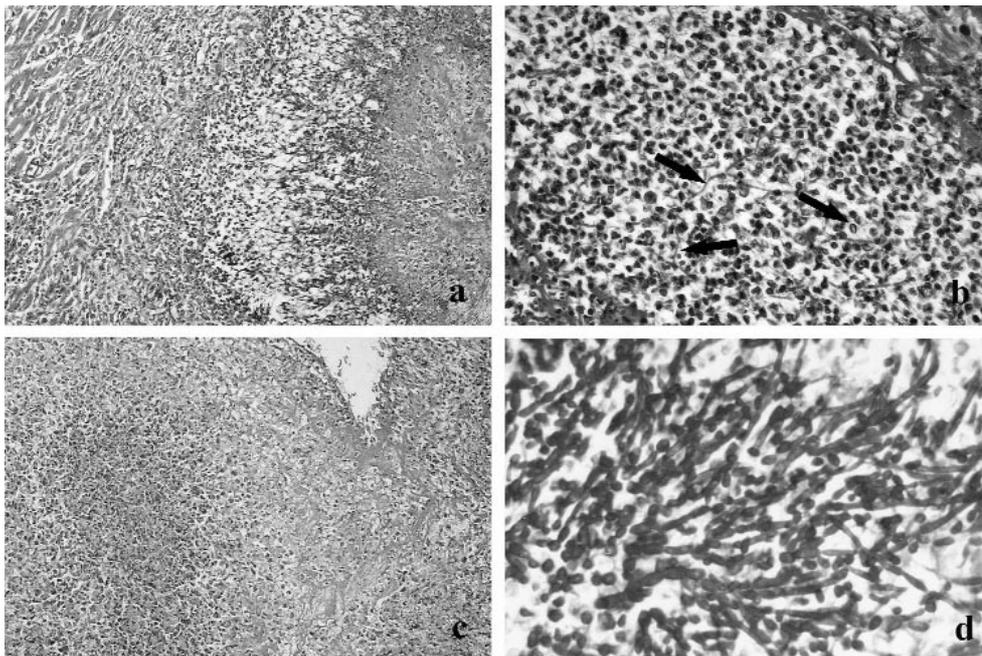


Figure 1. a: Epicardial surface of the myocardium. There is intense pyogranulomatous inflammation with widespread coagulation necrosis and many small developing *Candida* cells invading from the epicardium into the myocardium. Dog 1. H&E. x90. b: Pyogranulomatous pleuritis containing many inflammatory cells and small developmental forms of *Candida* cells. Dog 2. H&E. x180. c: Serosal surface of the jejunum. Pyogranulomatous lesion extending from the serosa to the omentum. Dog 3. H&E. x120. d: Pericardium showing PAS positive blastospores, long filamentous pseudohyphae, and hyphae. Dog 1. PAS. x360.

the capsule of the liver. Many of the organisms in the pericardium were observed to invade the myocardium.

In immunohistochemical preparations, *C. albicans* antigens were detected in the pericardium, epicardium, myocardium, liver, spleen and lymph nodes (Figure 2). The immunohistochemical reaction was more prominent in the pericardium, epicardium and myocardium than in the other organs. All developmental forms of *Candida* were seen by immunohistochemistry in the pericardium, epicardium, myocardium and liver. Only blastospores and budding forms were observed by immunohistochemistry in the spleen and lymph nodes (Figure 2). PAS and GMS were not able to demonstrate the agents in these organs.

Dog 2—A moderate lymphocytic-plasmacytic enteritis was observed in the duodenum and jejunum. Small focal pyogranulomatous inflammation was found in the liver, spleen, kidneys, lungs and peritoneum (Figure 1). These lesions were characterized by necrotic foci surrounded by a moderate infiltration of neutrophils, macrophages,

lymphocytes and some multinucleated cells. Some of these lesions were surrounded by fibrous tissue. The PAS and GMS stains revealed pseudohyphae, septate hyphae and blastospores, often budding and ranging from 3 to 5 μm in diameter in the centers of the pyogranulomatous lesions. Immunohistochemistry revealed numerous *C. albicans* antigens in these lesions (Figure 2).

