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Endoscopic Depiction and Treatment Evaluation of Spirocerca Lupi in Dogs

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Abstract: The present investigation has been dealt with *Spirocerca (S.) lupi* infested mongrel dogs. This parasitic disease is highly infective to human beings and carnivores. The diagnosis march has been comprised the lateral contrast thoracic radiographs, fecal examination, blood profile, endoscopic examination and histopathological sections of deep seated pinch biopsies. These infested dogs have been put under an adopted treatment with Ivermectin injection combined with oral prednisolone. The obtained results reveal absence of the pessimistic recognitions particularly after 3 weeks from the onset of treatment. Endoscopically the presented esophageal nodules are marked out in the distal third of infested dogs' esophagus as masses assigned into the esophageal lumen and fundus of stomach. The endoscopic outlook of *Spirocerca lupi* lesions has been considered an integral procedure of the diagnostic march and for evaluation of treatment follow up. The diagnostic procedures and the recommended treatment are the vet's guidance to care for *Spirocerca lupi* in dogs, hoping in future to prevent this disease from being spread among human beings and other carnivores.

Key words: Endoscopy · Esophagus · Stomach · Spirocercosis · Dogs

INTRODUCTION

Spirocercosis is caused by the spirurid nematode S. lupi that has a worldwide distribution in warm climates [1- 4]. The environmental conditions; including soil type and pH, temperature, rainfall, solar radiations may prolong the source of the intermediate hosts [5- 7]. This nematode is infective for many species including human beings and carnivores [2, 8, 9].

S. lupi is accepted in Veterinary Medicine as the cause of true malignancies in dogs. Aortic lesions and esophageal nodular granulomas are pathognomic lesions for this disease [10- 12].

The infective larvae are released into the stomach, penetrate its mucosa and begin a migration, reaching the thoracic aorta within 3 weeks [13- 15]. Most of the larvae leave the aorta approximately 3 months after infection and cross over to the esophagus. Following a prepatent period of 4 to 6 months required for granuloma formation, the deposited embryonated eggs pass into the esophageal lumen through small fistulous tracts. Depending on the female worm population, infected dogs may continuously or intermittently shed large numbers of eggs, frequently in excess of one million per day [5 -18].

The intramural granulomatous nodules of adult S. lupi are located in the distal thoracic esophagus, this infestation is usually subclinical. When it is clinically evident, it is characterized by regurgitation, odynophagia and excessive salivation [5, 6, 17]. The melena or dyspnea may be the sole presenting complaint [2, 3, 8, 9, 19].

Spirocercosis, either clinical or subclinical, is diagnosed by Teleman's sedimentation [2, 3, 19].

Normocytic, normochromic, non regenerative anemia and neutrophilic leukocytosis are clinicopathologic indicators of clinical spirocercosis [2, 3].

The radiographic feature associated with S. lupi-induced granuloma is a caudodorsal mediastinal mass [2, 3, 9].

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Esophagoscopy and sometimes gastroscopy are the diagnostic modalities of choice for direct visualization of *S. lupi* nodules [2, 3, 20].

The goals of the present scientific declaration are to provide an overwhelming endoscopic outlook of esophageal and gastric pictures of *S. lupi* that have been instituted during dogs' examination, also to overlay the methods approved for diagnostic importance and to highlight on the vet's practice for treating dogs suffering *S. lupi* which has a zoonotic importance for human beings.

MATERIALS AND METHODS

Dogs of the Study: The current investigation has been realized on 35 mongrel dogs affected with Spirocercosis, their age varied from eight months to five years, of unlike sex and different weight. They showed an imminent endoscopic pathological lesion in the esophagus and the stomach. Further 5 dogs are judged to have normal endoscopic viewing of upper gastrointestinal tract are considered as control group.

Dogs Preparation for Endoscopy: Each dog is fasted 24 hours for esophagoscopy and gastroscopy. The dogs are put under general injectable anesthesia which comprises atropine sulphate (0.05-0.1mg/kg.b.wt.), xylazine (1mg/kg.b.wt.) and ketamine (10-15mg/kg.b.wt.) or propofol (2mg/kg.b.wt.) [21].

