VETERINARSKI ARHIV 74 (2), 165-173, 2004

Atypical disseminated canine histoplasmosis - a case report

Petar Džaja[1], Željko Grabarević[2]*, Silvija Rakočević[3], Andrea Gudan[2], Ana Beck[2], Ozren Smolec[2], and Ružica Sabočanec[2]

[1]*Department of Forensic Veterinary Medicine, Faculty of Veterinary Medicine, University of Zagreb*

[2]*Department of General Pathology and Pathological Morphology, Faculty of Veterinary Medicine, University of Zagreb*

[3] *"GOLDI" Veterinary Station for Small Animals, Zagreb, Croatia*

DŽAJA, P., Ž. GRABAREVIĆ, S. RAKOČEVIĆ, A. GUDAN, A. BECK, O. SMOLEC, R. SABOČANEC: Atypical disseminated canine histoplasmosis - a case report. Vet. arhiv 74, 165-173, 2004.

ABSTRACT

This paper describes a case of disseminated histoplasmosis in a 13-year-old female, mixed-breed dog suffering from severe atopyc dermatitis that was treated with prednisolone. Blood laboratory evaluation showed anaemia, decreased haemoglobin level and decreased PCV. Blood smear examination revealed lymphopenia and neutrofilic left shift. Because of its poor health condition the animal was euthanized and necropsy was performed at our department. Macroscopically, deep haemorrhagic bilateral necrosis on both coxal tubers, miliary necroses dispersed through the liver, severe subcutaneous oedema and diffuse enlargement of spleen and lymph nodes were found. Tissue samples for histopathological examination were stained using the hematoxiline-eosine method, PAS stain and Grocott's methamine silver method for fungi. Histopathological examination of skin lesion, liver, spleen and lymph nodes revealed numerous enlarged histiocytes infiltrated with inztracytoplasmatical oval, 2-4 ěm large, yeast-like bodies. This intramacrophagic yeast was identified as fungus *Histoplasma capsulatum* and to our knowledge this is the first case of histoplasmosis in Croatia. The way of infection was very likely transepidermal through pre-existing skin lesions caused by atopy, because the lungs and intestines were devoid of any sign of histoplasmosis.

Key words: *Histoplasma capsulatum*, disseminated histoplasmosis, dog, Croatia

* Contact address:
Prof. dr. Željko Grabarević, Department of General Pathology and Pathological Morphology, Faculty of Veterinary Medicine, University of Zagreb, 10000 Zagreb, Heinzelova 55, Croatia, Phone: +385 1 2390 313; Fax: +385 1 2441 390; E-mail: zgrabar@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

Histoplasmosis is a disease caused by the diphasic and dimorphic fungus, *Histoplasma capsulatum* (Hc) with a saprophytic, infectious but non-pathogenic mould morphotype and a host-adapted, pathogenic yeast morphotype. There are three variants within the species: Hc *var. capsulatum*, Hc *var. duboisii*, and Hc *var. farciminosum*. The first variant causes classic histoplasmosis, the most common form of histoplasmosis in the world; the second causes African histoplasmosis, and the third causes epizootic lymphangitis of mules and horses (JONES et al., 1997; RETALLACK and WOODS, 1999). The classic form of the disease is globally distributed, but the most affected areas are endemic regions of the U.S.A., especially those bordering the Missouri, Ohio, and Mississippi Rivers, and South America (SELBY et al., 1967; TURNER et al., 1972; SILVA-RIBEIRO et al., 1987; COSTA et al., 1994; DEEPE, 2000; DE MEDEIROS MUNIZ et al., 2001). It should be emphasised that the Hc was also described in Italy (MANTOVANI, 1968), Austria (BAUDER et al., 2000), Australia (MACKIE et al., 1997), and Japan (SANO et al., 2001).

