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RESEARCH ARTICLE

Detection of multiple etiologies and comparison and investigation of pathological changes in small and large intestine lesions of dogs

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Köpeklerin ince ve kalın bağırsak lezyonlarında çoklu etiyolojilerin tespiti ve patolojik değişikliklerin incelenmesi ve karşılaştırılması

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Öz

Amaç: Bu çalışma, köpeklerin bağırsaklarında meydana gelen patolojik değişiklikleri araştırmak, yaygınlıklarını tespit etmek ve ölüm nedeni olarak rollerini belirlemek amacıyla yapılmıştır.

Gereç ve Yöntem: 100 ölü sokak köpeğin bağırsakları makroskopik, histopatolojik, mikrobiyolojik ve parazitik olarak incelendi. Parvovirüs ve distemper hastalıkları için immünohistokimyasal boyamalar da yapıldı.

Bulgular: Köpeklerin 61'inde kanama, 8'inde ülser, 36'sında parazit, 14'ünde invaginasyon ve 2'sinde yabancı cisim tespit edildi. Mikroskobik olarak epitellerde dejenerasyon, nekroz ve deskuamasyon, bakteri kolonileri, propriyada hiperemi, kanama, fibrozis ve yangısal hücre infiltrasyonları, kriptlerde dejenerasyon, dilatasyon, hiperplazi, lenfoid dokuda boşalma gibi histopatolojik bulgular gözlendi ve her birinin şiddeti skorlandı. 100 köpekten 90'ında köpek parvovirüs pozitif IHC reaksiyonu ve 27 köpekte de distemper virüsü pozitif reaksiyonu bulundu. Mikrobiyolojik olarak 58 köpekte *Campylobacter* spp., *E. coli*, *Enterococcus* spp., *Streptococcus* spp. belirlendi. Histopatolojik olarak; sırasıyla 29 akut, 51 kronik, 15 lenfositik ve plazmasitik enteritis; kalın bağırsaklarda 5 akut, 10 kronik, 6 diffuz ve 20 idiopatik mukozal kolitis tanımlandı.

Öneri: Köpeklerde, başta Parvoviral enteritis olmak üzere sindirim kanalı hastalıkları ve lezyonlarının birinci derecede ölüm sebebi olduğu ortaya konuldu. Genelde miks enfeksiyonların görüldüğü, sekonder etkenlerin enfeksiyona katılma dönemlerine göre lezyonların arttığı veya primer lezyonların maskelenmiş olduğu dikkati çekti.

Anahtar kelimeler: Hastalıklar, immunohistokimya, ince ve kalın bağırsak, köpek, patoloji

Abstract

Aim: This study was conducted for the purposes of investigating pathological changes in intestines of dogs, detecting prevalence of them and determining their role in being the cause of death.

Materials and Methods: The intestines of 100 dead stray dogs were examined in macroscopic, histopathologic, microbiologic, and parasitic aspects. Immunohistochemical stainings were also performed for parvovirus and distemper diseases.

Results: Hemorrhages in 61, ulcers in 8, parasites in 36, invagination in 14 and foreign objects in 2 of dogs were determined. Microscopically, histopathological findings such as degeneration, necrosis and desquamation in epithelium, bacteria colonies, hyperaemia, hemorrhages, fibrosis and inflammatory cell infiltrations in propria, degeneration, dilatation and hyperplasia in crypts, and depletion in lymphoid tissues were observed and their severity were scored. Canine parvovirus positive IHC reaction was found in 90 out of 100 dogs and canine distemper virus positive reaction was found in 27 dogs, also. Microbiologically, *Campylobacter spp., Escherichia coli, Enterococcus spp., Streptococcus spp.* are found in 58 dogs. Histopathologically, 29 acute, 51 chronic, 15 lymphocytic and plasmacytic enteritis; 5 acute, 10 chronic, 6 diffuse and 20 idiopathic mucosal colitis were diagnosed,respectively.