Dog 3—Two different lesion types were encountered in the small intestines. The first type was characterized by diffuse congestion and focal hemorrhages extending from the serosa through the muscular wall into the submucosa. The villi were either reduced in size or absent. The crypts were dilated and filled with necrotic debris. Some crypt epithelial cells were hyperplastic. In the lamina propria, there was an increase in mononuclear cells. The second type of lesion was characterized by hemorrhagic necrosis with many thrombi and leukocytic infiltration extending from the serosa through the musculature, submucosa and mucosa. Numerous pyogranulomas were observed in the

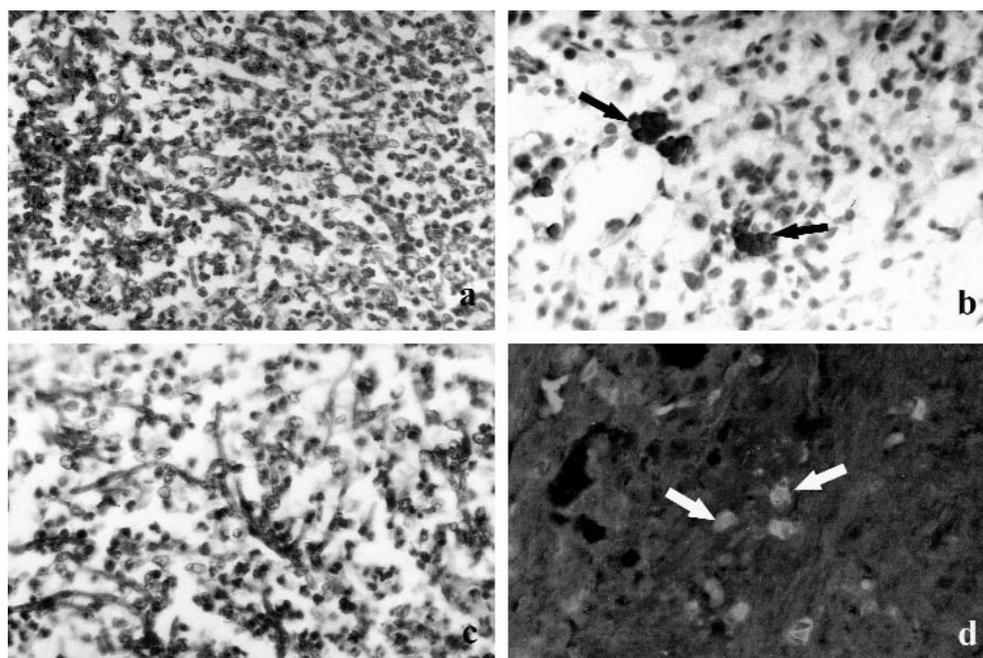


Figure 2. a: Immunohistochemical localizations of *Candida albicans* developmental forms located in the center of pyogranulomatous lesion on the pericardium. Dog 1. IP x360. b: Immunohistochemical demonstration *Candida* cells invading from the epicardium into the myocardium. Dog 1. IP x360. Inset: blastospores in the spleen with no recognizable inflammatory lesion. Dog 1. IP x360. c: Fungal developmental forms and inflammatory cells on the peritoneal surface of the liver. Dog 2. IP x360. d: Weak positive reaction for parvoviral antigens in macrophages in the lamina propria of the small intestine. Fluorescein antibody technique. Dog 3. x420.

peritoneum surrounding the necrotizing foci and in the adjacent segments of omentum (Figure 1). Similar pyogranulomas were also detected in the liver and kidneys. Peyer's patches were severely depleted of lymphocytes. In the spleen and lymph nodes, marked lymphocytolysis and lymphoid depletion were present.

Sections stained with PAS and GMS revealed numerous branching septate hyphae and pseudohyphae with small blastospores (Figure 1). In immunohistochemical sections, *C. albicans* organisms were demonstrated in the lesions in the intestines, liver and kidneys. Weak fluorescein staining for CPV-2 viral antigen was rarely recognized especially in the lymphocytes and macrophages in the lamina propria, and in reticular cells of both the mesenteric lymph nodes and spleen (Figure 2).

Microbiological findings: Smears prepared from the cultures were stained with lactophenol cotton-blue stain. Examination of these smears revealed yeast-like organisms with a diameter ranging from 2.5 to 3.0 µm. A young colony was inoculated onto Sabouraud medium, incubated for 48 h and examined directly. Ovoid or spherical chlamydospores of 8-12 µm were observed. According to the API 20C AUX (BioMerieux) system all the isolates from the 3 dogs were *C. albicans*.

Discussion

Candida species cause opportunistic localized infections in the skin, external ear canal, claw and urinary tract in various animal species (10-12). Systemic candidiasis can also occur and is seen mostly in calves and pigs (10). The causes of *Candida* infections were investigated in previous studies. In a raccoon, oral and nasal candidiasis as a result of adenovirus-induced immunosuppression was reported (13). In cats, infection with *Candida* species has been recognized as a cause of esophagitis and enteritis, especially in association with feline panleukopenia infection (14). In humans, localized and systemic candidiasis is reported due to the administration of broad-spectrum antibiotics, corticosteroids and immunosuppressive drugs (15). Diabetes mellitus, viral infections, and urinary and venous catheters were also reported as risk factors (12,13,16,17). A small number of reports indicate similar predisposing factors in dogs. In one report, systemic candidiasis in a Rottweiler puppy with parvoviral

enteritis was described (7). Multiple pyogranulomatous lesions in multiple organs were observed in that case. Suspected parvoviral infection associated intestinal candidiasis was also reported in a Dalmatian (5). In a diabetic Scottish terrier, administration of broad-spectrum antibiotics and corticosteroids together with urinary and venous catheter applications resulted in disseminated candidiasis (8). In the dogs in the current report, similar predisposing factors are thought to be the probable causes of the development of systemic *C. albicans* infection.

