

Epidemiology and Diagnosis of Feline Intestinal Lymphosarcomas in Egypt

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Abstract

Feline intestinal lymphosarcomas are mostly caused by Feline Leukemia Virus (FeLV). Unfortunately, there is no available vaccine for FeLV in Egypt. The diagnosis of feline intestinal lymphosarcomas depends upon abdominal palpation, x-rays examination, ultrasonography, direct ELISA and histopathology of masses excised during laparotomy. The recorded clinical signs in intestinal lymphosarcoma affected cats were variable including vomiting, fever, anorexia, ascites, anemia, dyspnea, constipation and emaciation. The affected lymph nodes were mesenteric, mediastinal and retropharyngeal. The prevalence of intestinal lymphosarcomas in the examined cats was 4.03 % (11 out of 273 cats). The prevalence was higher in queens than toms (2.93 % and 0.73 % respectively). The Siamese cats had higher prevalence than the Sherazy ones (2.56 % and 1.47 % respectively). X-ray films and ultrasonographic images performed on the eleven cats suffered from intestinal lymphosarcomas revealed ascities and abdominal masses. The comparison of ELISA and histopathology (of excised masses) results showed that 9 out of 11 intestinal lymphosarcoma affected cats were infected with FeLV that proved not all cases of intestinal lymphosarcoma were caused by FeLV. The sensitivity, specificity and accuracy of ELISA to diagnose intestinal lymphosarcoma in cats were 81.81 %, 100 % and 92 % respectively. Gross autopsy of the collected lymph nodes, livers, kidneys revealed that gross lymphadenopathy involving one or more nodes, hepatomegaly and kidney enlargement. Microscopically, the examined tissues specimens showed that the normal architecture of the examined lymph nodes, livers, and kidneys has been replaced by a diffuse infiltrate of both lymphocytes and lymphoblasts. The vast majority of the cells are small lymphocyte-type cells with round basophilic nuclei and a sparse rim of cytoplasm. The eleven intestinal lymphosarcoma affected cats exposed to abdominal exploratory surgery (laparotomy) died at one to three months post-surgery. It is concluded that the vaccination of kittens and cats against FeLV in Egypt is very important to prevent the highly fatal intestinal lymphosarcomas.

Keywords: Lymphosarcoma; FeLV; cat; diagnosis; Egypt.

Introduction

Feline Intestinal Lymphosarcoma is a malignant cancer often seen in older cats. The cancer usually originate in the lymph nodes located within tissues surrounding the intestines (mesentery) or within the lymphoid cells inside the intestinal walls themselves. Feline intestinal lymphosarcomas are caused by Feline leukemia virus (FeLV) and other non infectious causes as described by Mary and Wallace. Feline leukaemia virus is a gamma retrovirus affecting domestic cats and it was firstly detected in 1964 by electron microscopy (Jarrett *et al.*, 1964). Feline leukaemia virus can causes ane-

mia and lymphosarcoma, among other serious illnesses. The virus can also suppress the cat's immune system, affecting her ability to fight off bacteria, viruses and fungi that contribute to other serious health problems. The feline leukemia virus is spread through direct contact with an infected cat. High concentrations of the virus are found in saliva, while lower concentrations can be found in blood, urine and feces (Hardy *et al.*, 1976 and Pacitti *et al.*, 1986). The virus is most commonly transmitted through shared food and water bowls, and grooming of each other. Litter boxes, too, can be a source of transmission. The virus may also be passed from an infected momma cat to her kittens, either before birth or afterwards as she is nursing. The virus may be transmitted during breeding. Feline leukemia is species-specific and only affects

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felines. cats are mostly affected by lymphosarcomas (McGawin *et al.*, 2001). Intestinal neoplasms may result in intramural or intraluminal mechanical obstructions. They attack mostly the muscular layer of the intestinal wall and decrease the intestinal lumen (Hedlund, 1997).

