

# Intestinal T-cell lymphoma with eosinophilic infiltration in a cat: a cytohistopathological evaluation with immunophenotyping

Zahra Nikousefat,<sup>1</sup> Mohammad Hashemnia,<sup>2</sup> Moosa Javdani<sup>3</sup>

<sup>1</sup>Department of Clinical Sciences; <sup>2</sup>Department of Pathobiology, School of Veterinary Medicine, Razi University, Kermanshah; <sup>3</sup>Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

## Abstract

In the present study, we describe the cytohistopathological and immunohistochemical characteristics of the intestinal T-cell lymphoma in a seven-year-old male Persian cat, applying the World Health Organization (WHO) system for the classification of domestic animal lymphomas. In fine-needle aspiration, mesenteric lymph nodes contained many lymphocytes from small- to medium-size, a few lymphoblasts and plasma cells, and rare eosinophils suggestive of hyperplastic lymph node. Histopathologically, diffuse infiltration and proliferation of tumor cells in the large intestine mucosa with architectural distortion of the crypts to the tunica muscularis in the submucosa and mucosal ulceration were observed. The tumor cells were composed of small-to-intermediate-sized lymphocytes with round, monomorphic nuclei and scant-to-moderate cytoplasm. Eosinophils infiltration was present. The mitotic rate was low. Immunohistochemical analysis revealed that neoplastic cells were mostly and strongly positive for T-cell phenotype CD3+ but negative for B-cell phenotypes CD20- and CD79a-. On the basis of cytology, histopathology, and immunohistochemical findings, the present tumor was diagnosed as intestinal T-cell lymphoma, according to WHO histological classification. Applying the WHO classification system for the diagnosis of feline lymphomas is very useful and has high accuracy and consistency. Although cytological examination may assist in the evaluation of gastrointestinal disease, histopathological examination is necessary to establish a definitive diagnosis of alimentary lymphoma.

## Introduction

Lymphoma is the most common neoplasm in cats, accounting for 50-90% of all feline hematopoietic neoplasms. It is often classified grossly according to the organs or tissues affected (multicentric, alimentary, renal, mediastinal, or extranodal/miscellaneous). The gastrointestinal tract is the most common location for this neoplasm. Alimentary lymphoma is characterized by the infiltration of the gastrointestinal tract by neoplastic lymphocytes, with or without mesenteric lymph node involvement.<sup>1</sup> Previously, lymphoma was divided histopathologically into small-cell (lymphocytic [LL]; low grade) or large-cell (lymphoblastic [LBL]; high grade) types. Epitheliotropic intestinal lymphoma (EIL) is a subset of LL that is characterized by the infiltration of malignant T cells into the mucosal epithelium of the intestinal tract. Also, large granular lymphoma (LGL) is introduced as a different type that is characterized by the presence of natural killer T lymphocytes that have characteristic intracytoplasmic granules. Clinically, LGL has distinct entities with different clinical presentations, therapies, and outcomes. LL and LGL typically consist of T cells, and LBL consists of B cells.<sup>1-3</sup>

Fine-needle aspiration (FNA) and cytology of intestinal masses, enlarged mesenteric lymph nodes, or the liver may be used to diagnose lymphoma. These are relatively noninvasive and rapid diagnostic methods. However, the presence of inflammation and/or lymphoid reactivity may hinder a definitive diagnosis, and a histopathology of tissue biopsies may be necessary to confirm a diagnosis of lymphoma.<sup>1,2</sup>

In gastrointestinal lymphoma, controversy exists as to the proportion of B-cell versus T-cell tumors. Several studies have found that the majority of gastrointestinal lymphomas are of the B-cell phenotype. However, contradictory findings have been reported.<sup>4,5</sup>

In 2002, the World Health Organization (WHO) proposed a new classification system for domestic animal lymphomas, which was adapted from the human-based revised European American lymphoma (REAL) classification, in addition to the consolidated Kiel classification and the working formulation of the National Cancer Institute. With respect to previous systems, the WHO-REAL classification provides no separation between low-, medium-, or high-malignancy-grade tumors, except for follicular neoplasms. The identification of B- or T-cell lineage is critical, followed by a further classification of B-cell lymphomas on

Correspondence: Mohammad Hashemnia, Department of Pathology, School of Veterinary Medicine, Razi University, P.O. Box: 67156-85414, Kermanshah, Iran. Tel.: +98-83-38322599 / 38329540. E-mail: m.hashemnia@razi.ac.ir

Key words: Intestinal T-cell lymphoma, cytohistopathological features, cat, WHO classification system.

