Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 20 No. 3 Ser. No. 68 2019

# IRANIAN JOURNAL OF VETERINARY RESEARCH

**Scientific Report**

# Pemphigus erythematosus and cutaneous epitheliotropic lymphoma in a Labrador Retriever dog

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(Received 23 May 2018; revised version 29 Jan 2019; accepted 4 Feb 2019)

## Abstract

**Background:** Paraneoplastic pemphigus is an autoimmune blistering skin disease associated with concurrent neoplasia that is rarely observed in veterinary medicine. **Case description:** This case report presents a ten-year-old female Labrador Retriever dog with generalized seborrhea and alopecic, crusting and ulcerative lesions in the periocular and perioral regions, the lips and the groins. **Findings/treatment and outcome:** Hematology analysis showed a marked increase in the number of lymphocytes, while the rest of the values were normal. Skin biopsies were collected and the histological findings were consistent with pemphigus in association with an epitheliotropic lymphoma. Immunohistochemical analysis was performed. There was deposition of immunoglobulin G (IgG) in the basement membrane and in the intercellular space that corresponded with an immunostaining pattern characteristic of pemphigus erythematosus (PE). The lymphoma was positive to CD3 cells and was classified as a T-cell epitheliotropic lymphoma. **Conclusion:** To our knowledge, this is the first case of PE and cutaneous lymphoma coexisting in a dog, expanding the list of associations between immune-mediated diseases and cancer in dogs, and providing support to the eventual connection between autoimmunity and neoplasia in this species.

**Key words:** Autoimmunity, Dog, Epitheliotropic lymphoma, Pemphigus, Skin

## Introduction

Pemphigus can be classified into four types: pemphigus vulgaris (PV), pemphigus foliaceus (PF), pemphigus erythematosus (PE), and pemphigus vegetans (Pveg). Among these types it should be highlighted PE, which is thought to be a rare variant of PF characterized as either a benign form of PF or a crossover syndrome between PF and systemic lupus erythematosus. The lesions of PE are confined to the face, and consist of symmetric crusting, exudation and alopecia, and a characteristic depigmentation of the dorsal muzzle and planum nasale (Gross *et al*., 2005). This paper describes cutaneous lesions consistent with PE, in association with epitheliotropic lymphoma in a dog.

## Case description

A ten-year-old female Labrador Retriever dog was presented with generalized seborrhea and erythematous lesions affecting the face, the external ear canals, the trunk, and the extremities. In addition, alopecia and crusting lesions were described in the axillae and groins (Fig. 1), as well as in the periocular, perioral regions and along the lips (Fig. 2). Erosions, evolving to ulcers in some cases, were seen in the oral cavity, mainly within the gingiva. A hematology analysis was performed. All parameters were within the reference range, except lymphocytes, with an abnormally high value of 5.27 $\times 10^3/\mu L$ (Table 1). Serum biochemistry parameters were within normal limits (Table 2).

