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Pathological and Microbiological Investigations on Alimentary System Lesions of Dogs: Oral, Oesophagus and Stomach

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Abstract: Pathological changes along the alimentary tract (oral cavity, oesophagus and stomach with cardia, fundus and pylorus sections) were examined along with their frequency and roles in mortality among canines. This study was performed to determine the pathological changes, the incidence and the definition (macroscopic and microscopic) of these lesions in the alimentary tract of dogs. It also examined whether or not these lesions caused pathogenesis of secondary diseases or death. Total 100 dogs of various breeds aged from 1 week to 114 months (9 and a half years) were studied. For histopathological examinations, lesions in the organs were scored in detail as mild, moderate and severe. The macroscopic lesions identified were the types of infections generally found in the gastrointestinal system. Included in these observations were focal, greyish-red and white erosions in the tongue; hyperemia and haemorrhage in the oesophagus and hyperemia, bleeding, mucosal thickening, foreign bodies (in 3 dogs) and ascarids (in 5 dogs) in the stomach. On a microscopic level, severe histopathological observations included bacterial colonies, degeneration, desquamation and necrosis in the epithelium; hyperemia, haemorrhage and fibrosis in propria; degeneration, desquamation, dilatation and hyperplasia in glands and depletion in lymphoid tissue were observed and scored. Immunohistochemistry revealed the positive staining for Canine Parvovirus-2 (CPV-2) in 90 dogs, Canine Distemper Virus (CDV) in 27 dogs and 24 dogs with both of these viruses. Parasitological investigations revealed 5 dogs with ascarids. Results from pathoanatomical diagnoses demonstrated oesophagitis in 10 cases, acute gastritis in 11 cases, chronic gastritis in 17 cases and eosinophilic gastroenteritis in 1 case. Thus, the collective results suggest that primarily parvoviral enteritis but also other lesions and diseases in the gastrointestinal tract cause death in these dogs.

Key words: Alimentary system, diseases, pathology, immunohistochemistry, dog, Turkey

INTRODUCTION

Treating diseases of the gastrointestinal system is crucial in veterinary medicine because of the many physiological actions of this system such as glandular secretions, mucosal cell absorption and intestinal motility. The oral cavity and mucosa of the oesophagus are constantly enduring trauma such as perforations, contusions, erosions made by viruses, ulcers, inflammation, hyperplasia, parakeratosis and neoplasia. With uraemia, a previous study showed that small, erosive and ulcerative inflammation occurred inside the oral cavity and the edges of the ulcers were proliferative and hyperemic (Jubb *et al*., 2006). With reflux oesophagitis, hyperemia along with longitudinal erosions and ulcers occurs (Zarling, 1998). Oesophagitis is rarely caused by infections and is generally the result of irritating chemicals, foreign substances and regurgitation of gastric acid (Quinn *et al*., 1997; Mazaki-Tovi *et al*., 2002).

Gastritis among canines is common because of their irregular dietary habits. Due to low pH levels, gastritis caused by bacterial contamination is rare (Quinn *et al*., 1997). Acute gastritis is generally caused by the consumption of irritating substances, diet-related allergy, systemic viral infection and parasitism. Chronic gastritis usually results from long-term consumption of anti-inflammatory drugs, hepatorenal failures and infectious agents (Quinn *et al*., 1997; Webb and Twedt, 2003).

Chronic hypertrophic gastritis has been observed in dogs but the underlying rationale for this occurrence is unknown. Macroscopic hypertrophy of the gastric gyri was observed in the fundus region of the gastric mucosa

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and could be matched in the gyri of brain. With eosinophilic gastroenteritis, acites and thickening of the gastric and enteric wall were documented (Goto *et al*., 1983; Chira *et al*., 2005). Peptical ulcers which have been generally seen in adult canines, thoroughly localize on the pyloric antrum and the cranial parts of the duodenum (Jubb *et al*., 2006). *Toxocara canis* and *Toxascaris leonina* are frequently found in the small intestines of canines (Hendrix, 1998). However, there is one contradicting report for *T. canis* that states that these ascarids are rarely localized in the stomach of canines. Canine parvoviral infection occurs in 6-20 weeks old puppies because of their susceptibility to this virus. Stomach mucosa becomes congested, haemorrhagic and coloured with bile during parvoviral enteritis infection (Macartney *et al*., 1984; Jubb *et al*., 2006). McKnight *et al*. (2007) identified pseudo-cytoplasmic and intranuclear inclusions in the tongue epithelium of CPV-2 infected canines. Concurrently, a related study stated that sampling the canine tongue rather than the small intestine was better for diagnosing canine parvovirus. Infection of the gastroenteric epithelium occurs secondary to viremia has been reported. Canine Distemper Virus (CDV) infections of canines present as acute, subacute or chronic infections or they may not be seen clinically (Van Moll *et al*., 1995). With Canine Distemper (CD), mild congestion and aspirates (catarrhal or haemorrhagic) were observed in the gastrointestinal tract (Lan *et al*., 2006; Liang *et al*., 2007). Through histopathological and immunohistochemical examinations, diagnosis of the types of lesions and inclusion particles can be made. These examinations can also diagnose syncytial cells and the CDV antigen. Immunocolouring has been performed on the oesophagus and the multilayered leveled epitheliums of the tongue and gastrointestinal mucosa.