Acknowledgments: We thank the authorities of Veterinary School, Razi University for their cooperation. This work was supported, in part, by grants from Vice Chancellor for Research at Razi University (Grant No. 56837).

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 29 December 2015. Revision received: 18 February 2016. Accepted for publication: 26 February 2016.

This work is licensed under a Creative Commons Attribution NonCommercial 4.0 License (CC BY-NC 4.0).

©Copyright Z. Nikousefat et al., 2017  
Licensee PAGEPress srl, Italy  
Veterinary Science Development 2017; 7:6392  
doi:10.4081/vsd.2017.6392

the basis of their morphological characteristics. In contrast, T-cell lymphomas are classified on the basis of their anatomical location. The current WHO-REAL classification tends to group all intestinal lymphomas derived from T lymphocytes in a single class (intestinal T-cell lymphoma), suggesting that all these neoplasms have similar clinical characteristics and pathological patterns.<sup>6,7</sup>

In this report, we describe the cytological, histopathological, and immunohistochemical characteristics of intestinal T-cell lymphoma in a cat and classify it according to the WHO scheme.

## Materials and Methods

### Case history

A seven-year-old male Persian cat presented to a private small animal clinic with a history of weight loss for near four weeks. Abdominal palpation revealed diffuse, cord-like bowel thickening. An ultrasound showed the presence of intestinal thickening and mesenteric lymphadenopathy.

### Cytological evaluation

Cytological smears obtained by FNA of the mesenteric lymph node were air-dried, fixed, and stained by the May Grunwald Giemsa (MGG) procedure. The morphological classification criteria, as according to Fournel-Fleury *et al.*, were based on cell size (*small, medium, or large, i.e.*, having a nucleus smaller than, equal to, or larger than two red blood cells [RBCs]); the shape of the nucleus; the density of the chromatin; the number, size and distribution of the nucleoli; and the extension and basophilia of the cytoplasm.<sup>8</sup>

### Histopathological evaluation

The specimens were fixed in a 10% phosphate buffered formalin, processed routinely, embedded in paraffin, and sectioned at 5 mm. Sections were stained with hematoxylin and eosin (H&E). On H&E-stained sections, the size of lymphocytes was estimated relative to the measurement of RBCs. Specifically, RBC size was used as a unit of measurement compared with the nuclear size of the lymphocytes (small-to-medium lymphocyte nuclei were classified as having <2 times the RBC diameter; large lymphocyte nuclei were classified as having  $\geq 2$  times the RBC diameter).

### Immunological evaluation

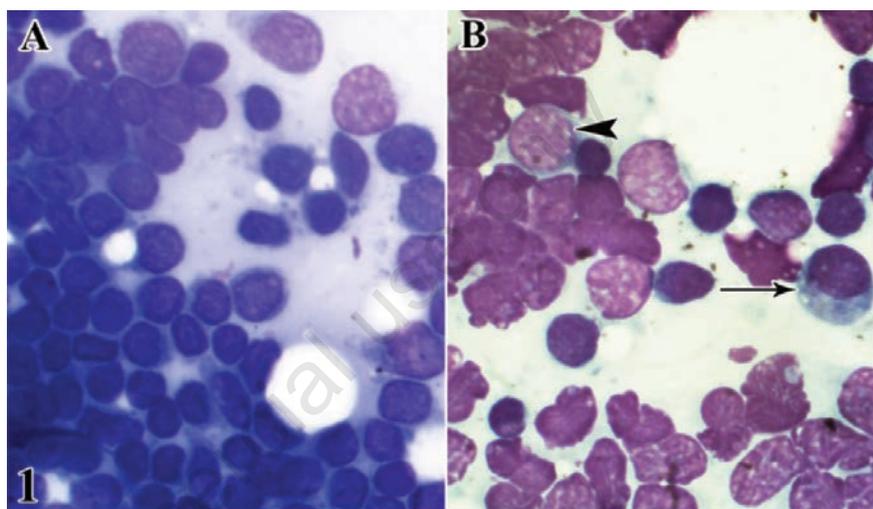
Our case was labeled immunohistochemically for B- and T-cell antigens on paraffin sections. Immunostaining was performed using CD3, CD20, and CD79a (DAKO, Denmark). Tissue sections were briefly cooked at 60°C and deparaffinized in