Diagnosis March: as shown in Fig. (2)

Lateral contrast radiographs are obtained from all dogs using Fischer x-ray unit, with radiographic settings ranged from 44-60 KV; 100 mAs at 0.1 second and 100 FFD [22].

Fecal samples are collected for fecal floatation test (Teleman's sedimentation) and examined under microscope for detecting any internal parasite eggs [7, 23].

Blood samples are collected on heparinized tubes from the cephalic vein for complete blood picture and the collected data were analyzed according to Kahn [24].

The dogs are put in left lateral recumbence for the standard procedure of upper digestive tract endoscopic examination. Moderate insufflations are employed during inspection of the esophagus and stomach [25]. During the endoscopic examination, the movies are computer recorded via Easy Cap connector.

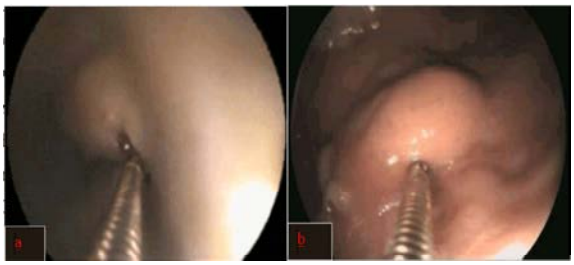

Fig. 1: The obtained biopsy sampling.
a: Esophageal biopsy sampling from the broad base of the founded nodule.
b: Gastric biopsy sampling from the founded polyp.

Histopathological Examination: Several superficial and deep seated punch biopsy samples are taken by using sterile biopsy forceps (Fig. 1). Those samples are fixed in 10% neutral-buffered formalin, routinely processed and stained with hematoxylin and eosin [26].

Treatment Trial: Each affected dog received ivermectin (600 µg/kg SC), two times at 14-day intervals, combined with oral prednisolone (0.5 mg/kg) given q12h for 2 weeks and then once daily for an additional week according to Mylonakis *et al.* [27].

Statistical Analysis: All the collected data are evaluated statistically by an ordinary ANOVA using GraphPad prism package program, using student t-test with the help of Office Excel package 2007 according to Smyth [28].

RESULTS

Clinical Observations: All the 35 clinically infected dogs were suffered from dysphagia, regurgitation. Out of them 10 dogs had suffered melena approximately 29% of the total number of proved positive to infestation of S. lupi.

Radiological Results: The different sizes of the esophageal nodules became more obvious after contrast radiography and appeared as radiolucent cauliflower masses surrounded by radiopaque zone of the barium contrast as shown in (Fig. 2.a).

Parasitological Results: In all infested dogs; the microscopic detection showed thick walled eggs with larva inside, the egg measured (mean length 34 µm x mean width 13 µm) as shown in (Fig. 2.b).

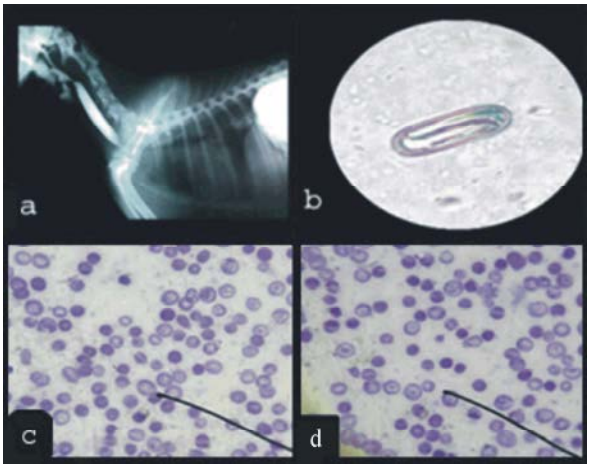

Fig. 2: Illustrating the obtained findings of the diagnostic methods

a: Lateral thoracic contrast radiography. Note: the caudodorsal mediastinal mass (black arrows) in the distal portion of esophagus.

b: Teleman's floatation showing Spirocerca lupi embryonated egg (1000x).

c: Macrocytic hypochromic anemia (black arrow).

d: Microcytic hypochromic anemia (black arrow).