Conclusion: It has been emphasized that alimentary tract diseases and lesions, mainly Parvoviral enteritis, are the first-degree cause of death in street dogs gathered in shelters. Generally, mixed infections were found, and it has been noticed that the lesions are increased, or primary lesions are masked according to participation period of secondary factors to infection.

Keywords: Diseases, dog, immunohistochemistry, pathology, small and large intestines

Introduction

Alimentary system diseases and lesions are among the most significant causes of death in dogs. In particular, the intestinal mucosa in stray dogs is exposed to damage such as acid burns, contusions and ruptures due to foreign objects, idiopathic ulcers, villous destructions caused by toxic chemicals, and acute, eosinophilic and chronic inflammations. Lesions such as invagination, volvulus, and mesenteric ischemia may also develop in the intestines (Carlton and McGavin 1995, Ceylan et al 2005, Brown et al 2007). Lymphocytic and plasmacytic enteritis (LPE) considered to be the most common cause of chronic diarrhea and vomiting in dogs and is an idiopathic chronic gastrointestinal disease (Garcia-Sancho et al 2005, Ohno et al 2006). In general, it is noted that the information relating to colon lesions is insufficient other than a few specific diseases. Idiopathic mucosal (lymphocytic and plasmacytic) colitis is one of the most common colitis types in dogs (van der Gaag 1988, Roth et al 1990). Viral infections such as canine parvovirus (CPV), canine coronavirus, canine rotavirus, and canine distemper virus (CDV) are defined as the main causes of severe enteritis in dogs (Webb and Twedt 2003, Haligur et al 2009). Having enteric, cardiac, and neurologic forms, parvovirus infection is an important and fatal viral disease, especially for young dogs. Acute fibrinous-necrotic or hemorrhagic enteritis is observed in the enteric form, as well as typical histopathologic lesions such as villous atrophy, degradation of propria, depletion of Peyer's Patches, necrosis, and dilatation in crypts (Macartney et al 1984, Agungpriyono et al 1999, Svara et al 2003, Tunca and Toplu 2007, Haligur et al 2009). Canine distemper is also a generalized and very contagious, fatal viral disease for canines, particularly those not vaccinated or having insufficient immunity (Lan et al 2006, Hammer et al 2007). Immune staining for CDV antigen is notified on tongue, oesophagus, stomach, and intestine (Liang et al 2007). Microscopically, lymphocytic and plasmacytic infiltration into propria, catarrhal enteritis, eosinophilic cytoplasmic and nuclear inclusion bodies in crypt epithelial cells have been observed (Lan et al 2009).

Among bacterial factors, *E. coli* forms significant part of facultative anaerobes available in the intestines in normal condition and it is notified that it is sometimes isolated from the feces of dogs with diarrhea (Drolet et al 1994, Beutin 1999, Holland et al 1999, Goffaux et al 2000). *Campylobacter jejuni* is isolated from dogs with bloody or mucous diarrhea and some dogs with no diarrhea, asymptomatic animals, and dogs with parvoviral enteritis or other viral infections also (McDonough and Simpson 1996, Brown et al 2007). Enterotoxemia is rare in dogs (Sasaki et al 1999) and generally formed by non-typed *Cl. perfringens* and *Cl. difficile* (Brown et al 2007). In relation to the parasites; cestodes such as Diphyllobothrium latum, Dipylidium caninum, Spirometra spp. and Taenia spp., ascarid infection in line with Toxocara canis and Toxoscaris leonina as well as sometimes giardiasis diarrhea are observed (Ayaz and Tınar 2006, Umur et al 2006).

Although there are some studies performed on various specific diseases in the alimentary system of dogs, no comprehensive research scanning the whole tract in the aspect of pathological changes is found. This study was aimed to examine all available lesions relating to infectious and/or non-infectious pathological changes as well as parvoviral enteritis and distemper diseases that occurred in small and large intestines of dogs, to make macroscopic and microscopic definitions by determining the intensity and extent of these lesions and to estimate their role in facilitating the emergence of secondary disease and being a cause of death.

