Scientific Research Publishing

Open Journal of Veterinary Medicine, 2017, 7, 175-183
http://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Is There Any Association between Intestinal Lymphoma and Coeliac-Like Disease in Prosimians? The Case of the Ring-Tailed Lemur (*Lemur catta*)

Camillo Sandri[1], Barbara Regaiolli[2*], Donatella Volpatti[3], Ernesto Pascotto[3]

[1]Department of Animal Health, Care and Management, Parco Natura Viva-Garda Zoological Park, Verona, Italy
[2]Research & Conservation Department, Parco Natura Viva-Garda Zoological Park, Verona, Italy
[3]Department of Food Science, Faculty of Veterinary Medicine, University of Udine, Udine, Italy
Email: *barbara.regaiolli@parconaturaviva.it

How to cite this paper: Sandri, C., Regaiolli, B., Volpatti, D. and Pascotto, E. (2017) Is There Any Association between Intestinal Lymphoma and Coeliac-Like Disease in Prosimians? The Case of the Ring-Tailed Lemur (*Lemur catta*). *Open Journal of Veterinary Medicine*, **7**, 175-183.
https://doi.org/10.4236/ojvm.2017.712019

Received: November 14, 2017
Accepted: December 25, 2017
Published: December 28, 2017

Abstract

Non-human primate species are considered as good models for human cancer research. Despite the relevant phylogenetic position of prosimians, few reports of neoplastic diseases have been described in these species. The current study investigated implication of an intestinal T-cell lymphoma in a 5-year-old female ring-tailed lemur (*Lemur catta*) hosted at Parco Natura Viva, an Italian zoological garden. First, a sub-occlusive thickening of the small bowel was found. Histologically, the lesion was caused by a malignant lymphoid infiltrate that was homogeneously CD3+. Moreover, inflammatory patterns peripheral to the lesion suggested a coeliac disease similar to that reported in human. A huge malignant lymphoid infiltrate was present also in the liver and spleen. Overall, the case suggests an etiopathological relationship between coeliac-like disease and intestinal T-lymphoma, as reported in several human studies. Findings from this study are useful to improve our knowledge on the occurrence of the T-lymphoma as well as to improve the husbandry and dietary protocol of prosimians in zoos.

Keywords

Lemur catta, Coeliac-Like Enteritis, Intestinal Lymphoma, Immunohistochemistry

1. Introduction

Non-human primates are similar to humans in terms of genetic evolution, immune

system, physiology and metabolism as well as other features [1] In addition, similarities in cancer genetics between humans and NHPs have been reported, particularly in great apes [2]. Therefore, NHP species are considered as good models for human cancer research. However, due to the low incidence of cancer in non-human primates and the reduced sample size compared to human studies, more data and reports need to be documented, particularly regarding spontaneous tumours [1]. Most studies of cancer in NHPs involved monkeys and great apes [3] [4]. Despite the relevant phylogenetic position of prosimians, few reports of neoplastic diseases have been described in these species [4]. Neoplasia in lemurs has been scarcely reported apart from some primary liver tumors [5] [6] [7] [8] and pulmonary tumors [9]. However, improving our knowledge on prosimian cancer would be useful to trace the evolution of this disease in humans. The current study focuses on a case of T-cell intestinal lymphoma in a female ring-tailed lemur (*Lemur catta*) hosted in Parco Natura Viva, an Italian zoological garden.