The final diagnosis of feline intestinal lymphosarcomas depend on abdominal palpation, X-rays examination, Ultrasonography, direct ELISA (enzyme-linked immunosorbent assay) and the Gold standard which is histopathology of tumours. No successful medications are proved till now to eliminate feline intestinal lymphosarcomas (Ettinger, 2003). The recent medications could be given only to relieve and treat symptoms such as upper respiratory infections, digestive problems and stomatitis. The commercially available vaccines for the causative virus (FeLV) are two, the first is a recombinant feline leukemia virus vaccine (rFeLV) and the second is an inactivated FeLV vaccine (FeLV-k). The two vaccines are protective against the FeLV infection in cats (Grosenbaugh *et al.*, 2004). Unfortunately, cats are not vaccinated with FeLV vaccine in Egypt. At the same time, no data are available about FeLV infection in Egypt that improve us to investigate this disease.

In this study, it was planned to study prevalence of FeLV infection among Cats, evaluation of ELISA test as a method of diagnosis, application of X-rays and ultrasonography as a diagnostic aid, removing lymphosarcoma surgically and investigating histopathological changes in lymphoid tissues and internal organs related to FeLV infection.

Materials and methods

Cats

Two hundred seventy three cats were examined representing different ages and both sexes. Each cat was examined clinically and by abdominal palpation to detect any abdominal mass. X-ray examinations, ultrasonography, FeLV direct ELISA and laparotomy, pathology and histopathology were done for cats had abdominal masses.

Samples

Blood, serum and tissue samples were collected for examination from cat suffered from abdominal masses.

Clinical examination

Two hundred seventy three cats were examined at teaching hospital of department of Medicine and Infectious diseases (in Faculty of Veterinary Medicine, Cairo University) by measuring body temperature, examination of conjunctival and buccal mucous membranes, examination of superficial and abdominal lymph nodes using palpation. The appetite, body condition, abdominal enlargement, respiratory manifestations, digestive troubles were recorded for each cat as a routine work at the teaching hospital. Breed, sex, age, previous medication and vaccination were also recorded for each cat.

X-ray examination

It was carried out on eleven cats suffered from abdominal masses in a lateral and ventro-dorsal positions at department of surgery, anesthesiology and radiology, Faculty of Veterinary Medicine, Cairo University.

Ultrasonography

It was carried out on eleven cats suffered from abdominal masses and it was also applied on the abdomen with the cat positioned in dorsal recumbency for each affected case at department of surgery, anesthesiology and radiology, Faculty of Veterinary Medicine, Cairo University.

Direct ELISA

Qualitative Enzyme Linked Immunosorbent Assay (ELISA) was carried out on twenty five sera of cats free and suffered from abdominal masses for detection of Feline leukemia virus (FeLV) (Synbiotics Corporation, USA).

Surgical interventions:

Laparotomy was carried out according to Patsikas *et al.* (2003). It was performed on eleven cats suffering from abdominal masses for surgical excision of the internal masses with the following steps:

Pre-Surgical Tests:

Preoperative tests depend in part on the age and general health of the animal.

Typically, radiographs, blood count, serum biochemical tests and a urinalysis.

Type of Anesthesia:

The procedure required general anesthesia to induce complete unconsciousness and relaxation. General anaesthesia premedication of cats was done by using atropine sulphate 0.1% in a dose of 0.05mg/kg body weight by subcutaneous injection. Then xylazine HCl (Rompun®2%) in a dose of 2 mg/kg body weight by intramuscular injection. General anaesthesia regimen was achieved by intramuscular injection of ketamine HCl in a dose of 10mg /kg body weight. Maintenance of general anaesthesia was applied by intramuscular injection of ketamine HCl in a dose of 5 mg/kg body weight.