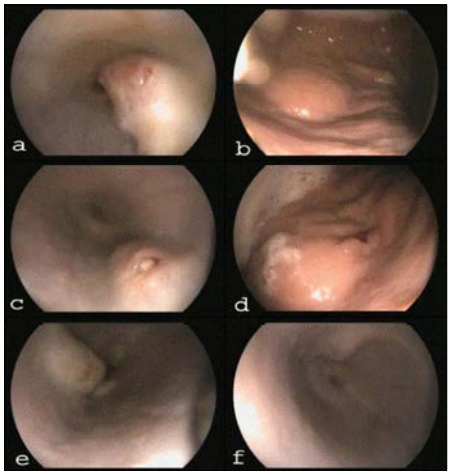

Fig. 3: Showing the endoscopic pictures of Spirocerca lupi lesions, biopsy and treatment evaluation

a: Esophageal nodule with ulcerated surface at 5 O'clock in the distal end of the esophagus.

b: Fundal nodule with smooth surface in the first part of the stomach.

c: Biopsy site after the sampling from esophageal nodule.

d: Biopsy site after the sampling from fundal nodule.

e: Regression of the esophageal nodule at 10 O'clock after one week from the treatment.

f: Regressed esophageal nodule at 12 O'clock after three weeks from the treatment.

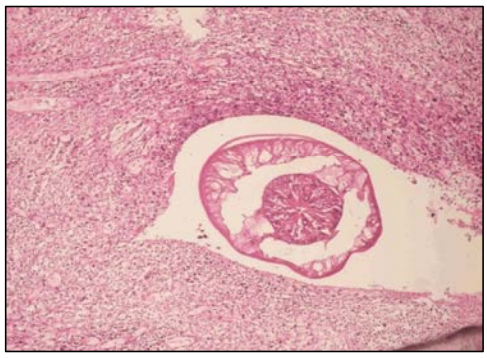

Fig. 4: Photomicrograph showing the S. lupi worm surrounded by severe inflammatory and fibrotic reaction (cross section stained with H & E X 100).

Hematological Results: The dog's blood film revealed macrocytic hypochromic anemia (Fig. 2.c), the dog's blood film of more advanced cases revealed microcytic hypochromic anemia (Fig. 2.d). The statistical analysis of haematological results proved insignificant reduction in Hb (g/dl) concentration, total RBC counts but the mean corpuscular hematocrit concentration (MCHC %) was below the normal ranges. The mean corpuscular volume (MCV %) was over the normal value of 23 dogs (65%) and MCV % of 12 dogs (35%) was below the normal ranges. Also, the differential leukocyte count of 12 dogs (35%) showed an increase in WBC count and neutrophilia.

Endoscopy Results: The dog's esophagoscopy revealed smooth surface nodule broad based protuberances (granulomas) with a nipple-like orifice with ulcerative surface in the distal end of thoracic part of esophagus, it blocked approximately 50% of the lumen (Fig. 3.a). Endoscopic examination of the dog's stomach revealed smooth surface nodule broad based protuberances in the fundal part of the stomach (Fig. 3.b). Endoscopic sampling was feasible and no recorded complications as perforation or hemorrhage were occurred, biopsies were picked up from esophagus and stomach nodules (Fig. 3.c&d). The nodule reduced into a smaller nodule one week after treatment (Fig. 3.e), then appeared as residual fold three weeks after treatment (Fig. 3.f).

Histopathological Results: The presence nematode evoked severs inflammatory reaction and fibrosis around it (Fig. 4). Microscopical examination of biopsy specimens revealed focal necrosis of mucosal epithelium replaced by inflammatory cells infiltration (Fig. 5.a), vacuolation of basal cells of mucosal epithelialium, oedema in lamina propria (Fig. 5.b). The submucosa showed necrosis of