Prior to 2005 the 5-year old female ring-tailed lemur did not show any clinical sign of illness. In March 2005 a 5-yr-old female ring-tailed lemur, belonging to a collection of 30 lemurs, was evaluated for apathy, in appetence and few episodes of regurgitation. She was thin (1.7 kg) and showed abdominal pain during palpation. Hematologic tests did not reveal abnormalities; thus a medical treatment with carprofen (2 mg/kg) and Joscina N-Butilbromuro was performed. Some weeks later, as no clinical improvement was noted, the lemur was anesthetized to perform a more detailed examination. A big firm mass was revealed in the epigastrium by the abdominal palpation and it was confirmed by radiographs: a large radiopaque mass about 3 - 4 cm in diameter. Two days later a laparotomy was performed: the mass completely occluded the intestinal wall and was removed through intestinal resection and anastomosis (**Figure 1**). Consequently to this surgery, the subject showed a slight recovery from the illness (lasting about 2 weeks) then it became again anorexic and did not accept fragmented/homogenized food. Therefore the lemur was submitted to a second surgery (one month after the first one), to which it did not survive. In this occasion internal organs (small intestine at two levels, kidney, liver, spleen, lung) were collected. Macroscopic evaluation of organs revealed: thickening and hypertrophy of the intestinal wall; presence of whitish sclerotic areas on the liver surface. The other organs did not show gross lesions.

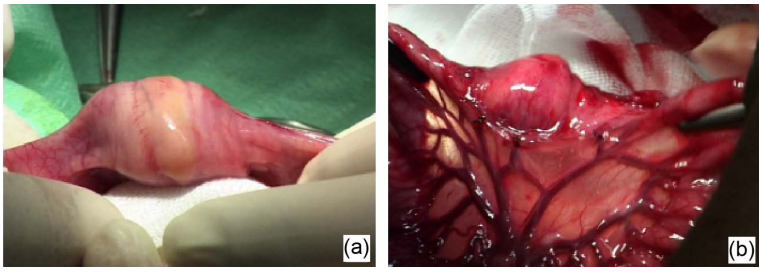

Figure 1. The mass that completely occluded the intestinal wall of the lemur.

2. Material and Methods

2.1. Histology

Samples of intestine (jejunum), liver, spleen, kidney and lung were collected, fixed in 10% buffered formalin or 80% ethanol (intestine only) for one month, then submitted to routine histological procedures. Sections of 5 μm were stained with Hematoxilin-Eosin, Masson trichrome, PAS, May Grunwald-Giemsa.

2.2. Immunohistochemistry (IHC)

To identify the lineage of the neoplastic lymphocyte population and the inflammatory infiltrating lymphocytes, sections were submitted to an immunoperoxidase based staining, employing the following antibodies: polyclonal rabbit anti-human CD3 (dil. 1:500, Dako, cod. A-0452), monoclonal mouse anti-human CD79*α* (dil. 1:100, Dako, cod. M-7050), monoclonal mouse anti-human CD20 (dil. 1:100, Dako, cod. M-0755). CD3 antibody allows the immune detection of T-lymphocytes derived neoplasms. CD79*α* antibody allows the immune detection of neoplasms deriving from B-lymphocytes at all stages of maturation. CD20 antibody is specific for B-cells precursors and mature B-cells, excluding plasma cells. After an incubation with secondary antibodies to rabbit or mouse immunoglobulins, reaction was developed by ABC-peroxidase and DAB. Counterstain was performed with Haematoxylin. To assess the cross-reactivity of wthe antibodies on lemur tissues, as well as the specificity of the reaction, sections of human healthy lymph node and skin, human skin with lymphocyte infiltration, lemur healthy spleen and skin, were submitted to the immunohistochemical evaluation.

3. Results

3.1. Histology

The intestine sample collected at first surgery revealed heavy changes of the intestinal wall in the area of the ring inspissation. The luminal surface was widely ulcerated, the *tonaca propria* was completely absent. There was a massive degeneration of the submucosal tonaca and a strong and exuberant connective new growth as well as a partial destruction of the muscular tonaca. The intestinal wall was heavily compromised and showed an intense lymphoid infiltrate, displaying a multifocal round and dense pattern (**Figure 2(a)**). Sometimes these lymphocyte aggregates were perivascular. The cellular infiltrate involved a hyperplastic reactive stroma, composed of immature connective tissue, strongly vascularized. Higher magnifications revealed the presence of micro-focal necrosis in the centre of some lymphoid foci (**Figure 2(b)**). The lymphoid cells were morphologically homogeneous. The nuclei were small (6 - 8 μm diameter), euchromatinic, with polygonal shape, and often presented indented membrane. They contained multiple, medium sized nucleoli and chromatin aggregates (**Figure 2(b)**). The cytoplasm tent to be diaphanous and slightly reticular. The mitotic index was high. At the edge of the ring-shaped lesion there was a peculiar inflammatory feature.

