Epitheliotropic cutaneous lymphoma (*mycosis fungoides*) with formation of nodal and distant metastases in a dog: a case report

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ABSTRACT: The presented case describes an interesting manifestation of epitheliotropic cutaneous lymphoma with formation of nodal and distant metastases in an 8-year-old cocker spaniel. Cutaneous lesions included multiple hypotrichous to alopetic foci, scales, erythematous plaques and multiple cutaneous nodules, often with superficial ulceration. The lesions were present predominantly on the neck, thorax, abdomen and hind legs. Clinically, the dog showed lethargy and there was an inappetence and a mild dyspnoe. Subsequent findings were generalized lymphadenopathy, fever, pallor of mucous membranes and tachycardia. Smear impression of cutaneous nodules contained degenerated neutrophils with phagocytized cocci and macrophages. Cytological examination of nodules (FNA) showed a predominantly round cell population, with a compound of histiocytoid cells mixed with cells of inflammatory infiltration. Histopathological examination of the skin was performed. There was infiltrate of large neoplastic round cells in the superficial and deep dermis, morphologically resembling histiocytes. In some tissue sections the neoplastic infiltrate was present only in the superficial dermis, composed of medium-sized lymphocytes with hyperchromatic round, oval to indented nuclei 1.5 red cells in diameter and a small amount of eosinophilic cytoplasm. Focal exulceration, formation of Pautrier's microabscesses in epidermis, and in some sections subepidermal and intraepidermal vesiculopustules and intraepidermal vesicles were present. Neoplastic infiltrate was CD3, CD18 and vimentin positive. Examination for CD79 and CD117 was negative. MHC II positivity was found only focally in cells of inflammatory infiltration in superficial dermis. Diagnosis of epitheliotropic cutaneous lymphoma (mycosis fungoides) was carried out. The response to the therapy of the disease was poor and the dog died two months after diagnosis. Necropsy revealed generalized lymphadenopathy, several white, fat-like nodules in heart muscle, lungs, esophagus and stomach, and mild hepatomegaly and splenomegaly. Multiple white disseminated foci were found in the spleen. Histopathological examination showed round cell, CD3 positive neoplastic infiltrate in heart, lungs, spleen, liver, lymph nodes, esophagus and stomach, morphologically corresponding with neoplastic infiltrate found in skin.

Keywords: cutaneous lymphoma; mycosis fungoides; immunohistochemistry; metastases

Epitheliotropic cutaneous lymphoma numbers among the tumors rarely diagnosed in dogs. Its incidence together with non-epitheliotropic cutaneous lymphoma accounts for 1% of all cutaneous neoplasia occurring in dogs (Gross et al., 2005). The tumor is found in aging dogs (mean age 9–12 years), and cocker spaniels and poodle breeds have a particular predisposition (Moore and Olivry, 1994). Immunophenotypically it is a T-cell lymphoma (Moore et al., 1994). It occurs in three forms – *mycosis fungoides*, pagetoid reticulosis and Sezary syndrome (Fontaine et al., 2009). Epitheliotropic cutaneous lymphoma is macroscopically markedly pleomorphous, and on the basis of clinical manifestation several forms can be distinguished (Moore and Olivry, 1994; Fontaine et al., 2009), which can be accompanied by the formation of nodal and distant metastases (Moore and Olivry, 1994; Hall, 2004). Microscopically the disease is characterized by the formation of Pautrier's microabscesses in epider-

mis and neoplastic infiltrate in dermis (Moore and Olivry, 1994).

Pagetoid reticulosis occurs in the localized (Woringer-Kolopp) and generalized form (Ketron-Goodman). Macroscopically, pagetoid reticulosis is characterized by the presence of similar cutaneous changes as in mycosis fungoides, while the microscopically neoplastic infltrate is characterized by prominent epitheliotropism. In dogs, the localized form (Woringer-Kolopp) occurs very rarely and the disease has slow progression. Generalized pagetoid reticulosis (Ketron-Goodman) is currently classified as a subtype of mycosis fungoides (Fontaine et al., 2009).