Factors that upset the normal endogenous microflora or disrupt normal mucosal barriers provide a means for *Candida* species to enter the body (6). Administration of broad-spectrum antibiotics is known to alter the normal bacterial flora and allow colonization of *Candida* species (18). Treatment with broad-spectrum antibiotics in all dogs in this report might be the cause of *Candida* outgrowth. However, generally treatment with broad-spectrum antibiotics without developing systemic candidiasis, as in the present case, indicates that some other factors may play a role in the development of systemic candidiasis including individual susceptibility and/or weakened immune system of the dogs due to some other factors such as stress and dietary insufficiency. Although antibiotic treatments in these cases only lasted for 1 to 3 weeks, systemic *Candida* lesions were observed in various organs. This was in accordance with the experimental study conducted by Kennedy and Volz (19). In their report, *C. albicans* was detected in the gut, liver, spleen and kidney 24 h after administration of the agents in penicillin-G treated hamsters. Corticosteroids are known to cause immunosuppression and, like antibiotics, may compromise the gastrointestinal tract microflora (3,8). Corticosteroid treatment in Dogs 1 and 2 in this study might also be predisposing factors for *C. albicans* infection.

CPV-2 infection is reported to be a predisposing factor for development of candidiasis in dogs (5,7). CPV-2 causes lymphocytolysis and epithelial cell necrosis, which may cause immunosuppression and allow secondary agents to invade the underlying tissues (7). In Dog 3, weak fluorescein staining for CPV-2 viral antigen was rarely seen in the lymphocytes of the lamina propria, and in reticular cells of both mesenteric lymph nodes and spleen. Although parvoviral intranuclear inclusions were

not observed in tissue sections, these inclusions are known to be most prevalent late in the incubation period and are not commonly encountered in animals after a period of clinical illness (8). The dog also had hemorrhagic diarrhea with intestinal lesions characteristic of CPV-2. These results, together with the overall pathological findings in this case, suggest that Dog 3 may have been infected with parvoviral enteritis as a primary lesion, leading to necrosis of the mucosa, colonization by *Candida* species, and then secondary systemic candidiasis.

Systemic *Candida* infections mostly affect the kidneys, liver and spleen (2). Involvement of myocardium and pericardium has been reported in experimental studies (2,3). Myocarditis, but not pericarditis, was observed in one case of natural systemic candidiasis (7). In humans, purulent pericarditis due to *Candida* infection has been reported after thoracic surgery (20). In the present study, Dog 1 had severe pyogranulomatous pericarditis and myocarditis and the most severe lesions were in the pericardium. *Candida* agents also invaded the myocardium. The only possible route of infection of these tissues would be hematogenous dissemination.

Diagnosis of *Candida* infection can be made based on the morphology of fungal elements within tissues when typical yeast forms are present (10). However, when hyphal forms predominate it can be difficult to distinguish *Candida* from other filamentous fungi such as *Aspergillus* and *Zygomycetes* (4). *Candida* species can also be diagnosed by culture (21). They can grow well on blood agar and can be isolated often from samples submitted for bacteriological analysis. *Candida* can be present on mucosal surfaces as a commensal organism (2), and therefore isolation of *Candida* by itself in culture does not necessarily indicate an infection. Immunohistochemical

and morphological identification in tissues with lesions may be necessary for final diagnosis (4). In this study, germ-tube formation and buds on malt agar confirmed that the agent in all 3 dogs was *C. albicans*. PAS and GMS revealed blastospores, pseudohyphae and true hyphae of *Candida* agents in tissues with lesions and confirmed the microbiological diagnosis. Immunohistochemistry also specifically identified the organisms as *C. albicans* in tissue sections. In Dog 1, while no agents were detected in the spleen by PAS and GMS, immunohistochemistry was able to show budding cells and blastospores. Therefore, the technique was especially useful in determining the agent in sections where no *Candida* species could be seen with other staining techniques.

In conclusion, disseminated candidiasis in 3 dogs with different clinical illnesses was investigated in this study. The agent was cultured and demonstrated by immunohistochemistry in tissue sections with lesions. Although a tentative diagnosis can be made based on the morphology of fungal elements within tissues and culture, immunohistochemistry was shown to be necessary to detect the infectious agent in all affected tissues. Administrations of antibiotics and corticosteroids as well as parvoviral infection were thought to be predisposing factors for *C. albicans* infection in these cases. Therefore, it is suggested that the possibility of systemic *Candida* infection must be considered when treating dogs with antibiotics and corticosteroids.