Although this systemic mycosis is described in humans and most mammals, especially in endemic areas, there is no evidence of direct transmission from animals to man, although it appears in animals and humans sharing the same environment. Concerning the clinics, there are two forms of the disease, benign and unapparent, which is characterised by pulmonary nodules, and a fatal, disseminated form which is a disease of the reticuloendothelial system with pulmonary and enteric subtypes (MARDI and STARK, 1980; JONES et al., 1997).

As mentioned above, Hc in the soil exists as a mould (mycelia and asexual spores) while in the mammalian host, because of the higher temperature of the host, it converts in a budding yeast which is essential for the infection (RETALLACK and WOODS, 1999). The most common means of infection is inhalation of the mycelia and microconidia, which become ingested by macrophages and transferred to the local lymph nodes. Subsequently, Hc disseminates to many different organs (DEEPE, 2000). Binding of Hc to monocyte is mediated via the CD 18 family of adhesion-promoting glycoproteins or integrins. After ingestion, Hc controls the intraphagosomal environment by maintaining acidity at pH 6.0-6.5, which

is important because such acidity inhibits the lysosomal acid hydrolases and, equally important, yeasts are still able to acquire iron from transferrin by a process that is dependent upon an acid environment (NEWMAN, 1999). It should be mentioned that the yeast can invade not only the monocytes but also the neutrophils (VANSTEENHOUSE and DENOVO, 1986) as well as eosinophils (CLINKENBEARD et al., 1988).

Therefore, the aim of this report is to present the first recorded case of canine histoplasmosis in Croatia with an atypical course of the disease and route of infection. This finding is also important because of its relevance for veterinary and human public health.

Case report

During March 2003, a euthanized 13-year-old female mixed-breed dog was sectioned at our Department. The animal had a history of atopyc dermatitis which was treated with prednisolone and a low allergy diet. During the last month prior to euthanasia (barbiturate overdose), the animal had been in poor health characterised by lethargy, anorexia and weight loss. Also, bilateral hemorrhagic-necrotical focal dermatitis unresponsive to therapy developed on the coxal tubers. Blood laboratory evaluation showed anaemia (RBC 4.06 x 10^{12} L), decreased haemoglobin level (7.9 g/L), and decreased PCV (25.4%), while examination of the blood smear revealed lymphopenia (5 %) and left shift (20% of unsegmented neutrophilic granulocytes). Liver enzymes (ALT /alanine transaminase/ and AST /aspartate transaminase/) showed no significant changes.

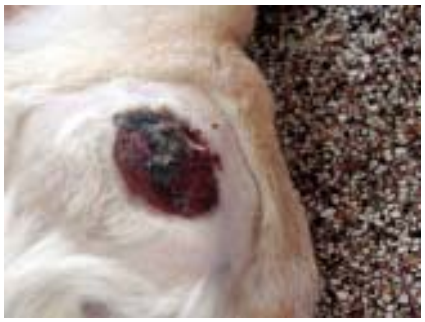

Fig. 1. Deep haemorrhagic necrosis of the skin

Fig. 2. Disseminated necrotic foci and fatty change of the liver

At necropsy, severe, deep, haemorrhagic bilateral necrosis were found on both coxal tubers (Fig. 1). Numerous, miliary, yellowish foci were found dispersed throughout the liver (Fig. 2). All lymph nodes and spleen were diffusely enlarged and severe subcutaneous oedema was observed in the animal. There were no macroscopical changes of the lungs and intestines.