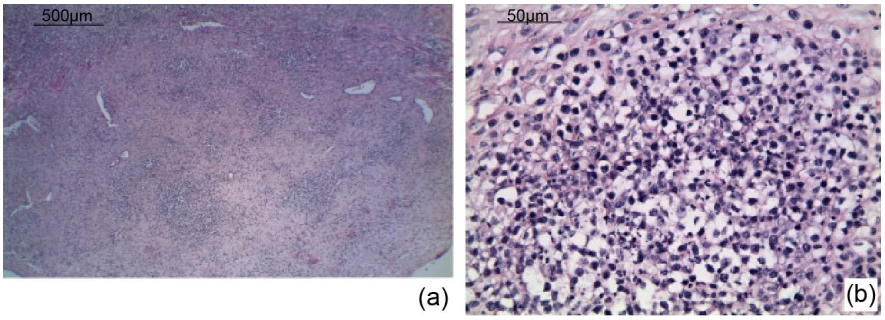

Figure 2. Compromised intestinal wall of the lemur: (a) intense lymphoid infiltrate, displaying a multifocal round and dense pattern. Magnification was set at 25X; (b) Nuclei of the lymphoid cells with multiple, medium sized nucleoli and chromatin aggregates Magnification was set at 200X.

Plasma cells and lymphocytes infiltration in the *lamina propria*; impressive villous atrophy and crypt hyperplasia with decreased villous height-to-crypt depth ratio; pronounced increase in the number of intraepithelial lymphocytes (IELs) (**Figure 3(a)** and **Figure 3(b)**). The primary lesion was identified as media cells intestinal lymphoma associated with enteritis, and the peripheral inflammatory pattern was similar to that described in the case of grade 4 Marsh-Oberhuber human coeliac disease (gluten-sensitive enteropathy). In this occasion the main internal organs (small intestine at two levels, kidney, liver, lung) were collected and processed for histology. The macroscopic evaluation of organs revealed: thickening and hypertrophy of the intestinal wall; presence of whitish sclerotic areas on the liver surface. The other organs did not show gross lesions. The histological evaluation of the intestine Haematoxylin-Eosin sections collected at two different levels at necroscopy, pointed out inflammatory alterations similar to those observed at the edge of the neoplastic lesion found in the first intestinal sampling. Intestinal specimens collected at two different levels of the intestine were similar. The villi were strongly atrophic and flattened (rarely branched or leaf-shaped) and the mucosa assumed peculiar colonic-like features (**Figure 3(c)**). The epithelial cells showed a karyorectic multi-focal necrosis and intraepithelial lymphocytes were abundant. The *tonaca propria* displayed an intense lymphocyte/plasma cells infiltrate, as well as areas of necrosis. At the base of the *muscularis mucosae* a slight lymphocyte infiltration was detectable. The *tunica muscularis* and *tunica serosa* presented foci of lymphocyte infiltration. The histopathological data concerning the intestine could suggest the presence of a malabsorption related-enteritis similar to the human coelic disease (4^{th} stage, Marsh classification). The intensity and the pattern of lymphocyte recruitment suggested the possible neoplastic progression of the lesion.