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References

1. Greene, C.E., Chandler, F.W.: Candidiasis, torulopsosis, and rhodotorulosis. In: Greene C.E., Infectious Diseases of the Dog and Cat. 2nd Ed., W.B. Saunders Co., Philadelphia, 1998: pp. 414-417.
2. Cole, G.T., Lynn, K.T., Seshan, K.R., Pope, L.M.: Gastrointestinal and systemic candidosis in immunocompromised mice. J. Med. Vet. Mycol., 1989; 27: 363-380.
3. Tunca, R., Haziroglu, R.: Pathological findings of experimental *Candida albicans* infection in guinea pigs. Ankara Üniv. Vet. Fak. Derg., 2004; 51: 133-142.
4. Carrasco, L., Bautista, M.J., de las Mulas, J.M., Jensen, H.E.: Application of enzyme immunohistochemistry for the diagnosis of aspergillosis, candidiasis and zygomycosis in three lovebirds. Avian Dis., 1993; 37: 923-927.
5. Ochiai, K., Valentine, B.A., Altschul, M.: Intestinal candidiasis in a dog. Vet. Rec., 2000; 146: 228-229.
6. Barker, I. K., Dreumel, A.A., Palmer, N.: Pathology of Domestic Animals, 4th edn. K.V.F. Jubb, P.C. Kennedy, N. Palmer. Academic Press, San Diego, 1993: pp 256.

7. Rodriguez, F., Fernandez, A., Espinosa de los Monteros, A., Wohlsein, P., Jensen, H.E.: Acute disseminated candidiasis in a puppy associated with parvoviral infection. *Vet. Rec.*, 1998; 142: 434-436.
8. Heseltine, J.C., Panciera, D.L., Saunders, G.K.: Systemic candidiasis in a dog. *J. Am. Vet. Med. Assoc.*, 2003; 223: 821-824.
9. Andrutis, K.A., Riggle, P.J., Kumamoto, C.A., Tzipori, S.: Intestinal lesions associated with disseminated candidiasis in an experimental animal model. *J. Clin. Microbiol.*, 2000; 38: 2317-2323.
10. Jungerman, P.F., Schwartzman, R.M.: *Veterinary Medical Mycology. Candidiasis*. Lea Febiger, Philadelphia, 1972: pp 61-74.
11. McKellar, Q.A., Rycroft, A., Anderson, L., Love, J.: Otitis externa in a foxhound pack associated with *Candida albicans*. *Vet. Rec.*, 1990; 127: 15-16.
12. Pressler, B.M., Vaden, S.L., Lane, I.F., Cowgill, L.D., Dye, J.A.: *Candida* spp. urinary tract infections in 13 dogs and seven cats: predisposing factors, treatment, and outcome. *J. Am. Anim. Hosp. Assoc.*, 2003; 39: 263-270.
13. Hamir, A.N., Mattson, D.E., Sonn, R.J., Stasko, J., Habecker, P.L.: Concurrent candidiasis, listeriosis and adenovirus infections in a raccoon (*Procyon lotor*). *Vet. Rec.*, 2000; 146: 320-322.
14. Langheinrich, K.A., Nielsen, S.W.: Histopathology of feline panleukopenia: a report of 65 cases. *J. Am. Vet. Med. Assoc.*, 1971; 158: 863-869.
15. Fraser, V.J., Jones, M., Dunkel, J., Storfer, S., Medoff, G., Dunagan, W.C.: Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. *Clin. Infect. Dis.*, 1992; 15: 414-421.
16. Fromtling, R.A., Abruzzo, G.K., Giltinan, D.M.: *Candida tropicalis* infection in normal, diabetic, and neutropenic mice. *J. Clin. Microbiol.*, 1987; 25: 1416-1420.
17. Sitges-Serra, A., Girvent, M.: Catheter-related bloodstream infections. *World J. Surg.*, 1999; 23: 589-595.
18. Ekenna, O., Sherertz, R.J.: Factors affecting colonization and dissemination of *Candida albicans* from the gastrointestinal tract of mice. *Infect. Immun.*, 1987; 55: 1558-1563.
19. Kennedy, M.J., Volz, P.A.: Ecology of *Candida albicans* gut colonization: inhibition of *Candida* adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infect. Immun.*, 1985; 49: 654-663.
20. Schrank, J.H., Dooley, D.P.: Purulent pericarditis caused by *Candida* species: case report and review. *Clin. Infect. Dis.*, 1995; 21: 182-187.
21. Euzeby, J.: The laboratory diagnosis of cutaneous and subcutaneous mycoses in animals. *Folia Vet. Lat.*, 1977; 7: 111-129.