Sezary syndrome is characterized by the presence of similar cutaneous changes as in mycosis fungoides, and the presence of tumor cells in blood. The disease is also characterized by intensive pruritus (Thrall et al., 1984; Foster et al., 1997; Fontaine et al., 2009).

Case report

An 8-year-old male cocker spaniel dog was presented to the clinic with an anamnesis of cutaneous problems, which had lasted for 2.5 months, and which included hypotrichous to alopetic foci, scales, erythematous plaques, multiple cutaneous nodules and intensive pruritus. On the surface of some nodules there was exulceration and the presence of crusts. The predominant localization of skin changes was on the neck, thorax, abdomen and hind legs. Other findings were generalized lymphadenopathy, fever, pallor of mucous membranes, dyspnoe, tachycardia and painful palpation of epigastrium. X-ray examination showed pleural effusion, mass in the cranial mediastinum, mild hepatomegaly and a mass of soft tissue opacity in the caudal retroperitoneum. USG and thoracocentesis were not performed by request of the owner. Initial laboratory examination included assesment of complete blood count (CBC) and biochemical profiling. Mild normocytic, normochromic, nonregenerative anemia, consistent with anemia of chronic disease, hypercalcemia and increased levels of AST and LDH, were present. Smear impressions were made from cutaneous lesions. Only degenerated neutrophils with phagocytized cocci and macrophages were found. Fine needle aspiration (FNA) of cutaneous nodules and prescapular lymph nodes was performed. Assesment of the aspirates showed a predominantly round cell population, morphologically resembling histiocytes. Biopsy of the lesions was performed under local anesthesia and samples were sent for histopathological examination.

Histopathological examination of the skin was performed. The samples were fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 μm, and stained with haematoxylin and eosin (HE). Focal exulcerations of epidermis with neutrophilic inflammatory reaction, orthokeratotic hyperkeratosis, irregular acanthosis and spongiosis were observed. Separation of epidermis and dermis with formation of subepidermal vesicles and vesiculopustules (vesicles focally also with intraepidermal localization) were seen in some sections (Figure 1). In the vesiculopustules, mixed infiltration was composed of neutrophils, well differentiated lymphocytes and macrophages. Focally there was necrosis of superficial epidermis (epidermis forming top wall of lesions). Further findings were intraepidermal cellular accumulations, morphologically corresponding to Pautrier's

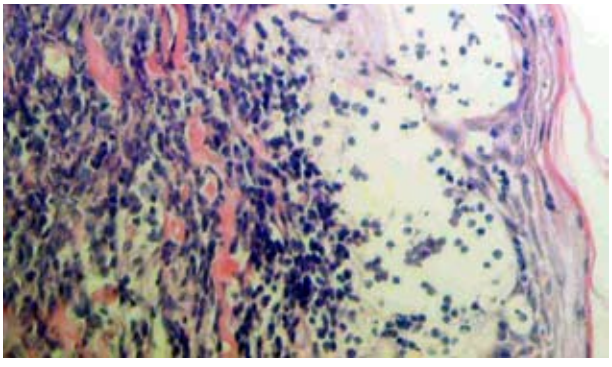

Figure 1. Subepidermal vesiculopustules; HE, 100×

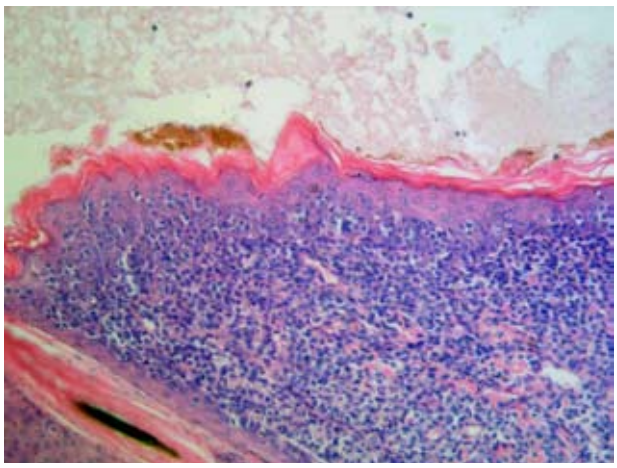

Figure 2. Skin, tumor infiltrate in dermis, Pautrier's microabscesses in the epidermis; HE, 200×