The liver parenchyma presented an evident architecture destructuration of the lobules. Moreover, the lobules showed areas of multifocal necrosis (detection supported by the Masson trichrome staining). Sometimes the necrotic areas were adjacent to the thickened Glisson membrane. An intense and diffuse infiltration of lymphoid cells was detectable, resembling those invading the *tonaca propria*

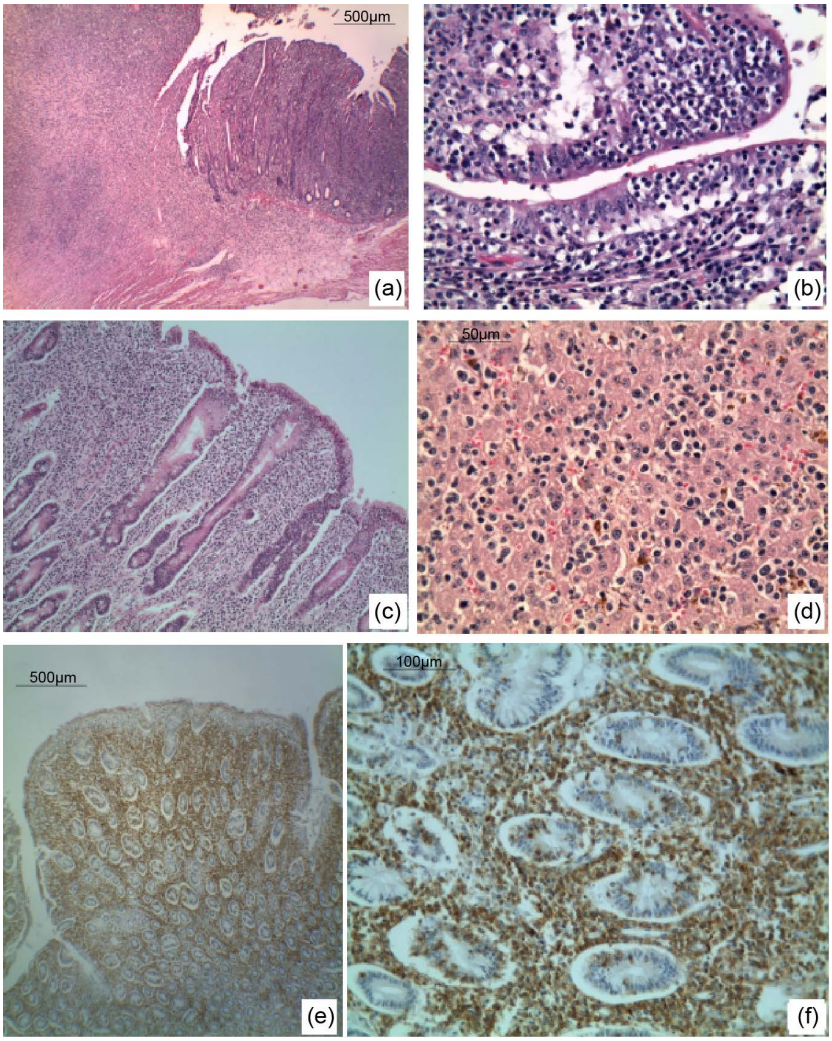

Figure 3. Histological images of the lemur tissues: (a) and (b) Intraepithelial lymphocytes; (c) Intestinal mucosa; (d) Infiltration of lymphoid cells in the liver parenchyma; (e) and (f) Infiltrating lymphocytes in the intestine sections. Magnification was set at 25X (a), 200X (b) and (d), 100X (c), 40X (e), 100X (f).

of the intestine (**Figure 3(d)**). Associated to these features there were also evident areas of hemosiderin accumulation, considered as a common finding in zoo lemurs. Lung parenchyma presented a diffuse oedema associated to areas of destructive emphysema. The apical part of the lung revealed an area of acute pneumonia, with infiltration of neutrophilgranulocytes, involving the apical part of the lung. The spleen was morphologically intact but it was involved in the recruitment of a strong infiltrate composed by lymphoid cells similar to those observed in gut and liver.