Researchers audited the scientific literature and discovered that there has not been a study that evaluates gastrointestinal lesions or estimates the importance and frequency of gastrointestinal diseases among canines. The aim of this study was to examine the pathological differences and determine the frequency of the lesions made by infectious and non-infectious agents in the gastrointestinal system of canines. These lesions are frequently observed and often lethal. In this study, lesions that occurred up to and including the stomach have been examined.

MATERIALS AND METHODS

This study was performed on canines of various age and race that were delivered over the span of a year for necropsy after death to Selcuk University, Faculty of Veterinary Medicine (Department of Pathology) mainly from the animal houses of the Konya Metropolitan Municipality. This study was approved by the ethical board of the Faculty of Veterinary Medicine of the Univercity of Selcuk, Konya, Turkey (Approval No. 2007/18). A total of 148 canine necropsies were executed however, only 100 dogs (51 male and 49 female) that had macroscopically invisible autolysis were reserved for this study. Following systematic necropsies in depth examinations of the gastrointestinal system were performed. Photographic documentation and sampling of the lesions were also performed on pathological regions. For microbiological diagnosis in this study, aspirates from jejunum and ileum were sampled. Intestinal cuts had been sampled and both ends of the cuts had been tied by strings and mesenterial lymph nodes were also sampled. In depth, parasitological examinations were not the focus of this study, thus parasites that were discovered during necropsies were taken to the Department of Parasitology at Selcuk University, Faculty of Veterinary Medicine for identification.

Histopathological examinations: In this study, tissue samples taken from oral cavity, oesophagus and regions of the stomach were fixed by 10% buffered formaldehyde solution. After common tissue processing procedures had been performed in an automatic tissue processor (Leica TP1020, Leica Microsystems, Nussloch, Germany), samples were coloured with Haematoxylin and Eosin (HE) and in proper cases with Periodic-Acid-Schiff (PAS) stain, van Gieson stain, May-Grunwald Giemsa stain, Lendrum's inclusion stain or Turnbull's blue (Luna, 1968). These samples were observed under a binocular light microscope (Olympus BX51, Tokyo, Japan). For noteworthy cases, microscopic images were photographed and transferred into a digital environment (Olympus DP12, microscopic digital camera systems, Tokyo, Japan). About 5 micron thick slices were isolated from all cases and placed on polylysined lams for immunohistochemical examination.

Differences in the HE coloured slices during histopathological examinations were scored as in Garcia-Sancho *et al*. (2005).

For overall evaluations, slices either had no lesions (-), a slight lesion/few cells (+), mild lesions/moderate number of cells (++) or severe lesions/many cells (+++). Differences amongst the epithelium, propria and submucosa were evaluated according to the presence and severity of lesions such as degeneration, necrosis, desquamation, erosion, ulcer, mononuclear cell infiltration, neutrophil and eosinophil infiltration,

Table 1: Microscopical findings, numbers and severity of observed findings in organs of alimentary tract

	Epythelium				Propria							Submucosa		
N:100	Degeneration necrosis desquamation	Inclusion	Bacterium	Erosion ulcer	Degeneration necrosis desquamation in glands	Inclusion in glands	MNC infilt.	Neutrophil eosinophil	Fibrosis	Hyperemia hemorrhage oedema	Dilatation in glands	Depletion lymphoid tissue	MNC infilt.	Neutrophil
Tongue	++ (1)	-	++ (2)	4	-	-	+ (1)	+ (2)	+ (1)	7	-	-	-	-
	+++ (4)						++ (3)	++ (1)	++ (1)					
								+++ (1)						
Oesophagus	++ (1)	-	++ (1)	-	-	-	+ (8)	+ (3)	++ (2)	15	-	-	-	-
	+++ (8)						++ (1)	++ (2)						
							+++ (1)							
Stomach	+ (10)	-	++ (1)	-	+ (3)	-	+ (47)	+ (4)	+ (4)	45	-		+ (2)	
Cardia	++ (12)				++ (1)		++ (4)		++ (2)			++ (1)		-
	+++ (8)				+++ (4)				+++ (1)					
Fundus	+ (14)		+ (3)		+ (19)		+ (66)	+ (10)	+ (31)				+ (7)	++ (1)
	++ (25)	-	++ (2)	-	++ (20)	3	++ (6)	++ (4)	++ (3)	87	4	-	++ (3)	+++ (1)
	+++ (44)				+++ (18)		+++ (3)	+++ (5)	+++ (1)					
Pylorus	+ (8)		++ (1)	-	+ (5)		+ (65)		+ (7)			+ (3)		
	++ (27)	1	+++ (1)		++ (4)	4	++ (16)	+ (14)	++ (6)	65	9		+ (3)	++ (1)
	+++ (34)				+++ (2)		+++ (1)	++ (4)	+++ (2)				+++(1)	