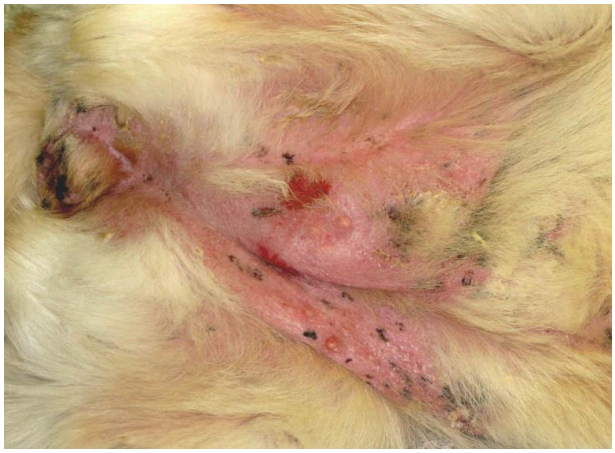

**Fig. 1:** Groin; dog. Alopecia, crusting and erosive lesions in skin

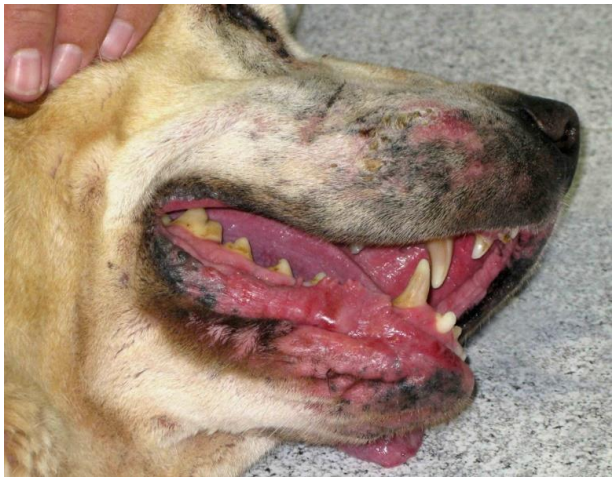

**Fig. 2:** Face; dog. Alopecic and crusting lesions in the perioral skin, and multifocal erosions in the lips

**Table 1:** Hematological results of the dog with cutaneous lesions

| Test | Value | Reference |
|---|---|---|
| Neutrophils | 3.43 $\times10^3/\mu L$ | 2.80-10.50 $\times10^3/\mu L$ |
| Lymphocytes | 5.27 $\times10^3/\mu L$ | 0.50-4.90 $\times10^3/\mu L$ |
| Eosinophils | 0.17 $\times10^3/\mu L$ | 0-1.3 $\times10^3/\mu L$ |
| RBC | 6.1 $\times10^6/\mu L$ | 5.5-8.5 $\times10^6/\mu L$ |
| HGB | 13.0 g/dl | 12-18 g/dL |
| HCT | 41.4 % | 37.0-55.0 % |
| MCV | 74.6 fL | 60-77 fL |
| MCH | 20.0 pg | 19.5-24.5 pg |
| MCHC | 33 g/dl | 30.0-36.9 g/dl |
| PLT | 302 $\times10^3/\mu L$ | 150-900 $\times10^3/\mu L$ |

RBC: Red blood cells, HGB: Haemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, and PLT: Platelets

**Table 2:** Serum biochemistry of the dog with cutaneous lesions

| Test | Value | Reference |
|---|---|---|
| GLU | 129 mg/dL | 74-143 mg/dL |
| CREA | 1.2 mg/dL | 0.5-1.8 mg/dL |
| BUN | 15 mg/dL | 7-27 mg/dL |
| TP | 6.7 g/dL | 5.2-8.2 g/dL |
| ALB | 3.6 g/dL | 2.3-4.0 g/dL |
| GLOB | 3.1 g/dL | 2.5-4.5 g/dL |
| ALT | 88 U/L | 10-125 U/L |
| ALP | 48 U/L | 23-212 U/L |

GLU: Glucose, CREA: Creatinine, BUN: Urea nitrogen, TP: Total protein, ALB: Albumin, GLOB: Globulin, ALT: Alanine aminotransferase, and ALP: Alkaline phosphatase

Skin biopsies from trunk, muzzle skin and oral mucosa were taken with a 6 mm punch biopsy and submitted to our laboratory. Histologic evaluation of skin lesions revealed severe changes involving both the dermis and the epidermis. Thus, a neoplastic infiltrate composed of round cells with a lymphoid appearance and arranged in a lichenoid pattern was seen (Figs. 3A-B). These cells presented a scant clear cytoplasm and a large round central nucleus, with one or two prominent basophilic nucleoli. Anisokaryosis and anisocytosis were mild. The mitotic count was low (approximately 2 mitoses per high-power field). In addition, intragranular pustules of approximately 1-2 mm were occasionally seen in the epidermis of the muzzle skin sample. Pustules contained some acantholytic keratinocytes and numerous non-degenerate neutrophils. Furthermore, subjacent to these pustules an interface dermatitis containing mature lymphocytes was observed (Fig. 4). One of these pustules was partially eroded and covered by a small serocellular crust. The oral skin sample showed areas of erosion of the epithelium with a mild suppurative exudate. The preliminary diagnosis was generalized epitheliotropic lymphoma and pemphigus affecting the skin on the face.