xylene. In order to antigen retrieval, the slides were inserted in Tris-EDTA, were heated, and then were cooled. The slides were put in a Tris buffer and, for endogenous peroxide activity inhibition, hydrogen peroxide 3% + methanol was used. The sections were incubated with selected monoclonal antibodies. Envision solution was used and diaminobenzidine chromogen was then used as a substrate. The sections were stained with hematoxylin. In the next step, they were washed with distilled water and

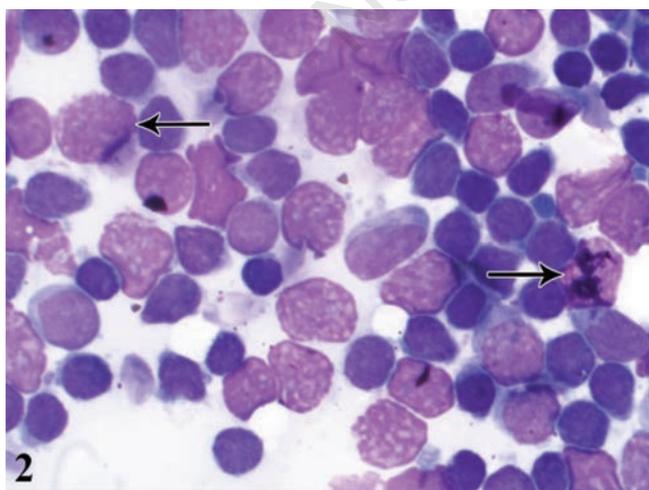
were then dehydrated with grading alcohol. Then, a Tris buffer solution was used for washing.

### Animal ethics

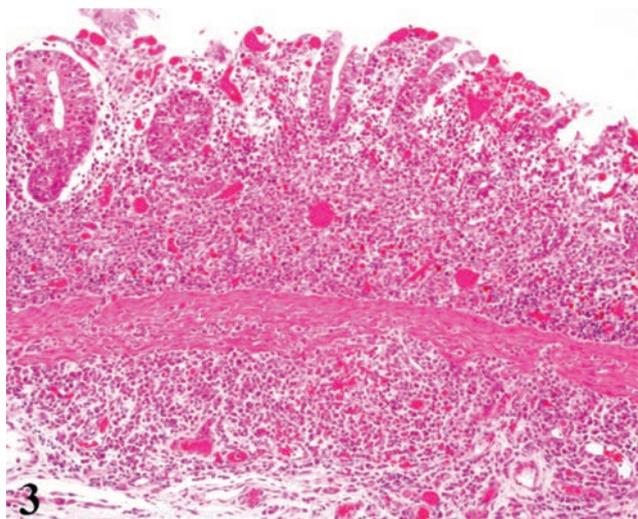
This study was performed under the approval of the state committee on animal ethics, Razi University, Kermanshah, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986 regarding the protection of animals used for experimental purposes were considered.



**Figure 1.** Fine-needle aspiration of mesenteric lymph nodes. A) Many lymphocytes from small- to medium-size; B) a few lymphoblasts (arrow head) and plasma cells (arrow) (MGG staining, 2500 $\times$ ).



**Figure 2.** Fine-needle aspiration of mesenteric lymph nodes. Rare mitotic figures (arrow) (MGG staining, 2500 $\times$ ).