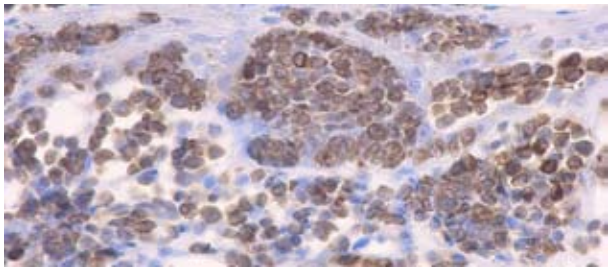

Figure 3. Pautrier's microabscesses, CD3 positivity; 400×

microabscesses and formation of serocellular crusts. In dermis and subcutis, there was diffuse, monomorphous, large round cell neoplastic infiltrate, morphologically resembling histiocytes. In the superficial dermis focally the cells were mixed with cells of pleomorphous inflammatory infiltration. Nuclei of neoplastic cells were large, round to oval, some nuclei were indented with finely to coarsely granulated chromatin and prominent nucleoli (1–3), while the cytoplasm was eosinophilic. Mitotic figures were frequent and often atypical. In some sections there was monomorphous neoplastic infiltrate only in the superficial dermis with the composition of medium-sized lymphocytes with round, oval to indented hyperchromatic nuclei 1.5 red cells in diameter with a small amount of eosinophilic cytoplasm. Pautrier's microabscesses were also present in the epidermis (Figures 2 and 3) and there was considerable infiltration of neoplastic cells to folicullar epithelium (Figure 4). Sebaceous and apocrine glands were massively infiltrated by neoplastic cells and the tumor showed infiltrative growth. Immunohistochemical examination was performed using monoclonal mouse anti-human CD79 (DAKO, dilution 1 : 25), polyclonal rabbit anti-human CD3 (DAKO, RTU), monoclonal anti-dog CD18 (P.F.Moore/dilution 1 : 10), polyclonal rabbit anti-human CD117 (DAKO/dilution 1 : 100), monoclonal mouse anti-human vimentin (DAKO/RTU) and monoclonal mouse anti-human MHC-II (DAKO, dilution 1 : 50) antibodies. For the detection of binding of primary antibody the EnVision (DAKO) detection system was used. DAB (Fluka) was used for visualization of the reaction. The samples were subsequently counterstained with Gill's hematoxylin. The cells showed positivity for CD3 and vimentin, variable positivity for CD18, focal positivity for MHC II of cells of mixed inflammatory reaction and negativity for CD79 and CD117. On the basis of performed examinations a diagnosis of epitheliotropic cutaneous lymphoma (mycosis fungoides) was made.

The therapy consisted of oral administration of prednisolone at a dose of 2 mg/kg SID, amoxicillin clavulanate 25 mg/kg BID, because of possible secondary infection, famotidine 1 mg/kg SID – as a prophylaxis against corticosteroid-induced gastroduodenal ulceration, furosemide 1 mg/kg SID and the local desinfection of cutaneous lesions. The response to the therapy was poor. The dog died two months after initial presentation to the clinic and necropsy was performed. Apart from the described cutaneous lesions (Figure 5) multiple nodules had formed in the mucocutaneous junctions of the oral cavity and annus and multiple nodules in the skeletal muscles of the abdominal