Overall evaluation: No lesion; + : Slight lesion; ++ : Mild lesion; +++ : Severe lesion; No. in the table indicates amounts of findings in cases; Mononuclear Cell infiltration (MNC): In each slice under ×20 oculus 5 different area has been selected and 1-20 cells mean +, 21-50 cells mean ++, >50 cells mean +++; Neutrophil and Eosinophil granulocytes infiltration: In each slice under ×20 oculus 5 different area has been selected and for mean value of neutrophil and eosinophi, l granulocytes 1-5 cells mean +, 6-20 cells mean ++ and >20 cells mean +++

fibrosis, inclusion particles, hyperemia, haemorrhage, oedema, dilatation of the glands, bacterial colonies in lumens and depletion of the lymphoid tissue. For all slices, 5 areas were selected under x20 oculus, the number of cells present was calculated and a related average was recorded. With respect to the mean of these 5 areas, infiltration of mononuclear cells, neutrophils and eosinophils on each organ was evaluated (Table 1). Glandular differences in the slices (degeneration, necrosis, desquamation, dilatation and hyperplasia) were classified as 10-20% (+), 21-50% (++) or >50% (+++). During the review of available literature, no other study related to this subject was found with objective criteria for modelling the scoring system, other than studies with subjective evaluations.

Immunohistochemical examinations: For immunohistochemical confirmation of the distemper and parvovirus infections, methods of Immunohistochemical techniques stated by Rhind (2001), Svara *et al.* (2003), Ramos-Vara (2005), Baum *et al.* (2007) and Buchwalow and Bocker (2010) were utilized. Antigen retrieval was performed in a microwave oven for the slices on polylysined lams that were sampled from tongue, oesophagus, stomach (cardia, fundus and pylorus) and cerebellum tissues in citrate buffer. Colouring has been done by Envision technique (Novolink™ polymer detection systems) via Shandon's manual colouring kit (Shandon Sequenza, ThermoShandon, Cheshire, England) at room temperature. Mouse monoclonal anti-canine distemper virus (1:200 dilution; Abcam ab20332) and mouse monoclonal anti-Canine Parvovirus (1:50 dilution; Abcam ab7669) were used. For chromogens, DAB (3,3'-diaminobenzidine, SkyTek Chromogen/substrate kit) was used for distemper while DAB and NovoLink™ DAB substrate buffer (Polymer) were used for parvoviral enteritis. Additionally for parvoviral enteritis, DAB Enhancer solution (Bond™ DAB Enhancer, AR9432) was applied. Reverse colouring was accomplished with Harris's haematoxylin. For both diseases, negative controls were coloured using identical colouring procedure except PBS was used instead of primary antibody. All the samples that were coloured were evaluated under light microscope.

Parasitological examinations: Parasites that were discovered inside the stomach during necropsies were transferred into a Petri case and species were identified. In depth analysis of parasitology among canines was not the aim of this study thus, parasitological examinations of gaita and gastric aspirates were not made.

RESULTS

Macroscopical findings: On the tongue of 3 dogs, macroscopic erosions with focal localization (greyish-red and white) were observed. In 1 canine, there were remnants of food in the pharynx while another canine had petechial haemorrhage in regions of the upper and lower oesophagus. Haemorrhage was observed in the cardia of 2 dogs in the fundus of 8 dogs and in the pylori of 3 dogs (Fig. 1).

In the stomach, 4 dogs exhibited thickening of the gastric mucosa, 5 dogs had parasites (*Toxocara canis*) and 5 dogs had foreign bodies such as bovine ear tag or metal (Fig. 2). In the stomach of a dog which obviously had a history of haemorrhage, dark black lesions (5 focal lesions; circular to linear) that were obviously blistered were observed throughout the entire pylorus, pyloroduodenal passage and also in the fundus (Fig. 3).