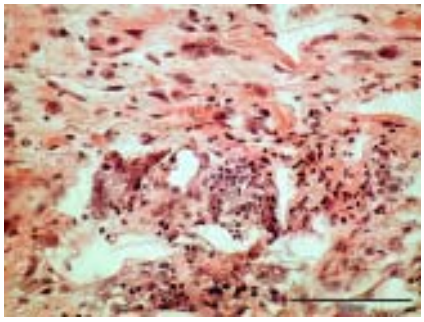

Fig. 3. Histiocytes filled with Hc at the periphery of dermal necrosis. H&E, scale bar = 100 μm

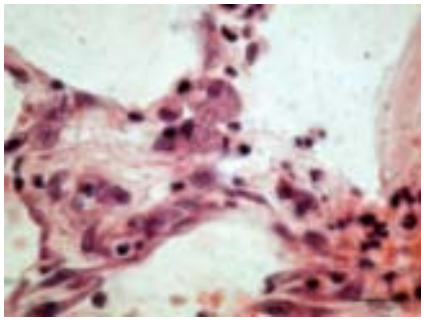

Fig. 3a. Detail of Fig. 3. H&E, scale bar = 20 μm.

During the section, tissue samples of the skin lesion, liver, lung, heart, spleen, kidney, small intestine, and brain were taken for histopathological examination. Samples were fixed in buffered formalin, embedded in paraffin, and cut into 5 μm-thick slices. They were stained using the hematoxilline-eosine (H&E) routine method, PAS stain and Grocott's methamine silver nitrate method for fungi (ARRINGTON, 1994). Histopathological examination of the skin lesion revealed deep haemorrhagic necrosis and severe histiocytosis. Histiocytes were enlarged and infiltrated with intracytoplasmatical oval, 2-4 μm-large, yeast-like bodies. In H&E stained sections the central, basophilic body was surrounded by an unstained zone with a thin cell wall (Figs. 3. and 3a.). This was particularly visible in the PAS stained sections (Fig. 4) in which organisms appear as dark rings. Grocott staining was positive for fungi, i. e. previously described intracytoplasmatical bodies stained black (Figs. 5 and 5a.). In the liver, severe interstitial hepatitis with hepatocytic fatty change and histiocytic interstitial infiltration were found. Macrophages

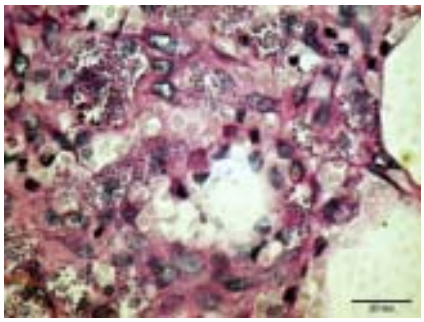

Fig. 4. PAS-staining of the skin lesions with histiocytes filled with Hc. PAS, scale bar = 20 μm.

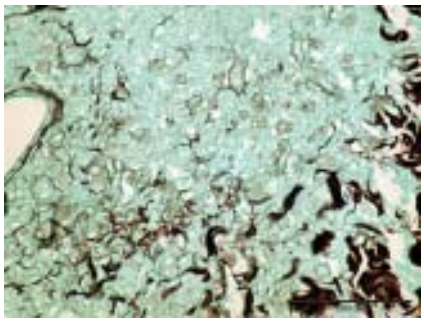

Fig. 5. Grocott staining of skin hystiocytes. Intracytoplasmatical Hc stained black. Grocott, scale bar = 100 μm.

were also filled with yeasts (Fig. 6). Reticular hyperplasia and histiocytosis with intracytoplasmatical fungal bodies were found in the spleen and lymph nodes (Fig. 7). Interestingly, heart, brain, kidneys, and especially lungs and intestines, displayed no pathological lesions which could be connected with previously described changes. The lesions of those organs was in the form of mild reversible degenerations (parenchymatous and hydropic degeneration of the heart and kidney, mild gangliocytic vacuolisation in the brain, mild intestinal mucosal atrophy, and, finally, focal alveolar emphysema and anthracosis of the lungs).