3.2. Immunohistochemistry

In order to identify the lineage of the neoplastic lymphocyte population and the inflammatory infiltrating lymphocytes, the histological sections were submitted to an immunoperoxidase based staining, using the following antibodies: polyc-

lonal rabbit anti-human CD3 (Dako, cod. A-0452); monoclonal mouse anti-human CD79*α* (Dako, cod. M-7050); monoclonal mouse anti-human CD20 (Dako, cod. M-0755). The CD3 antibody is specific for T cells and allows the immune detection of T lymphocytes derived neoplasms. The CD79*α* antibody is specific for B cells and allows the immune detection of neoplasms deriving from B lymphocytes at all stages of maturation. The CD20 antibody is specific for B cells precursors and mature B cells, excluding plasma cells. As positive controls, sections of human healthy lymph node, human skin characterized by lymphocyte infiltration and healthy lemur skin, were submitted to the same immunohistochemical procedure, using anti CD3, anti CD79*α* and anti CD20 as markers.In the intestine sections a relevant part of infiltrating lymphocytes (about 70%) was immunoreactive for the T-lymphocyte marker CD3 (**Figure 3(e)** and **Figure 3(f)**), revealing inflammatory features. Also numerous IELs were positive and a slight positivity was found also in the marginal part of the Auerbach plexus (granular aspect). The B-lymphocyte marker CD20 did not identify positive cells among the infiltrating lymphocytes. Only few granular cells localized in the epithelium were positive. The anti CD79 antibody was ineffective on lemur healthy spleen (positive control) and due to this lack of cross-reactivity was not considered suitable to evaluate the immunophenotype of infiltrating cells. The intestine sections of the neoplastic lesion, when treated with the CD3 antibody, revealed the positivity of all the neoplastic lymphocytes, becoming more intense in the cells localized marginally to the main lesion (**Figure 4(a)**). The CD20 antibody provided only a slight staining of some granular cells, as observed in the inflammatory lesions. The liver sections revealed a strong positivity of infiltrating cells to CD3, suggesting the presence of a T-lymphocyte population (**Figure 4(b)**). IHC confirms the diagnosis of T-cells intestinal lymphoma and a peripheral coeliac-like enteritis.

4. Discussion

Few studies have been investigating lymphoma in primates and reports of this tumor in lemurs are extremely rare [10]. Findings from the current study are important as they provide useful information on lymphoma in the *Lemur catta*,

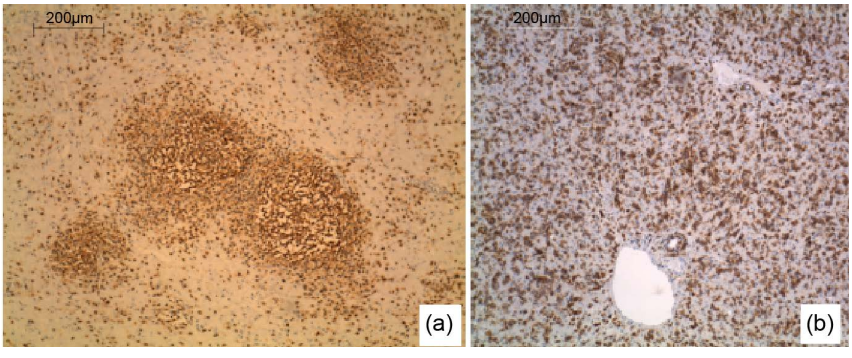

Figure 4. Intestine (a) and liver (b) sections of the neoplastic lesion treated with the CD3 antibody. Magnification was set at 40X.