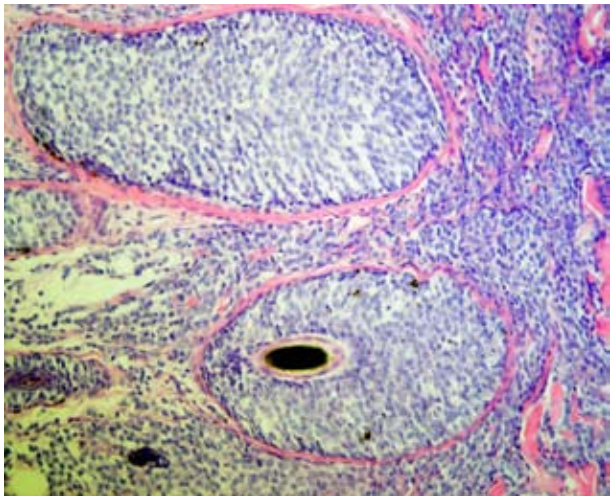

Figure 4. Infiltration of the wall of the hair folicules; HE, 200×

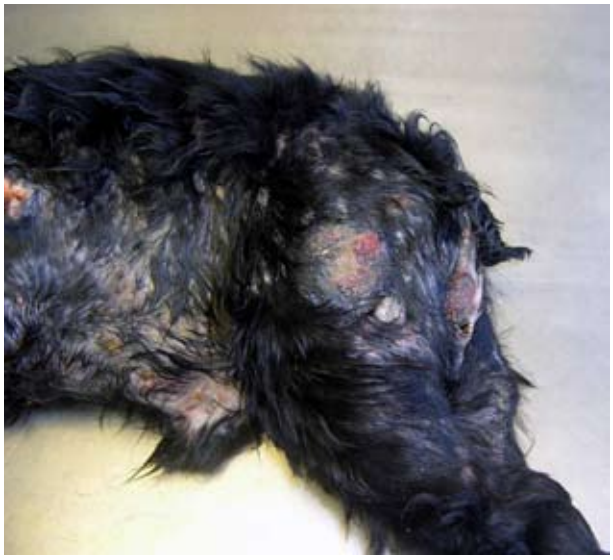

Figure 5. Epitheliotropic lymphoma, cutaneous hypotrichous to alopetic areas, skin nodules

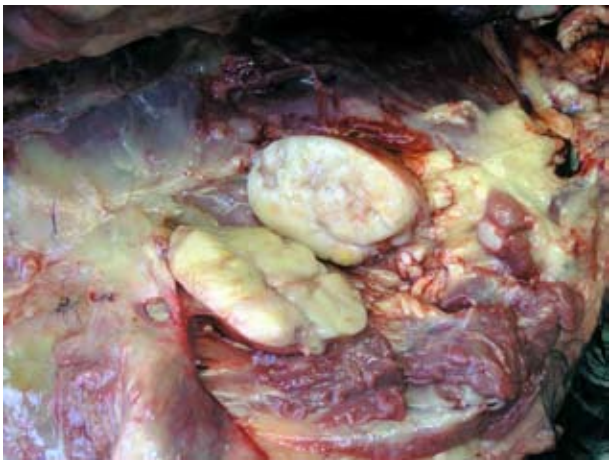

Figure 6. Enlargement of axillar lymph node (metastasis)

cavity associated with cutaneous nodules. There was generalized enlargement of lymph nodes, particullary prescapular, axillar, superficial inguinal and sternal lymph nodes. The surface of a cut section of the lymph nodes had a fat-like appearance (Figure 6). Several white fat-like nodules in the wall of esophagus, stomach, in lungs and heart muscle were found. One gastric nodule was superficially ulcerated (Figure 7). Further findings were mild hepatomegaly and splenomegaly and multiple white disseminated foci in the spleen. Histopathological and immunohistochemical examinations were performed using antibodies against CD3, CD79 and vimentin. There was neoplastic infiltrate in lymph nodes, heart muscle, lungs, esophagus, stomach, liver and spleen morphologically corresponding to the neoplastic infliltrate found in skin. The samples of lymph nodes, liver and spleen revealed diffuse neoplastic infiltrate effacing their morphological structure. In the stomach and esophagus,