**Figure 3.** Diffuse infiltration and proliferation of tumor cells in the large intestine (H&E; 250 $\times$ ).

## Results

### Cytological findings

The FNA of the mesenteric lymph nodes contained many lymphocytes from small to medium size, few lymphoblasts and plasma cells (Figure 1), and rare eosinophils suggestive of a hyperplastic lymph node. Rare mitotic figures were identified (Figure 2).

### Histological findings

Grossly, a prominent thickening of the intestinal wall and a narrowing of the lumen of the intestine with mucosal ulceration were observed. The cut surface of the mass was colored white to gray. On microscopic examination, diffuse infiltration and proliferation of tumor cells in the large intestine mucosa with architectural distortion of the crypts to the tunica muscularis in the sub-mucosa that lead to ulceration (Figure 3) were prominent. Morphologically, the tumor cells were composed of small-to-intermediate-sized lymphocytes (with a nuclear diameter <2 RBC diameters) with round, monomorphic nuclei and scant-to-moderate cytoplasm (Figure 4). Each tumor cell usually showed a distinct cell boundary. The nuclei had scattered or dense chromatin with small distinct nucleoli. Epitheliotropic behavior was evident. Eosinophils infiltration was present (Figure 5). The mitotic rate was low. The diagnosis was low-grade intestinal T-cell lymphoma, according to the WHO classification.

### Immunological findings

Immunohistochemical analysis revealed that neoplastic cells were mostly and strongly positive for T-cell phenotype CD3+ (Figure 6) but negative for B-cell phenotypes CD20- and CD79a- (Figure 7). Monomorphism of the lymphoid infiltrates was evidenced by diffuse CD3 expression. The neoplastic T lymphocytes infiltrated the epithelium, showing epitheliotropism.

### Discussion

The FNA cytology of lymph nodes is a valuable aid in achieving a diagnosis. Normal lymph nodes consist primarily of small mature lymphocytes (75-95% of all nucleated cells present). The remaining 5-25% of the cells are an admixture of the other various cell types but are primarily lymphoblasts and prolymphocytes, with lesser numbers of plasma cells and a few neutrophils and macrophages. In lymph nodes with lymphosarcoma, lymphoblasts typically make up more than 50% of the lymphoid cells.<sup>9</sup>

The presence of high numbers of only small lymphocytes (*i.e.*, the absence of the other cell types [lymphoblasts and prolymphocytes]) is suggestive of small-cell lymphoma. This type of lymphoma occurs rarely and generally must be diagnosed by histopathology.<sup>9</sup>

The alimentary form of lymphoma is a relatively common manifestation of this disease in the cats. The prevalence of different forms of feline lymphoma has changed

since the advent of widely available testing and vaccination for retroviral infection in this species. Although infection with the feline leukemia virus and the feline immunodeficiency virus are major risk factors for the development of lymphoma, cats with gastrointestinal (GI) lymphoma are usually negative for both viruses. Helicobacter infection and exposure to cigarette smoke may play a role in the development of feline GI lymphoma.<sup>3,5</sup>

In this study, we diagnosed low-grade intestinal T cell lymphoma in a seven-year-old male Persian cat, though there is a discrepancy between the cytological and histopathological findings in the mesenteric lymph nodes. Lingard *et al.* found that the cytological evaluation of enlarged mesenteric lymph nodes is misleading in establishing a diagnosis of low-grade alimentary lymphoma and that a histological assessment of lymph node architecture is required to distinguish between benign hyperplasia and neoplasia.<sup>10</sup>

In Gabor *et al.*'s study, 90% of alimentary lymphomas were intermediate or high grade.<sup>11</sup> In contrast, Fondacaro *et al.* found that 75% of alimentary lymphomas were low grade.<sup>12</sup> This raises the issue of the relative incidence of high-, intermediate-, and low-grade alimentary lymphoma. In a study conducted by Lingard *et al.*, procurement of cases led to roughly equal numbers of high-grade and low-grade alimentary lymphomas.<sup>10</sup> The observed variability in the prevalence of the three histological grades may be due to differences in geographic distribution, the methods used to procure biopsy samples, the grading systems used by