Material and Methods

Animals

The study was conducted on 100 dead stray dogs of different ages and breeds, 91 of which were from Municipality Animal Shelter, brought to Pathology Department of Veterinary Faculty, University of Selcuk during one year. Systematic necropsies of all dogs were performed, and their small and large bowels were examined in macroscopic, histopathologic, microbiologic and parasitic aspects. Also, immunohistochemical stainings were performed in aspect of canine parvovirus and distemper virus.

Histopathological examination

After fixation of all intestinal tissues belong to duodenum, jejunum, ileum, ileocecal valve, caecum, colon and rectum received from all dogs in 10% buffered formaldehyde solution, the samples were routinely processed, and the sections were stained with hematoxylin and eosin (HE) and then examined under light microscope.

Lesions observed in histopathological examinations in this study were scored as made by Garcia-Sancho et al (2005) and defined as; (-) no lesion, (+) mild, (++) moderate and (+++) severe lesions. All intestine sections were examined in aspect of epithelium, mucosa and submucosa; degeneration, desquamation, necrosis and ulcer, mononuclear cells, macrophage, neutrophil and eosinophil granulocyte infiltrations, fibrosis, atrophy, existence of bacteria colonies, inclusion bodies, hyperaemia, hemorrhage, oedema, dilatation and hyperplasia in crypts, depletion in lymphoid tissues, and each lesion was graded according to severities as specified in Table 1. Cell count was calculated by taking an average of 5 different areas under x20 objective magnification on each section. Changes in the crypts (degeneration, necrosis, desquamation, dilatation,

Table 1. Scoring the microscopic findings

Lesions / Scores	General Assessment	*Number of Mononuclear Cells	*Number of Neutrophil and Eosinophil Granulocyte	*Number of Macrophage	Impact Rate of Crypts
-	No lesion	0	0	0	< %10
+	Mild lesion/ few cells	1-20	1-5	1-10	%10-20
++	Moderate lesion/ average cells	21-50	6-20	11-30	%21-50
+++	Severe lesion/ Plenty cells	50 >	20 >	30 >	%50 >

*: Values indicate the average cell count in 5 different areas under x20 objective magnification on each section.

hyperplasia) were scored in percent according to the number of influenced ones among all crypts (impact rate) in tissue sections. No grading that was conducted with significant and clear criteria in this way and could be a model for the study was found other than the subjective assessments in reviewable sources.

Microbiological examination

Microbiological cultivation was performed to agar with 7% sheep blood containing MacConkey agar (Oxoid) and XLD agar (Biomerieux), perfringens selective agar (SPS agar, Merck), *Clostridium difficile* moxalactam norfloxacin selective supplement (Oxoid, SR0173) from the content of small bowel of dogs and incubated in an aerobe and anaerobe environment. Also, planting was carried out on agar with 7% sheep blood containing *Campylobacter* selective supplement (Skirrow, Oxoid SR0069E) and incubated in a drying oven with 10% CO2. Colony morphologies of isolated bacteria, their gramme staining properties, and identification with API 20 E and API 20 Strep (Biomerieux) were achieved in the microbiology laboratory of Konya Veterinary Control and Research Institute, Turkey.

Immunohistochemical examinations

For the purpose of immunohistochemical verification of parvovirus and distemper infections, immunohistochemical methods notified by Rhind (2001), Ramos-Vara (2005) and Buchwalow and Böcker (2010) were used. Antigen retrieval was performed in microwave oven within citrate buffer to sections received from polilizin coated lam from small and large intestine sections and cerebellum tissue. Staining with Envision technique (NovolinkTM Polymer Detection Systems) was done in Shandon's Manual Staining Set (Shandon Sequenza, ThermoShandon, Cheshire, England) and at room temperature. 1/200 dilution was applied to mice monoclonal antibody obtained against canine distemper virus (CDV antibody Abcam, ab20332) and 1/50 dilution was applied to mice monoclonal antibody obtained against canine parvovirus (CPV antibody Abcam, ab7669). DAB (3,3'-diaminobenzidine, SkyTek Chromogen/Substrate Kit) was used for distemper and DAB Chromogen and NovoLinkTM DAB Substrate Buffer (Polymer) were used for Parvoviral enteritis. Also, DAB Enhancer solution (BondTM DAB Enhancer, AR9432) was applied for parvoviral enteritis. Contrast staining was performed with Harris haematoxylin. In negative control, PBS was used instead of primary antibody.