Abdominal Exploratory Surgery:

Following anesthesia, the cat was placed on a surgical table, lying on his back. The hair was clipped over the middle of the abdomen, the skin was scrubbed with surgical soap to disinfect the area, and a sterile drape was placed over the surgical site. Using a scalpel to incise the skin at the middle of the abdomen to open the abdominal cavity. The abdominal organs were examined and evaluated. A celiotomy started with a ventral midline skin incision, which was long enough to access and visualize every abdominal organ from the liver to the bladder. In two cats, an incision from the xiphoid process to the pubis was done. After dissecting the subcutaneous tissue, the linea alba was tented and incised carefully with a scalpel blade. The dorsal aspect of the linea was palpated, cranially and caudally, to ensure that no adhesion or distended organ could be lacerated. The incision was carefully extended with scissors.

After a wound retractor was placed, the first organ investigated was the liver. Elevation of the xiphoid region was performed to better visualization of the diaphragm and carefully palpation of each liver lobe. The gallbladder was observed and very gently expressed to assess patency of the common bile duct. The stomach was observed and palpated, from the cardia to the pylorus. Elevating the duodenum allowed seeing the right limb of the pancreas. The duodenum and meso-duodenum was gently moved toward the left of the patient allowed seeing the right kidney, adrenal gland, ureter and

ovary. Seeing the right adrenal gland was tricky because of its cranial location. Then examination of the bowel, inch by inch was done. The thickness of the entire small intestine was appreciated. The mesenteric lymph nodes was observed and palpated. After reaching the cecum, the entire colon was then palpated. Gently retracting the colon and meso-colon toward the right of the patient was done to view and palpate the left kidney, adrenal gland, ureter and ovary. The spleen, omentum and left limb of the pancreas was inspected closely. The bladder, prostate, and uterus were visualized and palpated, depending on the patient's gender. Using this systematic approach for exploring the abdomen allowed a quick but thorough exam. After mass excision or any biopsies were taken, lavage the abdomen with warm, sterile saline was done. The abdominal incision was then closed with one or two layers of self-dissolving sutures (stitches). The outer layer of skin was closed with sutures; these need to be removed in about 7 to 10 days.

Aftercare:

Postoperative medication was given to relieve pain, which was judged in most cases to be moderate and can be effectively eliminated with safe and effective pain medicines. The home care requires reduced activity until the stitches are removed in 7 to 10 days. The suture line should be inspected daily for signs of redness, discharge, swelling, or pain.

Pathological and Histopathological examination

The excised masses representing lymph nodes, livers, and kidneys were obtained during laparotomy of lymphosarcoma suspected cats and they were grossly examined and then fixed in 10% Carson neutral buffered formalin. 3 to 5 µm sections obtained from blocks of paraffin wax were stained with hematoxylin-eosin (HE) and examined microscopically (Bancroft *et al.*, 1996).

Results

Clinical examination

Eleven cats out of 273 totally examined cats were suffered from intestinal lymphosarcoma. The recorded clinical signs were vomiting (V), fever (F), anorexia (Ano), ascites (As), anemia(An), dys-

pnea (D), constipation (C) and emaciation (E). The affected lymph nodes were mesenteric, mediastinal and retropharyngeal as illustrated in table (1).

The prevalence of intestinal lymphosarcoma

The prevalence in the examined cats was 4.03 %.

Table 1. Recorded clinical signs and affected lymph nodes in cats suffered from intestinal lymphosarcoma regarding breed, sex and age