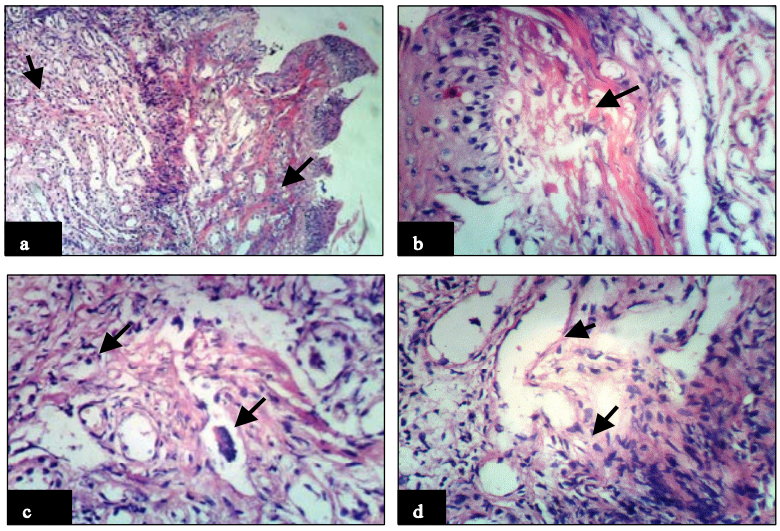

Fig. 5: Photomicrograph showing
a: Focal necrosis of mucosal epithelium replaced by inflammatory cells infiltration, necrosis of submucosal glands and periglandular fibroblasts proliferation (H & E X 100).
b: Vacuolation of basal cells of mucosal epithelialium and oedema in lamina propria(H & E X 400).
c: Necrosis of esophageal submucosal glands and periglandular fibroblasts proliferation (H & E X 100).
d: cystic dilatation of submucosal glands with marked fibrous connective tissue proliferation and inflammatory cells infiltration(H & E X 400).

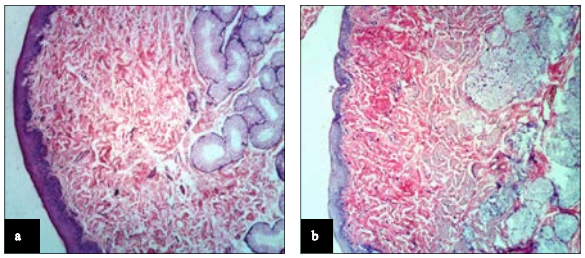

Fig. 6: Photomicrograph showing
a: Esophagus of control dog showing no histopathological changes (H&E X100)
b: Esophagus of dog after treatment showing no histopathological changes (H&E X 100).

esophageal glands, periglandular fibroblasts proliferation (Figs. 5.a & 5.c). Some examined sections revealed cystic dilatation of submucosal glands with marked fibrous connective tissue proliferation and inflammatory cells infiltration (Figs. 5.d).

Histopathological examination of esophageal sections of normal control dog as well as of dog after treatment revealed no histopathological changes with normal mucosal and submucosal layers (Figs. 6. a & 6.b). Histopathological examination of gastric sections of infected stomach revealed lymphoplasmocytic infilteration in lamina properia (Figs. 7).

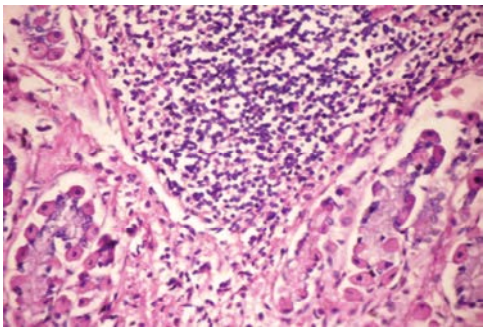

Fig. 7: Photomicrograph showing focal mononuclear cell aggregations in lamina propria (H&E400x).

DISCUSSION

Dogs are infected by ingesting third stage larvae-laden dung beetles as intermediate hosts or by feeding on reptiles, birds, rodents as transport hosts that have fed on beetles. S. lupi in human may vary in its life cycle, using dogs as the sole hosts or using a series of intermediate hosts ranging from beetles that eat fecal material to lizards, chickens, or mice [29]. No infected dog is met in the study with esophageal nodule, his age not less than eight months; this supports the statement of Bailey [5], Sen and Anantaraman [16], Fox *et al*. [17], Chhabra and Singh [30] and Lefkaditis [31].

The clinical diagnosis of canine spirocercosis, especially at early stages, is unreliable and difficult because symptoms vary considerably according to stage of disease, parasite burden, larval migration, aberrant localization and potential complications, thus overlapping a range of other diseases [2,9,32].