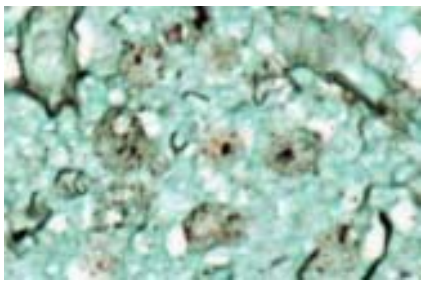

Fig. 5a. Computer-generated detail of Fig. 5. Black stained Hc is clearly visible in the cytoplasms of the histiocytes.

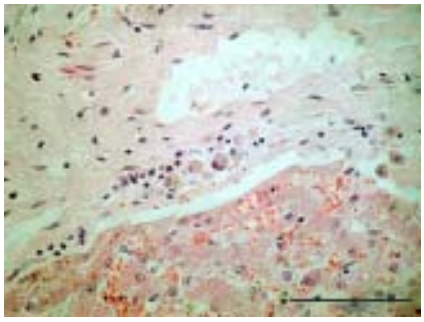

Fig. 6. Severe interstitial hepatitis with histiocytic infiltration and fatty change of the hepatocytes. H&E, scale bar = 100 μm.

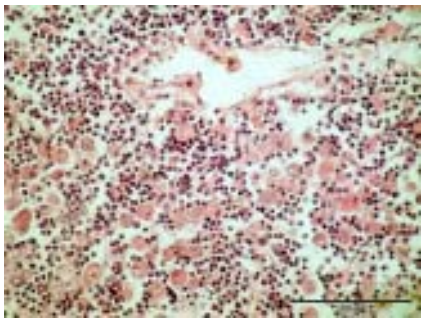

Fig. 7. Spleen histiocytosis. All histocytes invaded with *H. capsulatum*. H&E, scale bar = 100 μm.

Based on complete pathological data, the intramacrophagic yeast was detected as fungus *Histoplasma capsulatum* and the final diagnosis was disseminated histoplasmosis.

Discussion

Concerning the diagnosis of this disease, differentiation from other mycotic and protozoan organisms may be difficult. However, special histological stainings allow positive identification of the fungi, thus protozoan infections like *Leischmania donovani* and *Toxoplasma gondii* which also invade monocytes can be excluded. Although very sensitive PCR methods for diagnostic purposes have been developed (UEDA et al., 2003), it was shown that the Grocott method is almost equally sensitive for Hc identification as PCR (BIALEK et al., 2002). KOWALEWICH et al. (1993) reported a case concerning the identification of Hc in cytological specimens from pleural and peritoneal effusions using the PAS method. Differential diagnosis from other systemic mycoses include blastomycosis (*Blastomyces dermatitidis*) and cryptococcosis (*Cryptococcus neoformans*). However, differentiation is relatively simple because of their different morphology and pathology (LŐPEZ, 2001; JONES et al., 1997).

Our findings are in accord with other literature reports, in which anaemia (DENOVO, 1986; VANSTEENHOUSE and DENOVO, 1986) and skin infection (KAGAWA et al., 1998) was described. However, with regard to the route of the infection, it is interesting to note that we found no lung or

intestine lesions, which means that the inhalatory or enteral means of invasion could probably be eliminated. In our case, Hc probably invade the organism through pre-existing skin lesions. It is well known that immunosuppression caused either by diseases like cancer or AIDS (RETALLACK and WOODS, 1999) or by application of therapeutic immunosuppressive drugs such as prednisolone (CLINKENBEARD et al., 1988; RETALLACK and WOODS, 1999) could induce a fatal disseminated form of the disease. It should also be emphasised that African histoplasmosis has never been diagnosed in this region, while the last case of epizootic lymphangitis of horses was described in the region of former Yugoslavia in 1952 (JUKIĆ, 2003), predominantly in its southern parts (ZAHARIJA, 1978). To our knowledge, and despite a scrupulously performed literature review, this is the first finding of this disease in Croatia, and we have been unable to find any other literature data which describe disseminated histoplasmosis caused by skin lesion infection.