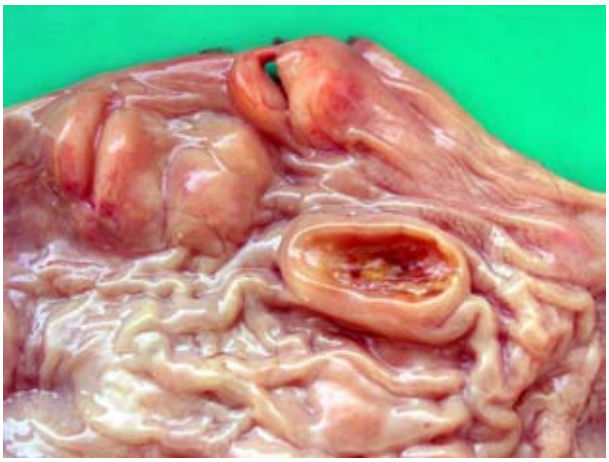

Figure 7. Metastasis in the gastric wall, one nodule with ulceration

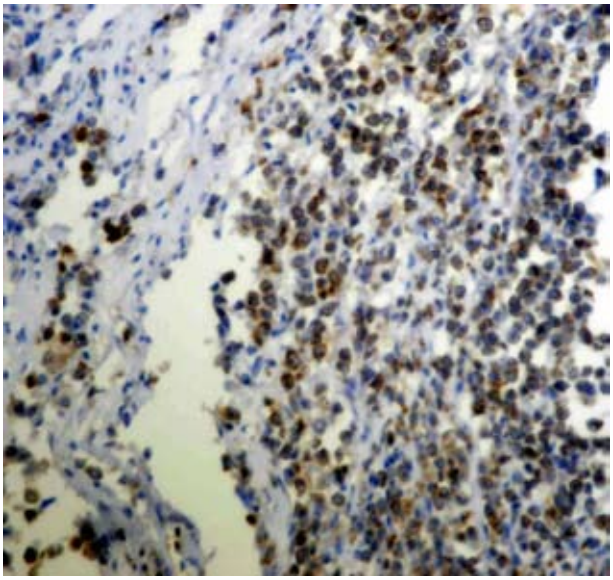

Figure 8. CD3 positive metastasis in the axillary lymph node; 200×

there was nodular neoplastic infiltrate localized in mucosa and submucosa, while the lung parenchyma also showed nodular infiltration; in the liver nodular to diffuse neoplastic infiltrate was present. In the samples taken from kidneys, pancreas and brain there was no neoplastic infiltration. Immunohistochemical examination showed positivity for CD3 (Figure 8) and vimentin, CD79 analysis was negative. On the basis of performed examinations a diagnosis of epitheliotropic cutaneous lymphoma *(mycosis fungoides)* with formation of nodal and distant metastases was made.

DISCUSSION

The presented case describes an interesting manifestation of epitheliotropic cutaneous lymphoma *(mycosis fungoides)*. Cutaneous lesions are generally markedly pleomorphous, characterized by exfoliative erythroderma, multiple patches, plaques and skin nodules. A mucocutaneous form involving oral mucosa is also described (Moore and Olivry, 1994; Fontaine et al., 2009). A variant of mycosis fungoides described as d'embleé, characterized by the presence of skin nodules without previous formation of skin patches and plaques has also been described (Gross et al., 2005), while the coexistence of different skin lesions has also been reported (Fontaine et al., 2009). In this case the changes seen macroscopically corresponded with this general description of the disease. The phase of skin patches and plaques is mi-

croscopically characterized by neoplastic infiltration of small and medium-sized lymphocytes with hyperchromatic nuclei, which have irregular borders. In the stage of skin nodules (tumor stage) neoplastic infiltration composed of cells resembling histiocytes has been encountered (Moore and Olivry, 1994). In this report neoplastic infiltration corresponded with the stages of patches, plaques and cutaneous nodules. The formation of subepidermal vesicles and vesiculopustules and separation of epidermis and dermis was interesting. The cause of these lesions is unknown, and we cannot rule out mechanical injury of the basement membrane due to intensive pruritus. The probable cause of formation of intraepidermal vesicles is spongiosis with subsequent alteration of intercellular junctions.