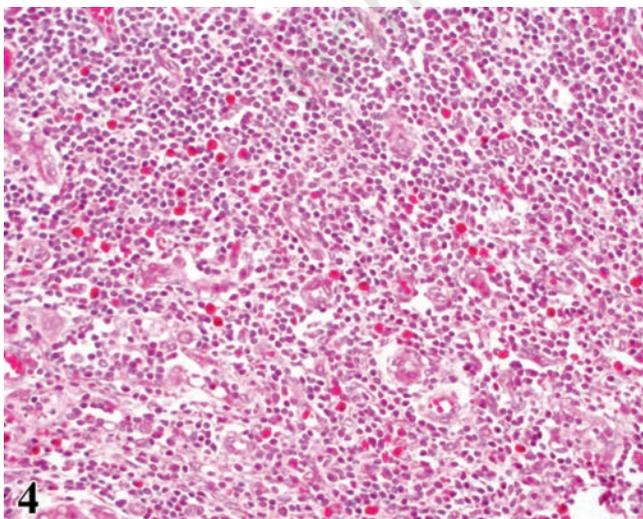


Figure 4. Small-to-intermediate-sized lymphocytes with round, monomorphic nuclei and scant-to-moderate cytoplasm (H&E; 500 $\times$ ).

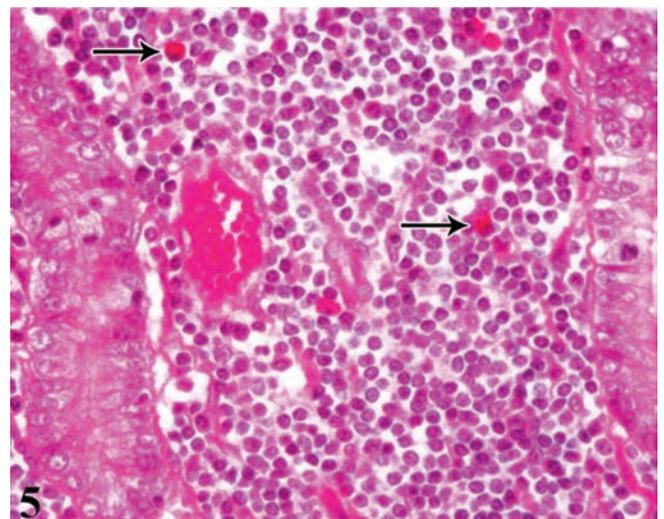
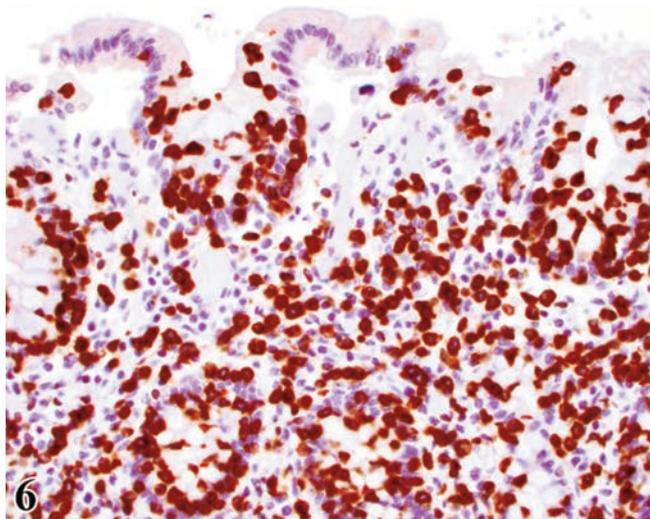
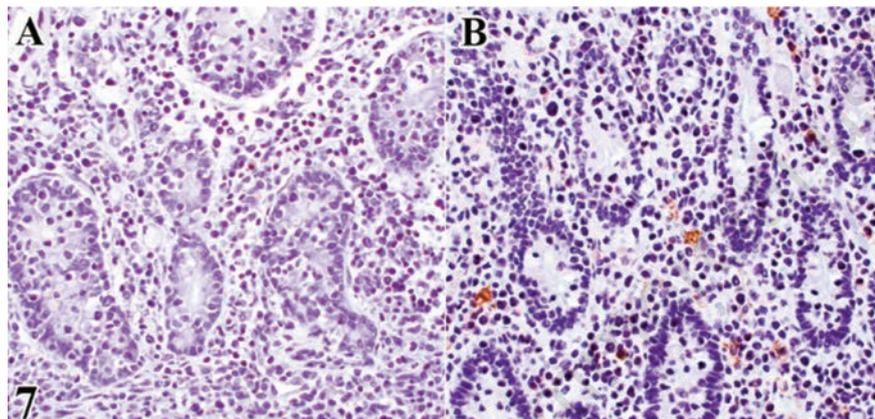