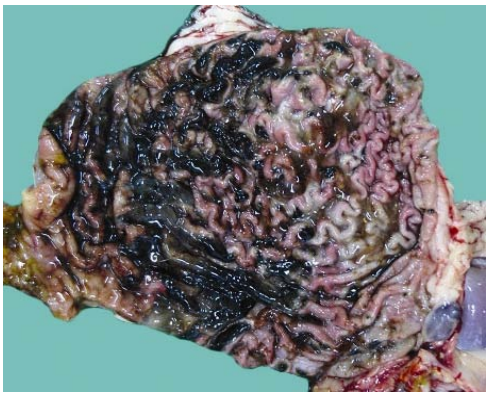

Fig. 1: Hyperemia and hemorrhage in stomach

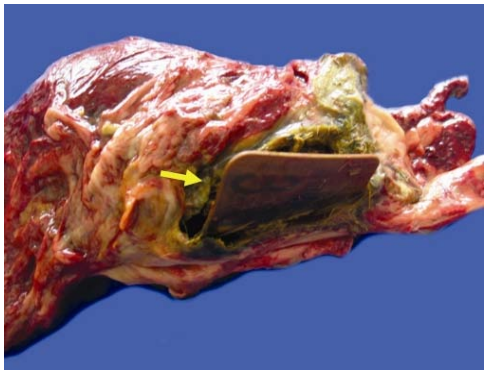

Fig. 2: Foreign body in the stomach (bovine ear tag) (arrow)

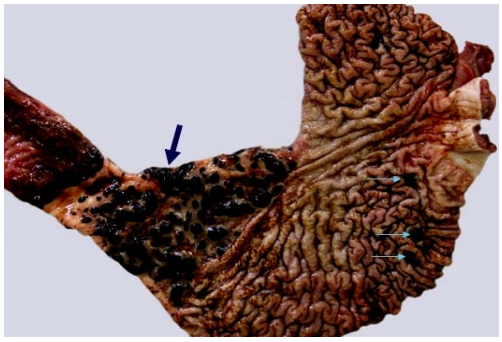

Fig. 3: Dark blackish-colored lesions significantly raised shaped a line or focal round in fundus (thin arrow) and in transition region to duodenum with all of pilorus region (thick arrow)

Dissection of these lesions revealed that coagulated blood (1 cm in diameter) had caused these lesions to the submucosa.

Histopathological findings: Results from the microscopic examinations of tongue, oesophagus and stomach regions (cardia, fundus and pylorus) of 100 dogs with related

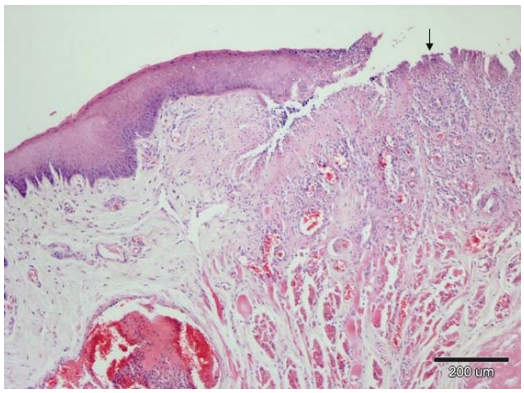

Fig. 4: Tongue. Erosion and ulcer (arrow), mononuclear cell infiltration, infiltration of neutrophil granulocytes

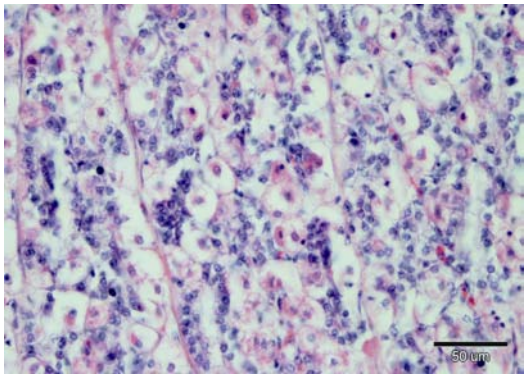

Fig. 5: Fundus. Hydropic and vacuolar degeneration in parietal cells

details are shown in Table 1. In one case, thrombosis in the propria of the tongue was observed. Erosion and ulcer were seen in tongue of 4 dogs (Fig. 4). Histopathological analysis diagnosed glossitis in 4 canines.

Necrotic oesophagitis was diagnosed in 3 dogs which was evidenced by the presence of diffusive necrosis in oesophagus epithelium and propria as well as infiltration by neutrophils, macrophages and mononuclear cells. Acute oesophagitis was diagnosed in 2 dogs because of the presence of neutro phil infiltration, macrophages and hyperemia. Chronic oesophagitis was diagnosed in 5 dogs because of an intense mononuclear cell presence and connective tissue hyperplasia.