Case	Breed	Sex	Age (years)	Enlarged LN	Clinical signs							
					V	F	Ano	As	An	D	C	E
1	Siamese	Queen	8	Mesenteric	+	+	+	+	+	-	-	+
2	Sherazy	Tom	2.5	Mesenteric	+	-	+	+	+	-	+	+
3	Sherazy	Queen	11	Mesenteric	+	+	+	+	-	-	-	+
4	Siamese	Queen	5	Mesenteric, mediastinal and retropharyngeal	+	+	+	+	+	+	-	+
5	Siamese	Queen	6	Mesenteric and mediastinal	+	+	+	+	+	-	-	+
6	Sherazy	Tom	4.5	Mesenteric, mediastinal and retropharyngeal	+	+	+	+	+	+	-	+
7	Siamese	Queen	9	Mesenteric, mediastinal and retropharyngeal	+	+	+	-	+	+	-	+
8	Siamese	Queen	3	Mesenteric	+	-	+	+	+	+	-	+
9	Sherazy	Queen	1.5	Mesenteric, mediastinal and retropharyngeal	+	+	+	-	+	+	-	+
10	Siamese	Queen	7	Mesenteric, mediastinal and retropharyngeal	+	+	+	-	+	-	-	+
11	Siamese	Tom	4	Mesenteric, mediastinal and retropharyngeal	+	+	+	+	+	+	-	+

(V):Vomiting , (F):Fever, (Ano):Anorexia,(As):Ascites,(An):Anemia,(D):Dyspnea,(C):Constipation and (E): emaciation

Considering sex, the prevalence was higher in queens than toms. It was 2.93 % and 0.73 % respectively as presented in table (2). The Siamese cats had higher prevalence than the Sherazy ones, the prevalence was 2.56 % and 1.47 % respectively as illustrated in table (3).

Table 2. Distribution of Lymphosarcoma in cats according to sex

Sex	Lymphosarcoma		Healthy	Total
	No	%		
Queen	8	2.93	190	198
Tom	3	0.73	72	75
Total	11	4.03	262	273

Table 3. Distribution of Lymphosarcoma in cats according to breed

Cat breed	Lymphosarcoma		Healthy	Total
	No	%		
Siamese	7	2.56	179	186
Sherazy	4	1.47	83	87
Total	11	4.03	262	273

X-rays examination and Ultrasonography :

X-ray films performed on the eleven cases revealed ascities and abdominal masses as illustrated in Fig. 1.

The ultrasonographic images showed abdominal masses associated with ascities in the eleven cats as illustrated in Fig. 2. Ultrasonographic findings included mixed echo-pattern localized masses associated with liver, spleen, stomach, intestine and mesenteric lymph nodes. Regional bowel hypomotility, decreased intestinal wall echogenicity and anechoic fluid filling the abdomen indicating ascities had also been demonstrated. In two cases there were hypoechoic thickening of the gastric wall (normal: <0.5 cm) and hypoechoic thickening of the intestinal wall (normal: <0.3 cm). Intestinal lesions were symmetrically thickened, but gastric lesions were asymmetric. Lesions were focal, multifocal, or diffuse. Loss of normal intestinal wall layering was observed in three cases. Normal

wall layering was more commonly preserved in cases of inflammatory disease than in neoplasia.



Fig. 1. Lateral abdominal radiographs of 2.5 years tom cat showed a large radio-opaque mass located in the ventral region of the cranial and extended to mid-abdomen that was displacing surrounding abdominal organs.



Fig. 2. B-mode sagittal scan of the caudal abdomen of 2.5 years tom cat revealed presence of anechoic free abdominal fluid (ascites), diffused thickening of the intestinal wall and a mixed echo-pattern mass associated with the intestinal wall.

ELISA :

The comparison of ELISA and histopathology (of excised masses) results showed that 9 out of 11 intestinal lymphosarcoma affected cats were infected with FeLV as presented in table (4). The sensitivity, specificity and accuracy of ELISA to diagnose intestinal lymphosarcoma in cats were 81.81 %, 100 % and 92 % respectively as in table (5), that means not all cases of intestinal lymphosarcoma were caused by FeLV.