Spirocercosis, either clinical or subclinical is diagnosed radiologically by the caudomediastinal mass which is not appeared with the plain radiography. Therefore, plain x-ray is not a good choice for diagnosis; this is concurrent with Mazaki-Tovi *et al*. [2], Mylonakis *et al*. [3] and Dvir *et al*. [9]. In spite of this, the present investigation has been proved that the contrast radiography facilitates the visualization of this esophageal mass by coating it by radiopaque layer; this is in agreement with Evans [33] who stated that barium swallow outlines the esophageal masses and demonstrates the diverticulum. Additionally, X-ray examination may not allow discrimination between tumors and other opaque soft tissue masses. Despite, histological evaluation of endoscopically obtained esophageal biopsies is reliable in diagnosis when tissue is examined with high microscopical magnification [2,34].

Moreover, negative fecal examinations do not rule out spirocercosis, so serial examinations of fecal samples are collected on consecutive days may be warranted to increase the diagnostic sensitivity in accordance to Mazaki-Tovi *et al*. [2] and Mylonakis *et al*. [3]. In addition to this, the spirocerca eggs are detected in the fecal samples after injection of ivermectin by three days; this supports the work of Seneviratna *et al.* [35] who stated that ova may still be detected up to two weeks after the institution of adulticide treatment.

Change in the blood profile with the spirocercosis occurs as a result of compensatory process mechanism which is resulted from iron deficiency. Therefore, the R.B.Cs becomes more lager and appeared as single large blood cells representing macrocytic hypochromic anemia. This is called a post-regenerative macrocytosis. With the time, continuation of iron deficiency the R.B.Cs becomes smaller size representing microcytic hypochromic anemia in accordance to Fry [36]. In the present study; the complete blood picture reveals mild regenerative anemia (macrocytic hypochromic anemia) of 65% of the presented dogs. Moreover, there is non regenerative anemia of 35% of those cases (microcytic hypochromic) approximately similar to the results stated by Mazaki- Tovi *et al*. [2] and Ranen *et al*. [32] who declared that the infected dogs showed mild anemia in almost 50% cases (normocytic normochromic anaemia) of early disease conditions. But, in advanced conditions when nodules transformed into neoplasia, microcytic hypochromic anemia became evident. The degree of anemia differs according to the severity of the infection and hemorrhage-induced iron deficiency which is primary or secondary gastrointestinal disease (neoplasia and ulceration) in accordance to Fry [36]. Neutrophilic leukocytosis is detected in 35% of the infested mongrel dogs with spirocerca oesophageal granulomas, although the clinico-pathological examination is nonspecific as stated by Mazaki-Tovi *et al*. [2], Mylonakis *et al.* [3] and Das *et al*. [37].The haematological tests exhibit non significant reduction in Hb (g/dl) concentration and RBC counts in the positive cases as mentioned by Das *et al.* [37].

Esophageal dysphagia is worsened by concomitant salivary gland necrosis associated with Spirocerca lupi infection in agreement with Mylonakis, *et al*. [3] and Ranen *et al*. [32]. Melena without hematemesis is observed in esophageal Spirocerca lupi granulomas of together with dysphagia due to mechanical obstruction and functional impairment due to ulceration of granulomas which leads to chronic hemorrhagic anemia [6,9,38,39].

Esophagoscopy and gastroscopy are the diagnostic modalities of choice for direct visualization of Spirocerca lupi nodules. Consequently, the distal part of the thoracic esophagus less than 10 cm from the cardia is the best place to visualize the parasitic nodules. Although esophageal neoplastic masses tend to be cauliflower or pedunculated shape, with smooth outline, this is in agreement with Mazaki-Tovi *et al*. [2], Dvir *et al*. [9], Lefkaditis [31] and Ranen *et al.* [32]. These nodules typically appear as broadbased protuberances with a nipple-like orifice from which a worm may occasionally protrude. They may be solitary, multiple, smooth, or ulcerated and they vary in size from an inconspicuous protrusion to a voluminous mass that obstructs most of the esophageal lumen in agreement with Mazaki-Tovi *et al*. [2] and Mylonakis *et al*. [3].