Results on the epithelium, propria and submucosa of all gastric regions (cardia, fundus and pylorus) for all dogs are shown in Table 1. In 37 of the 100 dogs, there was hydropic and vacuolar degeneration of parietal cells (Fig. 5) as well as coagulation necrosis (Fig. 6) in the propria of the fundus. In 9 dogs, calcification of parietal cells was also observed. Hyperemia and hemorrhage in

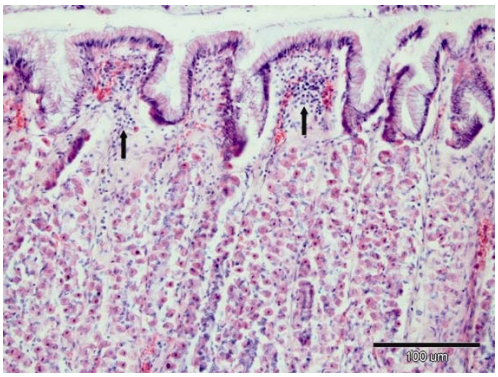

Fig. 6: Fundus. Mononuclear cell infiltration in propria (arrows) and necrosis in parietal cells

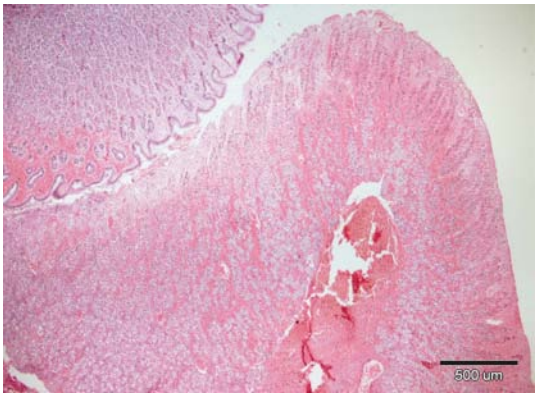

Fig. 7: Fundus. Severe hemorrhage in propria and submucosa

stomach were observed and shown in Table 1 (Fig. 7). Intracytoplasmic and intranuclear inclusion bodies were determined in gland epithelials (Fig. 8).

In the overall evaluation of stomach, acute gastritis was diagnosed in 11 dogs which was evidenced by neutrophil (Fig. 9) and macrophage infiltration, hyperemia and oedema. Chronic gastritis was diagnosed in 17 dogs because of intense mononuclear cell presence and fibrosis. Eosinophilic gastritis was diagnosed in a dog. Chronic atrophic gastritis was diagnosed in a dog due to observed diffusive or follicular lymphoid cell infiltration along with parietal cell loss, atrophy and mucosal slimness. Chronic hypertrophic gastritis was diagnosed in a dog because of hypertrophy of the fundus mucosa and mononuclear cell infiltration between glands of the propria and proprial oedema.

Immunohistochemical findings: Occurrences according to the organs of the immunohistochemical colouring results, the number of cases and concentration of colourings are shown in Table 2. It was observed that the most concentrated and numerous colouring was seen in

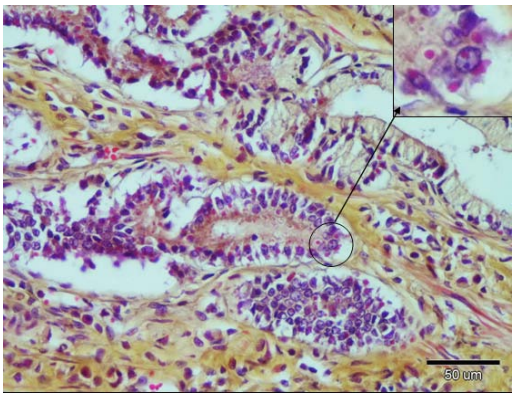

Fig. 8: Pylorus. Intracytoplasmic and intranuclear inclusion bodies in gland epithelium, Lendrum's stain

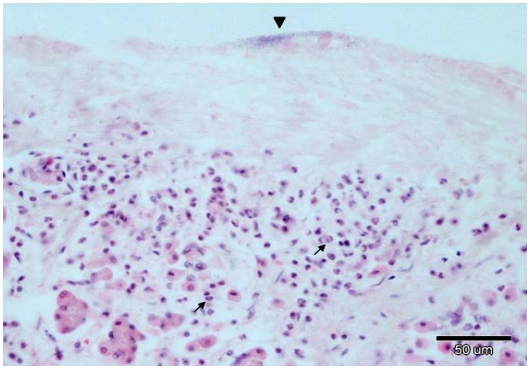

Fig. 9: Fundus. Bacterial colonies and necrosis in lamina epithelialis (arrow head), neutrophil granulocyte infiltration in propria (arrows)

Table 2: No. of cases that are immunohistochemically positive and the concentration of the stain according to organs

	Parvovirus (CPV)		Distemper (CDV)	
Organs	Amount of positive cases	Colouring degree	Amount of positive cases	Colouring degree
Tongue	-	-	-	-
Oesophagus	7	+5	-	-
		+++2		
Cardia	64	+55	23	+9
		++5		++4
		+++4		+++10
Fundus	90	+38	26	+10
		++27		++4
		+++25		+++12
Pylorus	65	+57	24	+8
		++6		++4
		+++2		+++12
Cerebellum	-	-	6	+1
				++1
				+++4

the fundus region for each disease. In this study, 90 of the 100 dogs were diagnosed with CPV-2, 27 dogs were diagnosed with CDV and 24 dogs had both CDV and