References

ARRINGTON, J. B. (1994): Bacteria, Fungi, and Other Microorganisms. In: Laboratory Methods in Histotechnology (Prophet, E. B., B. Mills, J. B. Arrington, L. H. Sobin, Eds.) American Registry of Pathology, Washington, D. C., pp. 203-234.

BAUDER, B., A. KUBBER-HEIS, T. STEINECK, E.S. KUTTIN, L. KAUFMAN (2000): Granulomatous skin lesions due to histoplasmosis in a badger (*Meles meles*) in Austria. Med. Mycol. 38, 249-253.

BIALEK, R., F. ERNST, K. DIETZ, L. K. NAJVAR, J. KNOBLOCH, J. R. GRAYBILL, G. SCHAUMBURG-LEVER (2002): Comparison of staining methods and a nested PCR assay to detect *Histoplasma capsulatum* in tissue sections. Am. J. Clin. Pathol. 117, 597-603.

CLINKENBEARD, K. D., R. L. COWELL, R. D. TYLER (1988): Identification of Histoplasma organisms in circulating eosinophils of a dog. J. Am. Vet. Med. Assoc. 192, 217-218.

COSTA, E. O., L. S. DINIZ, C. F. NETTO, C. ARRUDA, M. L. DAGLI (1994): Epidemiological study of sporotrichosis and histoplasmosis in captive Latin American wild mammals, Sao Paulo, Brasil. Mycopathologia 125, 19-22.

DEEPE, G. S. Jr. (2000): Immune response to early and late *Histoplasma capsulatum* infections. Curr. Opinion Microbiol. 3, 359-362.

DE MEDEIROS MUNIZ, M., C. V. PIZZINI, J. M. PERALTA, E. REISS, R. M. ZANCOPE-OLIVEIRA (2001): Genetic diversity of *Histoplasma capsulatum* strains

isolated from soil, animals and clinical specimens in Rio de Janeiro State, Brazil, by a PCR-based random amplified polymorphic DNA assay. J. Clin. Microb. 39, 4487-4494.

JONES, T. C., R. D. HUNT, N. W. KING (1997): Veterinary Pathology. Williams and Wilkins, Baltimore, pp. 519-522.

JUKIĆ, B. (2003): Tropske zarazne bolesti životinja. Veterinarski fakultet Sveučilišta u Zagrebu, Zagreb, pp. 297-305.

KAGAWA, Y., S. AOKI, T. IWATOMI, M. YAMAGUCHI, N. MOMIYAMA, K. HIRAYAMA, H. TANIYAMA (1998): Histoplasmosis in the skin and gingiva in a dog. J. Vet. Med. Science, 60, 863-865.

KOWALEWICH, N., E. C. HAWKINS, A. J. SKOWRONEK, F. A. CLEMO (1993): Identification of *Histoplasma capsulatum* organisms in the pleural and peritoneal effusions of a dog. J. Am. Vet. Med. Assoc. 202, 423-426.

LŐPEZ, A. (2001): Respiratory system, Thoracic Cavity, and Pleura. In: Thomson's Special Veterinary Pathology. (McGavin, M. D., W. W. Carlton, J. F. Zachary, Eds.) Mosby, St. Louis, pp. 125-195.

MACKIE, J. T., L. KAUFMAN, D. ELLIS (1997): Confirmed histoplasmosis in an Australian dog. Austral. Vet. J. 75, 362-363.

MANTOVANI, A., A. MAZZONI, L. AJELLO (1968): Histoplasmosis in Italy. I. Isolation of *Histoplasma capsulatum* from dogs in the province of Bologna. Sabouraudia 6, 163-164.

MARDI, M., D. R. STARK (1980): Disseminated canine histoplasmosis. Can. Vet. J. 21, 95-100.

NEWMAN, S. L. (1999): Macrofages in host defense against *Histoplasma capsulatum*. Trends Microbiol. 7, 67-71.