To better classify the type of cutaneous lymphoma an immunohistochemical analysis was performed. The

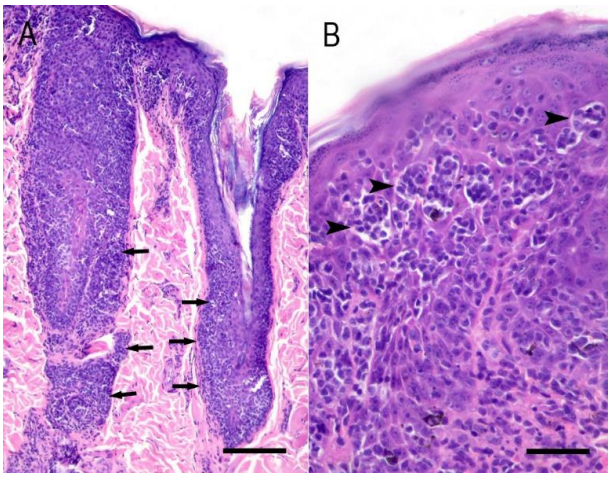


**Fig. 3:** Cutaneous epitheliotropic lymphoma. **A**: Skin biopsy specimen with an epitheliotropic lymphoma. Neoplastic cell infiltrate with a lymphoid appearance and arranged in a lichenoid pattern showing marked epitheliotropism (arrows) (H&E, scale bar, 100 μm), and **B**: The neoplastic cells are round, with lymphoid appearance, and form nests between the keratinocytes in the epidermis (arrowheads) (H&E, scale bar, 50 μm)

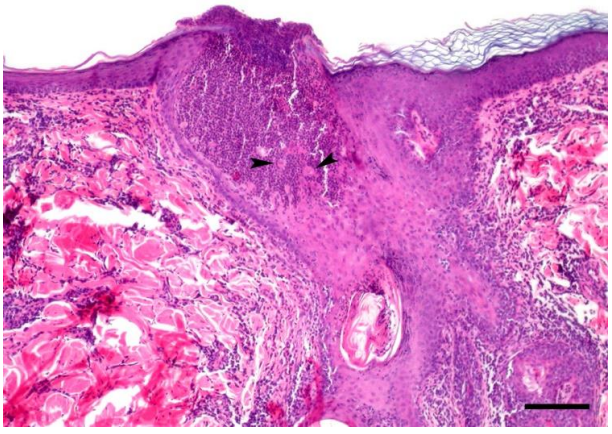

**Fig. 4:** Skin biopsy specimen with an intragranular pustule containing non-degenerate neutrophils and numerous acantholytic keratinocytes (arrowheads) (H&E, scale bar, 200 μm)

avidin-biotin peroxidase complex (ABC) method (Vector Laboratories, Burlingame, CA) was carried out using monoclonal commercial antibodies to CD3 and CD79a (Dako, Denmark). Positive and negative controls (normal canine lymph node) were introduced to achieve the reliability of the technique. Approximately 90-95% of the neoplastic cell population was CD3 positive (Fig. 5), whereas the CD79 antibody was negative. Based on these findings a diagnosis of epitheliotropic cutaneous lymphoma of T-cell origin was established. To confirm and classify the pemphigus suspicion, an immunohistochemical staining for immunoglobulin G (IgG) was done as previously described (Elmore *et al*., 2005; Jaber *et al*., 2013). Linear staining of the epidermal basement membrane together with staining of the intercellular space was observed, confirming the diagnosis of PE (Fig. 6).

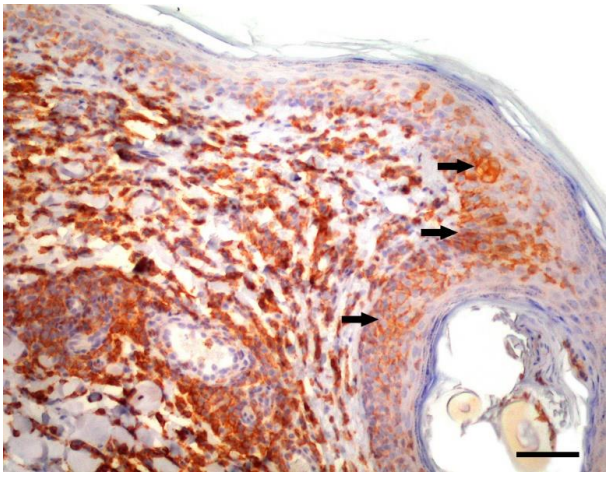

**Fig. 5:** Skin, epitheliotropic lymphoma. The neoplastic cells from Fig. 3 show positive cytoplasmic staining with CD3. Nests between the keratinocytes in the epidermis are indicated with arrows. Avidin-biotin peroxidase complex method, with Mayer's haematoxylin counterstain (scale bar, 100 μm)