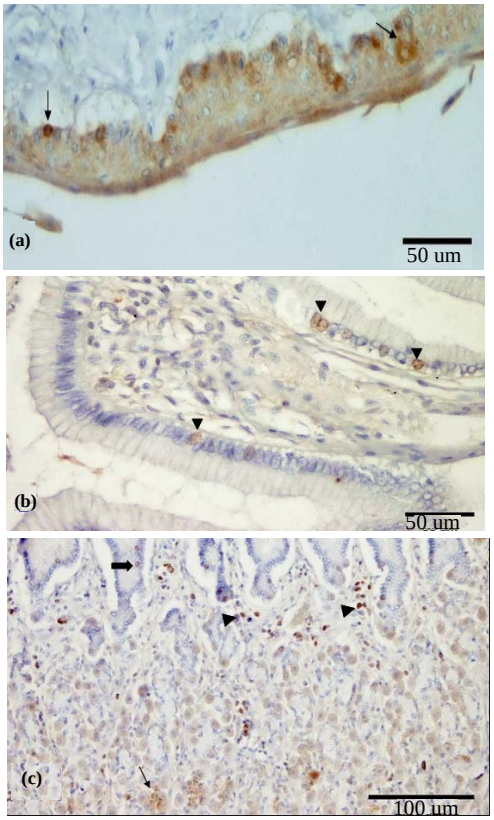

Fig. 10: a) Positive staining in oesophageal epithelium (arrows); b) positive staining in cardia epithelium (arrow heads) and c) fundus. Positive staining in lamina epithelialis (thick arrow), glands (thin arrow), mononuclear cells (arrow heads), CPV-2 and Streptavidin-biotin peroxidase

parvovirus. Immunocolouring revealed CDV antigens in 26 cases, however in one case it was noticed that the positive reaction had occurred in the cerebellum without colouring in the gastrointestinal system viscera. In 5 cases, colouring was observed in the gastrointestinal system and cerebellum. Immunocolouring had been applied to the cerebellum of all dogs but only 6 cases presented positive cerebellar reactions while 26 cases had positive reactions in the stomach. Thus, it was determined that distemper infection localizes to the gastrointestinal system more frequently than the nervous system among the subject canines. In the study, it was observed that 90% of cases are positive with parvovirus and 24 of the 27 distemper cases had parvovirus. In the diagnostic colourings of the CPV-2 antigen in the gastrointestinal system, positive reactions were observed as small, brownish granulated reactions in the cytoplasm of epithelial cells, glandular cells, mononuclear cells and macrophages (Fig. 10). CDV antigen was coloured

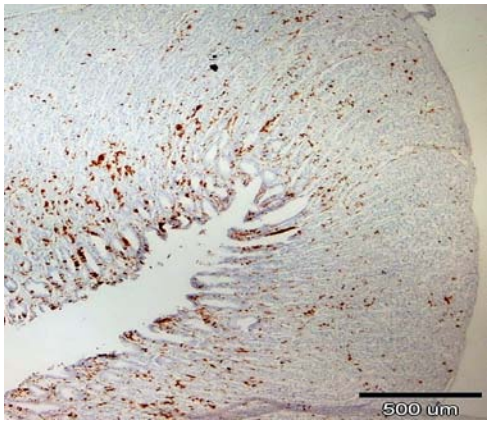

Fig. 11: Fundus. Positive staining in epithelialis and mononuclear cells, CDV and Streptavidin-biotin peroxidase

independently because of cytoplasmic shattering caused by the antigen retrieval technique. This colouring presented as yellowish, brown granulated forms in the nuclei and cytoplasm of gastrointestinal epithelial cells, the desquamated epithelial cells and the cytoplasm of macrophages (Fig. 11).

Parasitological findings: During the macroscopic examinations of organs, *Toxocara canis* was observed in the stomachs of 5 dogs.

DISCUSSION

In this full-scale study, pathological changes that occur in the gastrointestinal system organs (oral cavity to pylorus of stomach) in dogs were extensively defined and supported with microbiological and immunohistochemical analysis. Previous studies (field research and case reports) focused mainly on individual diseases and no full-scale pathological study on the gastrointestinal system has ever been performed. In this study, pathological changes in oral cavity, oesophagus and stomach are evaluated and considered along with parasitological, microbiological and immunohistochemical data. These results were compared to data from past literature. Oral eosinophilic granuloma can be considered when focal granuloma or screwed ulcer is observed in oral cavity tissue (Carlton and McGavin, 1995). Cyanosis in the buccal mucosa and tongue along with greyish-brown ulcer in cavum oral might present in uraemia cases (Jubb *et al*., 2006). In this study, macroscopic changes were only observed on tongue but no changes were observed on buccal mucosa, labia, gingiva and palatum. Focal erosions and ulcers were detected on tongue. Mono-nuclear cell infiltration around ulcers and