Figure 5. Infiltration of eosinophils in the large intestine (H&E; 1000 $\times$ ).



**Figure 6.** Neoplastic cells are positive by immunolabeling for T-cell phenotype CD3+ (1000×).



**Figure 7.** Neoplastic cells are negative for B-cell phenotypes CD20- (A) and CD79a- (B) (1000×).

pathologists, and increased awareness of the low-grade disease.

In our case, mucosal gastrointestinal eosinophilic infiltration was one of the most important histopathological findings. Paraneoplastic eosinophilic infiltrates such as hypereosinophilic syndrome (HES) were previously reported in cats by Lingard *et al.* and Barrs *et al.*<sup>10,13</sup> Also, Ozaki *et al.* reported alimentary T-cell lymphoma of dogs that was accompanied by eosinophilic infiltration.<sup>14</sup> In human medicine, HES is currently regarded as a group of disorders that are characterized by marked eosinophilia in the peripheral blood, tissues, or both, often without other identifiable causes. In our case, no peripheral blood eosinophilia was identified.

Eosinophil infiltration, however, has also been observed consistently in mast cell

tumors of intestinal origin. Thus, when a background of eosinophils is present in an intestinal round cell tumor, both T-cell lymphoma and mast cell tumors need to be considered in the differential diagnosis.<sup>10,13-15</sup>

In our case, the neoplastic cells were mostly and strongly positive for T-cell phenotype CD3+ but negative for B-cell phenotypes CD20- and CD79a-.

Lymphomas were classified as B-cell; T-cell; or non-B, non-T-cell lymphoma using immunohistochemistry to detect the expression of CD3 and CD79a. The lymphomas were further classified as mucosal lymphoma (infiltrate confined to the epithelium and lamina propria with minimal extension into the submucosa) or transmural lymphoma (infiltrates extending markedly into the submucosa and muscularis propria).<sup>16</sup>

It has been suggested that tumor phenotype in cats with GI lymphoma is associated with age. The reported hypothesis is that cats younger than ten years will likely have B-cell lymphoma, whereas cats older than ten years will likely have T-cell lymphoma.<sup>2</sup> The findings in the study did not support an association between age and phenotype.

Differentiating inflammatory bowel disease (IBD) from intestinal lymphoma in cats is a diagnostic challenge to both veterinary clinicians and pathologists. Food hypersensitivity and parasitism, both of which usually have an eosinophilic component, and endocrine, renal, or hepatic disease can cause similar signs. However, an accurate diagnosis is critical because disease outcome and therapy depend on it. The use of immunophenotyping to assess the phenotypic uniformity of a mucosal infiltrate or to test clonality would be a useful adjunct to histopathology. A monomorphic lymphocyte population supports a diagnosis of lymphoma, whereas a lymphoplasmacytic population supports a diagnosis of inflammation. In our case, monomorphism of the lymphoid infiltrates was evidenced by the diffuse CD3 expression that favors lymphoma. Furthermore, epitheliotropism describes the homing of neoplastic lymphocytes to the intestinal mucosal epithelium, and infiltration of the intestinal villus epithelium by lymphocytes in groups of five or more cells in direct apposition with one another is highly suggestive of lymphoma. In contrast to IBD, there are no reactive changes in the crypt or villus enterocytes to the presence of neoplastic lymphocytes and there is no mucosal congestion or edema. Intraepithelial T cells, as revealed in our case, are more easily identified in immunohistochemically labeled intestinal sections, compared to hematoxylin and eosin-stained sections.<sup>1,5,13,17,18</sup>

## Conclusions

In conclusion, we reported a case of feline low-grade T-cell lymphomas accompanied by eosinophilic infiltration and small lymphocytic cells epitheliotropism in the large intestine. Although cytological examination may assist in the evaluation of gastrointestinal disease, histological examination is necessary to establish a definitive diagnosis of low-grade alimentary lymphoma.