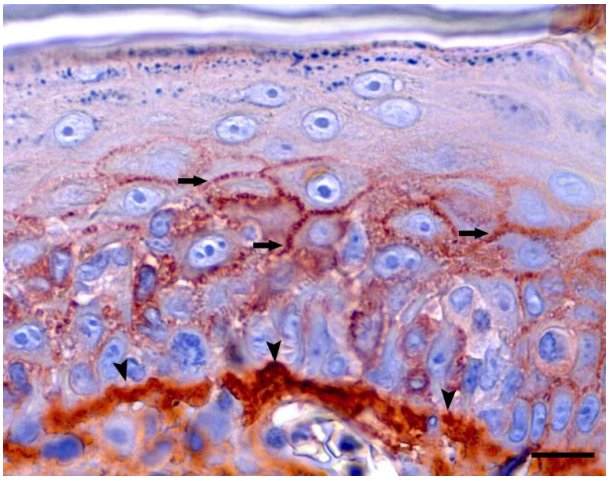

**Fig. 6:** Skin. IgG immunohistochemistry showing immunoglobulin deposition in the basement membrane (arrowheads) and in the intercellular space (arrows). Avidin-biotin peroxidase complex method, with Mayer's haematoxylin counterstain (scale bar, 16 μm)

## Discussion

The pattern of immunocomplex deposition observed in this paper was characteristic of PE (Haines *et al*., 1987; Gross *et al*., 2005; Bhang *et al*., 2008). Interestingly, this is the first case of PE and cutaneous lymphoma coexisting in a dog. It could represent two independent diseases affecting the animal simultaneously or an interrelation between the two processes.

Paraneoplastic pemphigus is an autoimmune blistering skin disease associated with concurrent neoplasia (Lemmens *et al*., 1998; Elmore *et al*., 2005; Hill *et al*., 2013; Wieczorek and Czernik, 2016). In human medicine, it has been associated with lymphoma, lymphocytic leukemia, poorly differentiated sarcomas and thymomas. In the two cases described in dogs, the disease was related with thymic lymphoma and splenic sarcoma. Histologically, paraneoplastic pemphigus and PE are very different. In the former, the development of suprabasilar vesicles without cellular content is the most characteristic lesion. In PE, subcorneal or intragranular pustules with acantholytic keratinocytes are consistently seen. Furthermore, PE has an interface component and only further immunohistochemical analysis showing deposition of IgG along the basement membrane with concurrent membranous staining of keratinocytes allows the final diagnosis (Gross *et al*., 2005; Bhang *et al*., 2008). These findings were consistent with the immunohistochemical results obtained in the present case. Despite this fact, it could not be completely ruled out that pustular lesions could represent a paraneoplastic phenomenon related to the cutaneous lymphoma.

Alternatively, the cutaneous lymphoma may have developed secondary to the autoimmune disease, as a result of a dysregulation of the immune system. In human medicine, lymphoma is the most common neoplasia associated with immune-mediated diseases. It has been observed in association with rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis or thyroiditis, amongst others (Keller *et al*., 1992). Many factors can account for the increased incidence of malignancy in autoimmunity, as diminished cell-mediated immunity leading to impaired immune surveillance or immunosuppressive drug treatment (Boumpas *et al*., 1986). However, in one study, increased transcriptional levels of c-myc, c-myb and c-raf proto-oncogenes in the lymphocytes of patients without neoplasia who had various autoimmune diseases were detected (Boumpas *et al*., 1986). This overexpression could eventually lead to malignant cellular transformation (Keller *et al*., 1992). It is important to highlight that lymphomas are heterogeneous neoplasm and treatment can be challenging (Cartagena Albertus *et al*., 2018).

In veterinary medicine several cases of immune-mediated disease and neoplasia have been described: thymomas and myasthenia gravis or polymyositis, immune-mediated thrombocytopenia and lymphoma, chronic lymphocytic leukemia or various solid tumors (Keller *et al*., 1992; Hill *et al*., 2013). There was also a

case of PF in association with systemic lupus erythematosus and subsequent lymphoma in a dog (Foster *et al*., 2000). Nevertheless, these observations have been based on small numbers of animals, without statistical validation. Broader studies about this type of pathological association would be necessary to positively reach a conclusion about their origin. Unfortunately, further information of this case could not be obtained since the owner decided to humanely euthanize the dog based on welfare grounds.

To our knowledge, this is the first case of PE and cutaneous lymphoma coexisting in a dog, expanding the list of associations between immune-mediated diseases and cancer in dogs, and providing support to the eventual connection between autoimmunity and neoplasia in this species.

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