Parasitic examination

Type and name of parasites observed macroscopically during necropsy were determined and recorded. Since a complete and comprehensive parasitological examination was not the aim of this study, no examination with detailed parasitological methods for feces or intestine content was performed.

Results

During macroscopic examination of small intestine, in addition to the hyperaemia found in most cases, it was determined that 19 dogs had common bleeding and bloody content (Figure 1A), 42 had petechial or echymotic hemorrhages in ileocecal valve (Figure 1B). Ulcers in greyish color with raised edges were seen in duodenum and jejunum of one dog and in ileum of five dogs. 13 of dogs had invaginations at jejunum and also 3 of them had at ileum (Figure 1C-1D). Fibrinous exudate in yellowish and greyish colours with sticky or watery density was found on the lumen and mucosa in the small bowels of 26 dogs (Figure 1E). In aspect of large bowels, it was seen that 2 of the dogs had bleeding in the caecum, 16 in colon and 7 in the rectum and 1 dog had foreign object in caecum (metal pipe part in 3 cm length). We have encountered invagination in 1 dog and a metal foreign object in 1 dog in colon, and one dog has possessed a megacolon filled with abundant contents.

Histopathological changes in mucosa and submucosa layers of all intestine segments are presented in Table 2 with details. As a result of the histopathological findings

