A case of systemic mycosis in a Hovawart dog due to *Candida albicans*

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ABSTRACT: *Candida albicans* is reported as the etiological agent of multi-systemic infections in dogs. A two-year-old female Hovawart dog was presented with marked alteration in its health condition characterised by weakness, fever, anorexia, abdominal pain, cachexy and generalized lymphadenopathy. A radiograph of the abdominal cavity showed several non-specific nodular lesions in the mesentery, ranging in size up to 10 cm in diameter. At necropsy, extensive enlargement of lymph nodes and the presence of numerous whitish to grey nodules of different sizes in several organs were evident. Histopathological examination revealed pyogranulomatous inflammation characterized by large areas of necrosis surrounded by neutrophilic granulocytes, macrophages, multinucleated giant cells, and a variable admixture of lymphocytes and fungi-like organisms in all affected organs. Numerous branching hyphae, subsequently identified by mycological cultivation as *Candida albicans*, were observed. A periodic acid Schiff (PAS) reaction to prove the presence of fungi in tissues was positive. Examination of tissue samples of affected organs using polymerase chain reaction (quantitative Real-Time PCR) and cultivation was negative for the presence of all members of the *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*.

Keywords: candidiasis; pyogranuloma; fungal disease; systemic infection; dog; PCR

*Candida albicans* is a natural inhabitant of the mucous membranes of the genital, alimentary and upper respiratory tract of animals (Greene and Chandler, 1998). This opportunistic yeast-like pathogen can cause localized infection in immunosuppressed patients, most often in those receiving long-term corticosteroid therapy, prolonged antimicrobial therapy, cytotoxic chemotherapy and patients with diabetes mellitus (Jones et al., 1997). Systemic candidiasis is rare in animals and there are scant reports in the literature describing multisystemic infection (Rodriguez et al., 1998; Heseltine et al., 2003; Brown et al., 2005; Kuwamura et al., 2006). The present report details a case of systemic mycotic infection in an apparently immunocompetent dog caused by *Candida albicans*.

Case description

A two-year-old female Hovawart dog was presented with a history of weakness, anaemia, fever and anorexia. Clinical examination revealed an enlargement of the submandibular lymph nodes. A fine needle biopsy was taken from the site of the submandibular lymph nodes. Cytological examination showed the presence of numerous neutrophils and the conclusion was purulent lymphadenitis. Based on the cytological result the dog was treated with *intra muscular* injections of high doses of antibiotics (streptomycin) and vitamin B. According to the owner, the patient showed an improvement in its health in the following two days, with intake of food and water, but still displayed persistent fe-
The dog was examined again 10 days after the initiation of antibiotic therapy and displayed a marked deterioration in its health condition with weakness, fever, anorexia, abdominal pain, cachexy and generalized lymphadenopathy. Radiography of the abdominal cavity showed several non-specific nodular lesions in the mesentery, up to a diameter of 10 cm. At the request of the owner euthanasia was performed.

At necropsy, the dog was in poor nutritional condition characterized by bony prominences with marked loss of subcutaneous and visceral adipose tissue and moderate atrophy of skeletal muscles. The subcutis and mucosal surfaces of the oral cavity and conjunctival sac were slightly icteric. On the ventral part of abdomen and thorax, a subcutaneous oedema was found. The mammary gland was swollen, with irregular nodular lesions visible on the cut surface with the presence of purulent exudate throughout the parenchyma. Extensive enlargement of lymph nodes (submandibular, retropharyngeal, inguinal, mediastinal, tracheobronchial, mesenteric, splenic, hepatic, renal), often with altered architecture due to the presence of numerous whitish to grey nodules of different size, was evident (Figure 1).

In several organs of the abdominal and thoracic cavity (kidneys, adrenal glands, liver, spleen, serosal surfaces of gastrointestinal tract, mesentery, peritoneum, lungs and myocardium) similar nodular formations as in the lymph nodes of different sizes were found (Figure 2). On the cut section of the nodules there were often large necrotic foci with haemorrhages, containing variable amount of purulent exudate. A moderate amount of fibrinopuru-
lent effusion was present in the abdominal cavity. Petechial and ecchymotic haemorrhages were diffusely present on the peritoneum as well as on the serosal surfaces of the intestines and throughout the omentum. Other pathomorphological findings included lung hyperaemia and dilatation of the right heart ventricle.