Table 4. Comparison of ELISA according to histopathological changes of excised masses

	ELISA		Total
	Positive	Negative	
Histopathological changes of excised masses (lymphosarcoma)	Positive	9 (T+)	11
	Negative	0 (F+)	14
Total	9	16	25 (n)

(T+): True positive, (T-): True negative, (F+): False positive, (F-): False negative and (n): total number

Table 5. Statistical evaluation of ELISA

ELISA statistical parameter	Value (%)
Sensitivity	81.81
Specificity	100.00
Accuracy	92.00

Abdominal Exploratory Surgery

The eleven intestinal lymphosarcoma affected cats exposed to abdominal exploratory surgery (laparotomy) died at one to three months post-surgery.

Pathology and histopathology

Gross autopsy of the collected lymph nodes, livers, kidneys revealed that gross lymphadenopathy involving one or more nodes (Fig.3), hepatomegaly with pale yellow discoloration and infiltration may be nodular or diffuse in livers, nodular or diffuse infiltration mainly affecting renal cortex and changes may be subtle so always compare with contra-lateral kidney , although disease usually bilateral.



Fig. 3. Enlargement of mesenteric lymph node excised from the same tom cat.

Microscopically, the examined tissues specimens showed that the normal architecture of the examined lymph nodes, livers, and kidneys has been replaced by a diffuse infiltrate of both lymphocytes and lymphoblasts. The vast majority of the cells are small lymphocyte-type cells with round basophilic nuclei and a sparse rim of cytoplasm. The lymphoblasts characterized by round or oval nuclei with fine and evenly dispersed chromatin.(Fig. 4, 5 and 6).

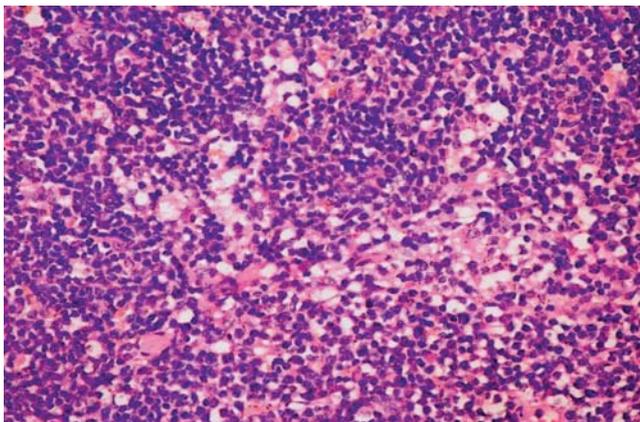


Fig. 4. Variable sized lymphocytes and lymphoblasts infiltrating and replacing normal tissue of lymph node (H&E X400).

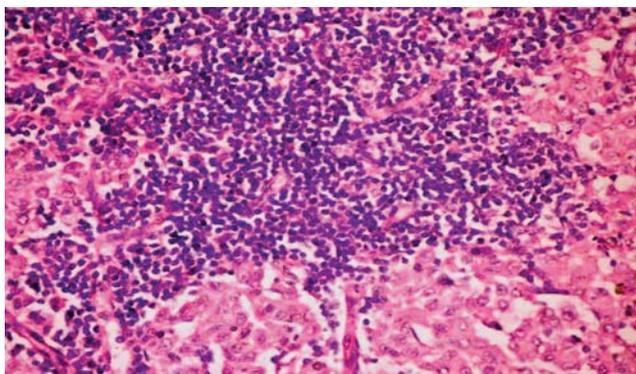


Fig. 5. Variable sized lymphocytes and lymphoblasts infiltrating and replacing normal tissue of liver (H&E X400).

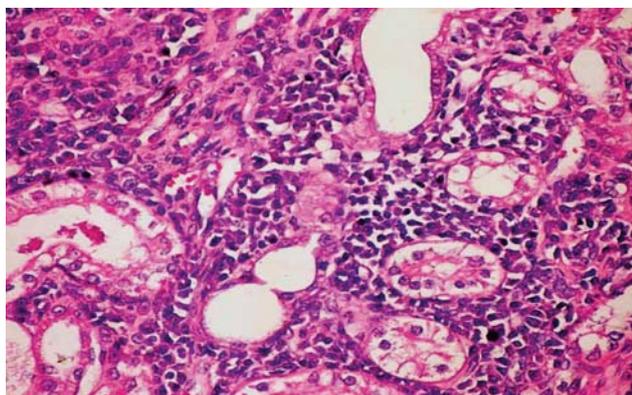


Fig. 6. Variable sized lymphocytes and lymphoblasts infiltrating and replacing normal tissue of kidneys (H&E X400).