highlighting an association between this tumor and coeliac disease. Basing on previous literature, humans with coeliac disease have been found to be at higher risk to develop small-bowel non-Hodgkin's lymphoma [11] [12] [13] [14] [15]. This study highlighted that similar association may exist also in lemurs, which are the most phylogenetically distant primates from our species. In particular, in human pathology enteropathy-type T-cell lymphoma is a recognized complication of gluten-sensitive enteropathy [15] [16] [17] [18]. Tumour cells probably derive from a subset of intraepithelial lymphocytes (IELs), as suggested by their ability to invade crypt epithelium [19]. The routine application of immunohistochemical staining for lymphocyte markers such as CD3 has been proposed as a mean to better evaluate the number and distribution of IELs and the lymphocytes composing the lymphoma associated to the enteropathy [20] [21]. The immunophenotyping approach has been already proposed by Pye *et al.* [10] in a lemur affected by intestinal lymphoma, resulting as a B cell (CD79+) lymphoma enriched with a T cell (CD3+) infiltrate [4]. In our experience a relevant part of infiltrating lymphocytes in the intestine, most of the lymphocytes composing the intestinal neoplasm, and the liver lymphoid infiltrate, were immunoreactive for the T-lymphocyte marker CD3. These findings reinforce the diagnostic hypothesis proposed by the authors. In comparative terms, the role of dietary related disorders in captive lemurs should be considered as possible aetiology. Although the causative agent is still uncertain, a gluten-sensitive enteropathy should be considered. The possible sources of dietary gluten in captivity are biscuits, flours and other accidental foods [22]. In 2005, although not allowed in the zoo, interactions between visitors and lemurs were frequent, due to the enclosure design. Although the lemur diet was made of fruits and vegetable and no gluten-food was provided, uncontrolled food items were given to the lemurs by the visitors. In 2010, all lemur groups were moved in a new area, in large naturalistic enclosure with no possibility for visitor-animal interactions.

5. Conclusion

In conclusion, findings of this study might be useful not only to improve our knowledge on the occurrence and evolution of lympho-plasmacellular sub-clinical enteritis in primates but also to improve the husbandry and dietary protocol of prosimians in zoos.

Acknowledgements

The authors wish to thank Carla Calligaro (DIAL, Univ. of Udine) for the technical support on histological and immunohistochemical procedures. Special thanks should be given to Parco Natura Viva veterinarians for their help in the lemur surgery and to Caterina Spiezio, the Head of the Research & Conservation Dept. of Parco Natura Viva for her collaboration in manuscript preparation and revision. Finally we are grateful to Dr. Cesare Avesani Zaborra, Parco Natura Viva CEO, for allowing this study to take place.

References

[1] Xia, H. and Chen, C. (2011) Progress of Non-Human Primate Animal Models of Cancers. *Zoological Research*, **32**, 70-80.

[2] Puente, X.S., Velasco, G., Gutiérrez-Fernández, A., Bertranpetit, J., King, M.C. and López-Otín, C. (2006) Comparative Analysis of Cancer Genes in the Human and Chimpanzee Genomes. *BMC Genomics*, **7**, 15. https://doi.org/10.1186/1471-2164-7-15

[3] Beniashvili, D.S. (1989) An Overview of the World Literature on Spontaneous Tumors in Nonhuman Primates. *Journal of Medical Primatology,* **18**, 423-437.

[4] Remick, A.K., Van Wettere, A.J. and Williams, C.V. (2009) Neoplasia in Prosimians: Case Series from a Captive Prosimian Population and Literature Review. *Veterinary Pathology*, **46**, 746-772. https://doi.org/10.1354/vp.08-VP-0154-R-FL

[5] Brygoo, E.R., Levaditi, J., Destombes, P. and Guillon, J.C. (1964) Adeno Cancer with Bronzed Cirrhosis in a *Lemur macaco*. *Bulletin de la Societe de pathologie exotique et de ses filiales,* **57**, 228-233.

[6] Chang, J., Wagner, J.L. and Kornegay, R.W. (1979) Spontaneous Cholangiocarcinoma in a Ring-Tailed Lemur (*Lemur catta*). *Laboratory Animal Science*, **29**, 374-376.

[7] Wolshein, P., Petzold, D.R. and Brandt, H.P. (1996) Hepatocellular Carcinoma in a Lemur (*Varecia variegata rubra* × *variegata*). *Deutsche Tierarztliche Wochenschrift*, **103**, 180-183.