Notably, endoscopic pinch biopsies may also be misleading; they usually detect superficial inflammation rather than a deep-seated neoplastic process. Consequently, surgically obtained esophageal biopsies are essential for reliable histopathologic evaluation of the tissue inflammatory or neoplastic response to the parasite [2,9,32].

The endoscopy is limited in differentiating between an advanced granuloma and neoplasms. Moreover, PCR-based techniques are simple, fast and more convenient and reliable than other methods and the necessary instruments and consumables have ready availability and application for laboratories [40].

Histopathologically; S. lupi associated with actively dividing fibroblasts. These fibroblasts are located between numerous immature capillaries, peripheral to the worms and their migratory tracts in agreement with Bailey [5], Moulton [41] who stated that S. lupi associated esophageal nodules are composed predominantly of fibrocytes with ample mature collagen at initial stage where with the time of nodular maturation; actively dividing fibroblasts become predominant. These fibroblasts are located between numerous immature capillaries, peripheral to the worms and their migratory tracts. Therefore, development of esophageal osteosarcomas and fibrosarcomas has been reported in dogs infected with Spirocerca lupi, particularly in endemic areas, whereas there is an extensive fibrous tissue proliferation. The nodular lesions showed different histological pictures according to their constituents and their stage of development. Nodules showed diffuse thickening in the oesophageal wall with fibrous tissue accumulation, mature fibrocytes and intervening lymphoplasmacytic cell. Large numbers of newly formed blood vessel is seen in the loosely arranged fibrous layer giving the appearance of granulation tissue. Large numbers of neutrophils, fewer lymphocytes, plasma cells and macrophages are commonly found in between the diffusely arranged connective tissue layers as recently in accordance to Das *et al.* [37].

It is proposed that non-neoplastic S. lupi nodules could be divided into two stages: an early inflammatory stage, where the nodule is characterized histologically by fibrocytes and abundant collagen and a preneoplastic stage, where the nodule is characterized by the presence of activated fibroblasts (more mitoses and a greater proportion of fibroblasts that showed some degree of atypia) and reduced collagen. Both stages are characterized by lympho-plasmacytic inflammation. The neoplastic cases generally had less inflammation; the inflammation was predominantly suppurative and the foci of suppuration were typically confined to necro-ulcerative areas in the tumour [42,43].

The medical treatment is preferred about the surgical intervention due to surgical removal of esophageal nodules or tumors have been largely unsuccessful due to the frequently extensive nature of the lesions and the postsurgical complications, this is in agreement with Fox *et al*. [17], Fincher *et al.* [44] and Colgrove [45]. However recently, the excision of esophageal tumors using a partial esophagectomy technique, with or without complementary doxorubicin-based chemotherapy appears to substantially prolong survival time and improves quality of life in dogs with S. lupi-associated sarcomas [32,46].

The administration of doramectin (200 µg/kg SC) at 14-day intervals for three treatments is effective in achieving clinical remission in all cases and eliminating esophageal nodules 70% of dogs; increasing the dose to 500 µg/kg PO daily for an additional 6-week period led to the complete disappearance of parasitic nodules in the remaining 30% of dogs. So, the doramectine is not give satisfactory percentage result in comparsion to ivermectin within two weeks as stated by Berry [47], in addition to, the experimental studies indicate that when the Ivermectin is used with higher doses it has wide spectrum of activity as denotes by Lefkaditis [31] and Paradis [48], so that, the implemented treatment in the present study was Ivermectin (600 µg/kg SC), administered twice at 14-day intervals, combined with oral prednisolone (0.5 mg/kg) for 2 weeks and then once daily for an additional week, led to negative results of fecal testing and almost complete nodular regression in 100% of treated dogs; complete clinical remission is accomplished in 85% of the dogs, implying the presence of residual esophageal dysfunction in agreement with Mylonakis *et al.* [27].

CONCLUSIONS

The present declaration gives rise to the various diagnostic procedures adopted to diagnose the S. lupi in dogs and stated the recommended line of treatment for such parasitosis hoping to guide the veterinarians to diagnose and treat such disease as well and pave the way for its prevention as worldwide spreading zoonotic disease and participating a major role in human health and welfare.

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