Discussion

Feline intestinal lymphosarcomas may cause different tumours in cats, mainly lymphoma and leukaemia, but also other non-haematopoietic malignancies. Feline intestinal lymphosarcomas -induced lymphomas are among the most frequent tumour forms of the cat; myeloproliferative disorders are less common and not always associated with FeLV infection (Francis *et al.*, 1979a; Louwerens *et al.*, 2005).

Different forms of lymphoma have been classified according to its most frequent anatomic location: 1) The thymic or mediastinal form. 2) The alimentary form, where tumour cells are associated with organs of the digestive tract. 3) The multicentric or peripheral form, which affects lymph nodes. 4) The atypical or extranodal form, presenting with solitary tumours in kidneys, CNS, or skin.

In some cases, lymphoma is disseminated with multiple organ and site involvement. (Hardy *et al.*, 1970; Reinacher and Theilen 1987). Liver, spleen, bone marrow, blood and/or nonlymphoid organ involvement are associated with a poor prognosis (Vail and Thamm, 2005).

It is also possible for cats to develop some forms of lymphoma with no known or detectable association with FeLV infection, which carries a better prognosis (Vail and Thamm, 2005).

Clinical signs of feline were recorded and varied in relation to tumour location as previously recorded by (Hardy *et al.*, 1976; Hardy *et al.*, 1973; Hedlund, 1997; Dvorak *et al.*, 1999; Mary and Wallace). In the beginning, cat showed depression, anorexia and lethargy, which can lead to vomiting and diarrhoea. Additional symptoms include emaciation, dehydration, melaena, haematemesis, anaemia, fever, icterus, and abdominal effusions. Anaemia detected in the FeLV infected cats could be explained by haemolysis related to secondary opportunistic infections such as *Mycoplasma haemofelis* or to immune-mediated destruction (Scott *et al.*, 1973; Kociba, 1986). Feline intestinal lymphosarcomas can interfere with a haem transport protein (Cotter, 1979; Quigley *et al.*, 2000), which directly results in a non-regenerative anaemia. Non-regenerative anaemias may be caused by chronic inflammatory mechanisms, myelodestruction, myelosuppression.

The emaciation in lymphosarcoma affected cats could be explained by (i) decreased uptake of food,

(ii) the rapidly multiplying cancer cells consume most of the body energy, (iii) As the intestinal lymphosarcoma progresses, diarrhea and vomiting developed and result in dehydration, (iv) The intestine lose its ability to absorb nutrients and becoming mechanically blocked by rapidly growing lymphosarcoma, (v) Prerenal azotemia due to dehydration caused by repeated vomiting and diarrhea, (vi) Mechanical obstruction of the intestine by lymphosarcoma results in flourishing in aerobic and anaerobic microflora, leading to de-conjugation of bile acids, hydroxylation of fatty acids and production of bacterial metabolites that are toxic to epithelial cells, (vii) Lymphosarcoma alter carbohydrate, lipid and protein metabolism, impair parameters of electrolytes, minerals and internal environment, and change in endocrine and immunological functions lead to emaciation (Feney *et al.*, 1982).