Neoplastic infiltrate is composed in the majority of cases of CD3+/CD8+ T-lymphocytes, while in some of the presented cases immunophenotype CD3+/CD4–/CD8– is found (Moore et al., 1994). In the case of cutaneous lymphoma immunohistochemical examination is usually necessary, due to the morphological resemblance of this neoplasia with other round cell cutaneous tumors (Gross et al., 2005). In our case the positivity for CD3 confirmed a T-cell immunophenotype. In tissues processed with the paraffin technique further typing (differentiation of CD4 and CD8 T-lymphocyes) is impossible (Gross et al., 2005). Antibodies for this typing are available only for frozen sections (Moore et al., 1994). In cutaneous lymphoma hypercalcemia has been described, which arises due to the production of parathormone related peptide (PTH-rP) by neoplastic cells (Bhang et al., 2006). In our case hypercalcemia was proven by biochemical examination. The presented case is also interesting due to the formation of multiple nodal and distant metastases. Epitheliotropic cutaneous lymphoma is generally classed among the diseases with rare and late formation of metastasis (Czach et al., 2000; Hall, 2004). Distant metastases in different organs, such as liver, spleen, kidneys, CNS, lungs and diaphragm are described (Moore et al., 1994; Czach at al., 2000; Bennett at al., 2005). The formation of distant metastases is described in dogs with mycosis fungoides and Sezary syndrome (Fontaine et al., 2009). Sezary syndrome is characterized by, as well as skin lesions, the presence of neoplastic cells with an atypical cerebriform appearance of the nucleus in peripheral blood (Sezary cells) (Thrall et al., 1994; Foster et al., 1997). In our case Sezary cells were not present.

Several treatments can be used for epitheliotropic lymphoma, such as peroral differentiating agents, topical therapies, radiation therapy, and single-agent or polychemotherapy (Apisarnthanarax et al., 2002). Usually treatment is based on standard chemotherapy regimens used for other lymphoproliferative diseases or is derived from treatment of human epitheliotropic lymphoma (Tzannes et al., 2008). The therapy is only palliative and aims to reduce pruritus, the severity of lesions and associated pain (Guaguere et al., 2008). In some cases local therapy can be beneficial. In extremely localized lesions which are rare in dog radical surgery, radiotherapy, topical glucocorticoids, topical retinoids or mechlorethamine can be used (Hoppe et al., 1987; Apisarnthanarax et al., 2002; Burg and Dummer, 2000). Mechlorethamine is no longer recommended because of its cancerogenic effect (Guaguere et al., 2008). Prednisolone can be administered as a sole agent but mostly it is combined with other products (Scott et al., 2001) like cyclophosphamide and vincristine (COP protocol), while cytosine arabinoside can also be added to this regimen. Other drugs used for systemic combination chemotherapy are azathioprine, chlorambucil, L-asparginase, and doxorubicin (Guaguere et al., 2008). Liposome-encapsulated doxorubicin or PEG-asparaginase is better at penetrating into tumors and has lower systemic toxicity (Tzannes et al., 2008). Synthetic retinoids (isotretinoin and etretinate) can be used alone or in combination with glucocorticoids. In dogs, retinoids are usually well tolerated (White et al., 1993). Lomustin is now the treatment of choice, and can be used alone or in combination with glucocorticoids or as part of a polychemotherapy regimen (Moore et al., 1999). Dacarbazine was used in one case of a dog with a solitary cutaneous epitheliotropic lymphoma lesion and regional lymph node involvement, and complete remission was achieved (Lemarie and Eddlestone, 1997). Recent studies report the use of recombinant human interferon alfa-2a in the treatment of mycosis fungoides (Tzannes et al., 2008). This can be used as a sole agent or in polychemotherapy regimens. It is considered one of the most effective single agents for treatment of *mycosis fungoides* in human medicine (Olsen, 2003). Systemic therapy can be combined with localized radiation but the extent of the lesions has to be considered. In large lesions there is significant risk of deep-tissue side effects from hypofractionated megavoltage radiotherapy (Tzannes et al., 2008). The prognosis for

mycosis fungoides is poor, and the average survival time varies from several months to two years after diagnosis (Fontaine et al., 2009).

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