Subsequently, tissue samples were collected for histopathological examination. Samples were fixed in buffered 10% neutral formalin, dehydrated and embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm, and stained with haematoxylin and eosin (H&E) and the PAS reaction for the detection of fungal elements in tissues. Tissue samples from all the affected organs were taken for mycological and mycobacterial culture and direct detection of selected mycobacterial species (all members of *Mycobacterium tuberculosis* complex, *M. avium* subsp. *avium* and *M. avium* subsp. *hominissuis*) using one-tube nested PCR and quantitative Real-Time PCR.

Histopathological examination revealed pyogranulomatous inflammation in all the examined organs characterized by large areas of necrosis infiltrated and surrounded by neutrophilic granulocytes, epithelioid macrophages and multinucleated giant cells. A variable admixture of lymphocytes and plasma cells were also present at the periphery of pyogranulomas. Throughout the pyogranulomas, there were numerous, pale staining, oval yeast cells and branching hyphae of fungal elements. The PAS reaction to prove the presence of fungi in affected tissues was positive with fungal elements staining deeply eosinophilic (Figure 3).

All samples were cultured onto blood agar, desoxycholate citrate agar (DCA) and Sabouraud’s agar. Incubation of blood agar was performed both aerobically and anaerobically at 37 °C. Sabouraud’s media were incubated aerobically at 25 °C. Isolates of fungi were obtained from all examined tissues and were subcultured onto Sabouraud’s media and rice extract agar. They were identified based on their macro/micromorphology and biochemical properties were tested by Auxacolor 2 (BIO-RAD, France). All isolates were identified as *Candida albicans*.

Direct microscopy of the tissue samples for the presence of acid-fast bacilli was negative. One gram of each tissue sample was homogenized and decontaminated as previously described by Matlova et al. (2003). One hundred microlitres of sample suspension was inoculated into three test tubes with HEYM (one HEYM medium with Mycobactin J) and cultured at 37 °C for two months. Cultures were checked during the first three weeks (to see possible contamination) and subsequently at the end of the incubation period. Cultivation did not reveal the presence of viable mycobacterial species.

DNA from the tissue samples of the affected organs was isolated using a slightly modified Blood and Tissue Kit protocol (Qiagen, Hilden, Germany) as previously described by Slana et al. (2010). Isolated DNA was examined using one-tube nested PCR (Malamite, Moravske Prusy, Czech Republic) and by two independent quantitative Real-Time PCRs (Slana et al., 2008, 2010). Examination for the presence of the specific insertion sequence IS6110 for all eight members of *Mycobacterium tuberculosis*...
complex (M. tuberculosis, M. bovis, M. africanum, M. canettii, M. microti, M. pinnipedi, M. bovis BCG, M. caprae) was negative. Detection of the insertion sequences (IS901 and IS1245) specific for Mycobacterium avium was also negative. Based on the results of histopathological findings and mycological cultivation, systemic mycosis due to Candida albicans was determined to be the final diagnosis.

**DISCUSSION AND CONCLUSIONS**

Candidiasis mainly affects mucosal surfaces. Under normal conditions, many potentially pathogenic fungi do not cause systemic infection. This occurs mainly due to immunosuppression caused by iatrogenic manipulations (long-term administration of corticosteroids, antibiotics and cytotoxic drugs) or in patients with diabetes mellitus (Jones et al., 1997). There are a limited number of cases in the literature reporting systemic candidiasis. The disease usually occurs as a result of various underlying immunosuppressive processes (Holoymoen et al., 1982; Clercx et al., 1996; Chen et al., 2002; Rogers et al., 2009). Systemic candidiasis has been described in dogs previously treated with corticosteroids and antibiotics (Heseltine et al., 2003; Tunca et al., 2006). It has also been induced artificially, with the administration of a challenge dose of Candida albicans in dogs that were made neutropenic by cyclophosphamide (Ruteh et al., 1978; Ehrensaft et al., 1979; Weber et al., 1985; Khosravi et al., 2009). According to one study, paroviral infection in a puppy can also be linked to this condition (Rodriguez et al., 1998). To our knowledge, there are only three reports in the literature documenting systemic candidiasis in dogs, without the presence of an underlying condition (Brown et al., 2005; Kuwamura et al., 2006; Chen et al., 2008). In the present case, there was no evidence of pathological lesions in the oral cavity, upper respiratory tract or genito-urinary tract. There was no history of penetrating wounds or trauma and the dog showed no clinical signs of underlying disease, e.g., infections, metabolic disorders, malnutrition or neoplastic disease. There was also no history of immunosuppressive drug therapy and the dog was fully vaccinated. Therefore, the precise pathogenesis of systemic candidiasis in the dog and the initial site of the infection in this case remain unknown.

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