neutrophil infiltration due to bacterial contaminations were observed. The researchers propose that oral eosinophilic granulomas were due to the irregular forms of these tongue ulcers. Severity of hydropic degeneration and necrosis on the epithelium is considered to vary according to the nature infection. In a dog, uraemia was considered because of the discovery of pitch black coloured oral mucosa, ulcer and mineralization on the lateral sides of tongue, hyperemia and haemorrhage in stomach and nephrosclerosis in the kidneys. Hyperemia on mucosae and haemorrhagic areas in the upper and lower parts of the oesophagus were observed. Studies have stated that hyperemia or longitudinal erosions and ulcers with entire dilatation of oesophagus are present in reflux oesophagitis (Jubb *et al*., 2006). In addition, Mazaki-Tovi *et al*. (2002) reported that oesophageal and gastric granulomas were detected in oesophagus during necropsies of 14 out of 50 dogs with spirosarcosis. In this study, only in 2 cases were hyperemia and haemorrhage detected macroscopically and these are thought to result from gastric acid or other enzymes like pepsin that had affected the mucosa because of reflux and because no oesophageal granuloma was observed during the study. Hyperemia, haemorrhage and in a dog, colonies of bacterium were observed microscopically. It was reported that oesophagitis rarely occurred because of infection but was mostly related to irritating chemicals, foreign bodies or gastric acid (Quinn *et al*., 1997; Mazaki-Tovi *et al*., 2002). In this study, however hydropical and vacuolar degeneration, necrosis on epithelium, mononuclear cell infiltration in propria and severe degeneration with necrosis in submucosa were observed histopathologically. These pathological changes were thought to originate from CPV-2 (7 cases) infection which was demonstrated by CPV-2 positive reactions during immunohistochemical analysis.

During ultrasonographical screening of dogs with experimental pyloric stenosis, it was shown that narrowing of the pyloric lumen occurred after the 3rd day. In this study, foreign bodies (bovine ear tag, junk, etc.) were detected in the pylori of 5 dogs, thus gastric contents could not be transferred to the intestinal lumen because of the blockage. In 1 of these 5 dogs, hypertrophy of the tunica muscularis and lamina muscularis of the pylorus occurred. It was assumed that pyloric stenosis had occurred because of the narrowing of pyloric lumen. In other dogs, foreign bodies (ear tag, junk, metal, etc.) blocked food passage and irritated gastric mucosa. However, macroscopic examination showed that these mucosae were only hyperemic thus, it was recently supposed that foreign bodies could be consumed with foods. Furthermore, it was stated that foreign bodies were observed in dogs with distemper or rabies and in this study, a dog with a foreign body in its stomach was diagnosed with distemper upon histopathological and immunohistochemical examination. Gastric dilatation and volvulus frequently occur in canines (Jubb *et al*., 2006). In this study, however gastric dilatation and volvulus were not observed in dogs, contradicting this previous report. Nevertheless, stomachs of the subject dogs were generally empty due to the overcrowded environments and the insufficient nutrition conditions of the shelters.

Many previous reports state that the underlying mechanisms for chronic eosinophilic gastrointestinal disease are not fully understood and this disease is typically considered idiopathic (Kleinschmidt *et al*., 2006; Ohno *et al*., 2006; Kobayashi *et al*., 2007). Kalantar *et al*. (1997) stated that irregular thickening of the entire gastric mucosal gyri might occur. Thickening of the mucosa along with chronic inflammatory cell infiltration, glandular atrophic changes and interstitial fibrosis combined with eosinophil infiltration in the mucosa and submucosa were observed in eosinophilic gastritis (Goto *et al*., 1983; Chira *et al*., 2005; Jubb *et al*., 2006). In this study, researchers observed that a single dog with eosinophilic gastroenteritis had foreign body (junk, wood, etc.), parasites and a thickening of the mucosa. The cause of the eosinophilic gastroenteritis could not be identified. It was speculated that eosinophilic gastroenteritis in this dog might result from parasitism because of the significant presence of *Dipylidium caninum* and *Toxocara canis*. It was proposed that parvoviral enteritis occurs mostly among puppies between 6 and 20 weeks of age and the stomach mucosa could become congested, haemorrhagic and coloured with bile (Macartney *et al*., 1984; Jubb *et al*., 2006). In this study of parvoviral enteritis, dog age ranged from a few weeks to 10 years old. McKnight *et al*. (2007) identified psuedocytoplasmic inclusions along with intranuclear viral inclusions on the lamina epithelialis in dogs with CPV-2 infections. Hullinger *et al*. (1998) reported basophilic intranuclear inclusion particles in the tongue and the oesophagus. In this study, no inclusion particles or positive reactions were observed during histopathological and immunohistochemical examinations of tongue but in 7 cases, positive reactions were detected in oesophagus. After immunohistochemical colouring, it was observed that antigens of CPV-2 are coloured as brownish granules with variable concentrations in the nuclei of oesophageal, cardial and pyloric epithelial cells; nuclei and cytoplasm of glandular epithelial cells and cytoplasm of mononuclear cells, macrophages and desquamated epithelial cells. These data are in line with results from previous studies