## References

1. Waly NE, Gruffydd-Jones TJ, Stokes

- CR, et al. Immunohistochemical diagnosis of alimentary lymphomas and severe intestinal inflammation in cats. *J Comp Pathol* 2005;133:253-60.
2. Pohlman LM, Higginbotham ML, Welles EG, et al. Immunophenotypic and histologic classification of 50 cases of feline gastrointestinal lymphoma. *Vet Pathol* 2009;46:259-68.
3. Gieger T. Alimentary lymphoma in cats and dogs. *Vet Clin Small Anim* 2011;41:419-32.
4. Jackson ML, Wood SL, Misra V, et al. Immunohistochemical identification of B and T lymphocytes in formalin fixed, paraffin embedded feline lymphosarcomas: relation to feline leukemia virus status, tumor site and patient age. *Can J Vet Res* 1996;60:199-204.
5. Patterson-Kane JC, Perrins Kugler B, Francis K. The possible prognostic significance of immunophenotype in feline alimentary lymphoma: a pilot study. *J Comp Pathol* 2004;130:220-2.
6. Valli VE, Jacobs RM, Parodi AL, et al. Histological classification of hematopoietic tumors of domestic animals. Washington, DC: Armed Force Institute of Pathology, American Registry of Pathology; 2002.
7. Cesari A, Bettini G, Vezzali, E. Feline intestinal T-cell lymphoma: assessment of morphologic and kinetic features in 30 cases. *J Vet Diagn Invest* 2009; 21: 277-279.
8. Fournel-Fleury C, Magnol JP, Bricaire P, et al. Cytohistological and immunological classification of canine malignant lymphomas: comparison with human non-Hodgkin's lymphomas. *J Comp Pathol* 1997;117:35-9.
9. Cowell RL, Dorsey KE, Meinkoth JH. Lymph node cytology. *Vet Clin Small Anim* 2003;33:47-67.
10. Lingard AE, Briscoe K, Beatty JA, et al. Low-grade alimentary lymphoma: clinicopathological findings and response to treatment in 17 cases. *J Feline M Surg* 2009;11:692-700.
11. Gabor LJ, Canfield PJ, Malik R. Immunophenotypic and histological characterization of 109 cases of feline lymphosarcoma. *Aus Vet J* 1999; 77: 436-441.
12. Fondacaro JV, Richter KP, Carpenter JL, et al. Feline gastrointestinal lymphoma: 67 cases (1988-1996). *Eur J Comp Gastroenterol* 1999;4:5-11.
13. Barrs VR, Beatty JA, McCandlish IA, et al. Hypereosinophilic paraneoplastic syndrome in a cat with intestinal T cell lymphosarcoma. *J. Small Anim Pract* 2002;43:401-5.
14. Ozaki K, Yamagami T, Nomura K, et al. T-Cell lymphoma with eosinophilic infiltration involving the intestinal tract in 11 dogs. *Vet Pathol* 2006;43:339-44.
15. Samoszuk M, Ramzi E, Cooper DL. Interleukin-5 mRNA in three T-cell lymphomas with eosinophils. *Am J Hematol* 1993;42:402-4.
16. Moore PF, Rodriguez-Bertos A, Kass PH. Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality. *Vet Pathol* 2012;49:658-68.
17. Kiupel M, Smedley RC, Pfent C, et al. Diagnostic algorithm to differentiate lymphoma from inflammation in feline small intestinal biopsy samples. *Vet Pathol* 2011;48:212-22.
18. Moore PF, Woo JC, Vernau W, et al. Characterization of feline T cell receptor gamma (TCRG) variable region genes for the molecular diagnosis of feline intestinal T cell lymphoma. *Vet Immunol Immunopathol* 2005;106:167-78.