Abdominal palpation reveals solid masses, thickened intestinal loops or mesenteric lymphadenopathy. The cats with alimentary lymphosarcoma usually present with gastrointestinal signs that have frequently been present for several weeks. The masses visible in native X-ray pictures are associated with accumulation of gases and liquids proximal to the site of obstruction (Klimes *et al.*, 2000). The abdominal cavity was palpated soft, palpable and painless. The intestinal lymphosarcoma were circumscribed or irregular solid masses that were found in the centre of the abdominal cavity. The retropharyngeal and popliteal lymph nodes were swollen. The clinical signs of alimentary lymphosarcoma may confuse with that of partial obstructions caused by intussusception or a linear foreign body. The differentiation is mostly depending on laparotomy and histopathological examination of mass or excised intestinal loop wall samples (Klimes *et al.*, 2000).

The prevalence of lymphosarcoma in the examined cats (273) were 4.03 % (11) as showed in tables 2 and 3. The incidence was higher in Siamese than Sherazy cats and it was 2.56 % and 1.47% respectively. The incidence was 2.93 % and 0.73 % in the examined Queen and Tom cats respectively. Age range of the lymphosarcoma affected cats were from 1.5 year to 11 years as presented in table (1), the presence of intestinal lymphosarcoma in adult and old cats is observed and that is agree with that mentioned in (The Merck Veterinary Manual, 2006).

Radiographs (x-rays) showed the presence of a

radio-opaque fluid filling the abdomen indicating ascities and a large radio-opaque mass located in the abdominal cavity displacing surrounding abdominal organs (Fig.1).

Ultrasound images clearly showed the presence of anechoic free abdominal fluid (ascities), a mixed echo-pattern mass dorsally located to the spleen and associated with the intestinal wall and diffused thickening of the intestinal wall (Fig. 2).

The false negative of ELISA (2 out of 11) could be explained by: 1-FeLV is a very immunosuppressive retrovirus that has been linked to the development of lymphosarcoma in both FeLV test-positive and FeLV test-negative cats (Mary and Wallace). 2- Idiopathic lymphadenopathy that is a non-neoplastic peripheral lymphadenomegaly reported in young cats (usually 1-2 years of age). Clinical signs of fever and generalized lymphadenomegaly (nodes 2-3 times normal) are similar to lymphosarcoma. Affected cats test negative for FeLV. Histologically, lymph nodes from affected cats resembled lymphosarcoma (Mary and Wallace).

The eleven intestinal lymphosarcoma affected cats exposed to abdominal exploratory surgery (laparotomy) died at one to three months post-surgery. We think the cause of death is the metastasis of lymphosarcoma cells to develop new masses in the vital organs especially these cats did not exposed to chemo or radio-therapy.

Intestinal lymphosarcoma can only be confirmed with histopathology of excised lymph nodes or affected organs biopsies. As illustrated in figures 4, 5 and 6, variable sized lymphocytes and lymphoblasts infiltrating and replacing normal tissues of lymph node, liver and kidney. The lymphosarcoma affected mesenteric lymph nodes were swollen as showed in Fig. 3.

The association between malignant lymphoma (lymphosarcoma) and FeLV is recorded in our study in nine cats out of eleven that agrees with that recorded by Ettinger *et al.* (1995), they mentioned that Lymphoma is the most common malignancy diagnosed in cats. Lymphoma in young cats occur most frequently following infection with FeLV or to a lesser degree feline immunodeficiency virus (FIV). These cats tend to have involvement of lymph nodes, spine, or mediastinum. Cats with FeLV are 62 times more likely to develop lymphoma, and cats with both FeLV and FIV are 77 times more likely.

Lymphoblastic lymphosarcoma were detected

in tissues of mesenteric lymph nodes, liver and kidney that agrees with that recorded by (Matz, 2007), who stated that gastrointestinal lymphoma classified into low grade, intermediate grade, and high grade. Low grade types include lymphocytic and small cell lymphoma. High grade types include lymphoblastic, immunoblastic, and large cell lymphoma. Low grade lymphoma is only found in the small intestine, while large grade can commonly be found in the stomach. It is concluded that the vaccination of kittens and cats against FeLV in Egypt is very important to prevent the highly fatal intestinal lymphosarcomas.

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