(Svara *et al*., 2003; Romero *et al*., 2007; Sezer *et al*., 2009). The researchers observed intense positive reactions in glandular epithelium but other studies reported that intense positive reactions occur initially in mononuclear cells (Svara *et al*., 2003). Here in 90 of the 100 dogs examined were diagnosed as CPV-2 positive and this rate far exceeded expectations. Notably during the histopathological examinations, it was observed that few pathological findings related to this infection were present. Thus, the direct roles and effects of these agents on the clinical presentation of the disease are debatable. In 24 cases, CPV-2 and CDV were found concurrently and in these cases might be the result of immunodepression in which one viral infection predisposes the subjects to infection by other bacterial or viral agents. Moreover, based on the bacteriological culture results from intestines which will be discussed in this research, various bacteria were isolated in 58 dogs. Therefore, researchers propose that mostly mixed infections occurred in these dogs.

Canine Distemper Virus (CDV) presents with swollen condition in tonsils; enlargement together with necrosis in the cortex and medulla of the regional lymph nodes and mild congestion and haemorrhage in the gastrointestinal tract along with severe dehydration (Lan *et al*., 2006; Liang *et al*., 2007; Rodriguez-Tovar *et al*., 2007). In this study, dogs with distemper did not present these specific traits, however hyperemia in intestinal mucosa and haemorrhage along with dilatation of lymph nodes were observed. In microscopic evaluations of CDV positive dog tissue, eosinophilic cytoplasmic and nuclear inclusion particles were observed in the epithelium of gastric glands (Kubo *et al*., 2007; Lan *et al*., 2009). The researchers observed inclusions in the stomach that were more significant and intense than those in the intestines. Viral inclusions in clinical cases were reported in peripheral blood cells, especially in lymphocytes (McLaughlin *et al*., 1985) however, no inclusion particles or immunocolouring were observed in peripheral blood cells during the histopathological and immunohistochemical examinations. There are no specific methods for diagnosing distemper histopathologically. One study (Liang *et al*., 2007) proposed that diagnosis can be accomplished by an immunohistochemical screen for CDV antigen combined that is further supported by histopathological examination, i.e., identifying the type of lesions and detecting the inclusion particles and syncytial cells. Immunological diagnosis was reported for gastric mucosal epithelium and the multilayered epitheliums of tongue and oesophagi (Liang *et al*., 2007). The researchers report severe (+++) immunohistochemical positive reactions in the gastric mucosa and glandular epithelium, however none were present on tongue and oesophagus. Thus, the researchers propose that the stomach is the main target for this particular infection. Previous research stated that *Toxocara canis* which is a canine ascarid is generally found in small intestines and rarely localized in the stomach. Another study showed that infestations are more severe in dogs under a year old or even limited to puppies <6 months (Lloyd *et al*., 1991). Oncel showed that the rate of infestation decreases with age. Contrary to these previous findings, the results demonstrate that ascarids are present at various ages. Moreover, contrary to reports that eosinophilic gastroenteritis is associated with ascarids (Chira *et al*., 2005), the researchers report that dogs with ascarids exhibit very few to no eosinophils. In the pathoanatomical macroscopic and microscopic examinations, 11 acute forms of gastritis, 17 chronic types of gastritis, chronic atrophic and hypertrophic gastritis and eosinophilic gastroenteritis were examined. Parasitological examination revealed ascarids in the stomachs of 5 cases.

The leading cause of death was diseases of the alimentary tract in dogs brought as dead to necropsy to the Department of Pathology and provided the majority of the shelters. Among these diseases found in these dogs, parvoviral enteritis was the most abundant (with an occurrence rate of 90%), possibly due to insufficient variolation of street dogs and maternal antibody insufficiency. Even though, this disease was so abundant in dogs taken from the streets, the primary cause of death was probably due to secondary infections caused by the predisposing nature of parvoviral enteritis. The common difficulties associated with clinical and pathological examination of mixed infections were considered during this study and it is accepted that techniques that validate the presence of these agents in tissue like immunohistochemical colouring and PCR are necessary. This study should encourage similar future research in this topic.

CONCLUSION

Finally, the researchers propose that in mass living units such as shelters, newly arrived canines should be temporarily quarantined and variolated for distemper and parvovirus in order to reduce the incidence of these diseases in the mass population.

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