[8] Porter, F., Goens, S.D., Brasky, K.M. and Hubbard, G.B. (2004) A Case Report of Hepatocellular Carcinoma and Focal Nodular Hyperplasia with a Myelolipoma in Two Chimpanzees and a Review of Spontaneous Hepatobiliary Tumors in Non Human Primates. *Journal of Medical Primatology*, **33**, 38-47. https://doi.org/10.1111/j.1600-0684.2003.00048.x

[9] Wadsworth, P.F., Gopinath, C. and Jones, D.M. (1980) Mammary Neoplasia in Ring-Tailed Lemurs (*Lemur catta*). *Veterinary Pathology*, **17**, 386-388. https://doi.org/10.1177/030098588001700313

[10] Pye, G.W., Bennett, R.A., Terrell, S.P., Ginn, P.E., McSherry, L.J. and Alleman, A.R. (2000) T-Cell Rich B-Cell Lymphoma in a Ring Tailed Lemur (*Lemur catta).Journal of Zoo and Wildlife Medicine*, **31**, 388-393. https://doi.org/10.1638/1042-7260(2000)031[0388:TCRBCL]2.0.CO;2

[11] Holmes, G.K., Stokes, P.L., Sorahan, T.M., Prior, P., Waterhouse, J.A. and Cooke, W.T. (1976) Coeliac Disease, Gluten-Free Diet, and Malignancy. *Gut,* **17**, 612-619. https://doi.org/10.1136/gut.17.8.612

[12] Williamson, R.C., Welch, C.E. and Malt, R.A. (1983) Adenocarcinoma and Lymphoma of the Small Intestine. Distribution and Etiologic Associations. *Annals of Surgery*, **197**, 172-178. https://doi.org/10.1097/00000658-198302000-00008

[13] Mathus-Vliegen, E.M. (1995) Lymphoma in coeliac disease. *Journal of the Royal Society of Medicine*, **88**, 672-677.

[14] Koo, V., Armstrong, A. and Harvey, C. (2002) Coeliac Disease Presenting with Colonic Lymphoma. *Ulster Medical Journal,* **71**, 136-138.

[15] Holmes, G.K. (2002) Coeliac Disease and Malignancy. *Digestive and Liver Disease,* **34**, 229-237. https://doi.org/10.1016/S1590-8658(02)80198-0

[16] Cellier, C., Delabesse, E., Helmer, C., *et al.* (2000) Refractory Sprue, Coeliac Disease, and Enteropathy-Associated T-Cell Lymphoma. *The Lancet*, **356**, 203-208. https://doi.org/10.1016/S0140-6736(00)02481-8

[17] Freeman, H., Lemoyne, M. and Pare, P. (2002) Coeliac Disease. *Best Practice & Research*: *Clinical Gastroenterology*, **16**, 37-49. https://doi.org/10.1053/bega.2002.0264

[18] Rooney, N. and Dogan, A. (2004) Gastrointestinal Lymphoma. *Current Diagnostic Pathology*, **10**, 69-78. https://doi.org/10.1016/j.cdip.2003.10.001

[19] Ruskone-Fourmestraux, A. and Rambaud, J.C. (2001) Gastrointestinal Lymphoma: Prevention and Treatment of Early Lesions. *Best Practice & Research*: *Clinical Gastroenterology*, **15**, 337-354. https://doi.org/10.1053/bega.2000.0177

[20] Mino-Kenudsona, M., Brown, I. and Lauwers, G.Y. (2005) Histopathological Diagnosis of Gluten-Sensitive Enteropathy. *Current Diagnostic Pathology*, **11**, 274-283. https://doi.org/10.1016/j.cdip.2005.05.002

[21] Dickson, B.C., Streutker, C.J. and Chetty, R. (2006) Coeliac Disease: An Update for Pathologists. *Journal of Clinical Pathology*, **59**, 1008-1016. https://doi.org/10.1136/jcp.2005.035345

[22] Mowry, C. and Campbell, J.L. (2001) Nutrition. In: *Ring-Tailed Lemur (Lemur catta) Husbandry Manual*, America Association of Zoos and Aquariums.