RETALLACK, D. M., J. P. WOODS (1999): Molecular epidemiology, pathogenesis, and genetics of the dimorphic fungus *Histoplasma capsulatum*. Microbes and Infection 1, 817-825.

SANO, A., Y. UEDA, T. INOMATA, M. TAMURA, T. IKEDA, K. KAMEI, A.KIUCHI, Y. MIKAMI, K. NISHIMURA, M. MIYAJI (2001): Two cases of canine histoplasmosis in Japan. Jap. J. Med. Mycol. 42, 229-235.

SELBY, L. A., R. W. MENGES, R. T. HABERMANN (1967): Survey for blastomycosis and histoplasmosis among stray dogs in Arkansas. Am. J. Vet. Res. 28, 345-349.

SILVA-RIBEIRO, V. L., M. F. FERREIRA-DA-CRUZ, B. WANKE, B . G A L V A N O - CASTRO (1987): Canine histoplasmosis in Rio de Janeiro: natural and experimental infections. J. Med. Vet. Mycol. 25, 319-322.

TURNER, C., C. D. SMITH, M. L. FURCOLOW (1972): Frequency of isolation *Histoplasma capsulatum* and *Blastomyces dermatitidis* from dogs in Kentucky. Am. J. Vet. Res. 33, 137-141.

UEDA, Y., A. SANO, M. TAMURA, T. INOMATA, K. KAMEI, K. YOKOYAMA, F. KISHI, J. ITO, Y. MIKAMI, M. MIYAJI, K. NISHIMURA (2003): Diagnosis of histoplasmosis by detection of the internal transcribed spacer region of fungal rRNA gene from a paraffin-embedded skin sample from a dog in Japan. Vet. Microbiol. 94, 219-224.

VANSTEENHOUSE, J. L., R. C. DENOVO (1986): Atypical *Histoplasma capsulatum* infection in a dog. J. Am. Vet. Med. Assoc. 188, 527-528.

ZAHARIJA, I. (1978): Zarazne bolesti domaćih životinja. Školska knjiga, Zagreb, pp. 625-630.

Received: 14 Jula 2003
Accepted: 15 March 2004

DŽAJA, P., Ž. GRABAREVIĆ, S. RAKOČEVIĆ, A. GUDAN, A. BECK, O. SMOLEC, R. SABOČANEC: Atipična diseminirana histoplazmoza psa. Vet. arhiv 74, 165-173, 2004.

SAŽETAK

Članak opisuje pojavu diseminirane histoplazmoze u kuje, križane, stare 13 godina koja je zbog teškog oblika atopičnog dermatitisa bila liječena prednisolonom. Laboratorijskim pretragama krvi utvrđena je anemija, smanjenje količine hemoglobina, smanjenje vrijednosti hematokrita, limfopenija i skretanje krvne slike u lijevo. Zbog lošeg zdravstvenog stanja životinja je eutanazirana te je obavljena razudba. Makroskopski su uočene duboke hemoragične bilateralne nekroze kože u području bočnih kvrga, opsežni edem potkožja i difuzno povećanje slezene i limfnih čvorova. Uzorci tkiva za histopatološku pretragu bili su obojani H&E, PAS i Grocottovom metodom metaminskog srebra za bojanje gljivica. U koži, jetri, slezeni i limfnim čvorovima histopatološki su utvrđeni brojni povećani histiociti s introcitoplazmatskim uklopinama gljivica veličine 2-4 μm, koje morfološki odgovaraju gljivici *Histoplasma capsulatum*. Prema našim spoznajama ovo je prvi opis ove gljivične infekcije u Republici Hrvatskoj. S obzirom na to da u plućima i crijevima nisu utvrđeni znaci histoplazmoze, infekcija je najvjerojatnije nastupila transepidermalno preko već postojećih kožnih lezija uzrokovanih atopijom.

Ključne riječi: *Histoplasma capsulatum*, diseminirana histoplazmoza, pas, Hrvatska