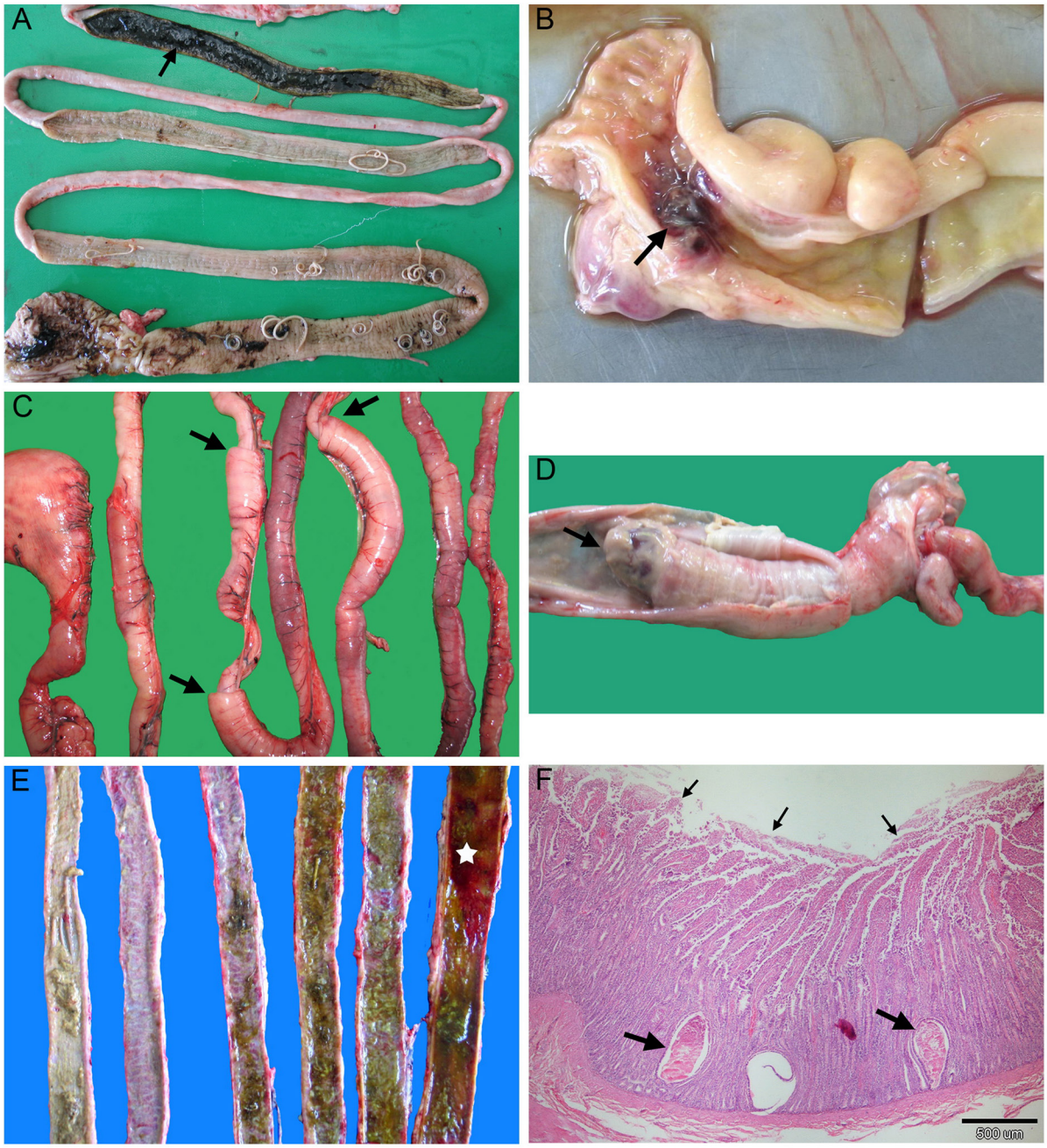

Figure 1. A) Parasites in duodenum and jejunum, widespread bleeding in ileum (arrow). B) Local hemorrhage in ileocecal valve (arrow). C) Invaginations in jejunum (arrows). D) Dissected invagination in ileum, necrosis in mucosa (arrow). E) Sticky and fibrinous exudate in small intestines, hyperaemia in mucosa (star), and F) Duodenum. Desquamation in the surface epithelia (thin arrows), dilatation in crypts, necrosis and desquamation in crypts (thick arrows). Hematoxylin and eosin (HE)

in intestines, the cases in which epithelial degeneration, desquamation, hyperaemia, oedema, neutrophil granulocyte and macrophage infiltrations in propria are preponderant, were described as acute; mononuclear cell infiltrations and fibrosis/atrophy formed cases as chronic; and the cases formed with only dense lymphocyte and plasma cell accumulations in mucosa were defined as lymphocytic and plasmacytic enteritis (LPE). Accordingly, acute enteritis was found in 29 cases in small bowel in total, chronic enteritis in 51 cases, LPE in 15 cases, and eosinophilic enteritis in 1 case in Table 3. The highlighted microscopic findings in brief are as follows: Villous atrophy is found in 26 dogs at duodenum, bacterial colonies in 32 dogs, mononuclear cell infiltration in 94 dogs, dilatation and degenerations in crypts in 64 dogs (Figure 1F). In jejunum, villous atrophy is found in 46 cases, bacteria in 29 cases, coagulation necrosis extending to propria in 4 cases, mononuclear cells in 94 cases, neutrophil infiltration in 48 cases, lesions in crypts in 57 cases, and lymphoid depletion in 14 cases (Figure 2A-2B). It is drawn attention that lesions are more common and severe in the

Table 2. Microscopic findings, counts and severity observed in the each intestine parts

Findings N=100	Total Number	Duodenum	Jejunum	Ileum	Ileocecal Valve	Caecum	Colon	Rectum
Degeneration and Desquamation	99	+2 ++21 +++64(87)	+5 ++19 +++67(91)	+1 ++18 +++69(88)	+8 ++17 +++62(87)	+10 ++21 +++44(75)	+5 ++24 +++64(93)	+20 ++33 +++26(79)
Mononuclear Cells Infiltration	98	+13 ++30 +++51(94)	+19 ++35 +++40 (94)	+20 ++33 +++43 (96)	+ 34 ++45 +++13 (92)	+21 ++39 +++18(78)	+20 ++64 +++10(94)	+ 27 ++51 +++4 (82)
Neutrophils	63	+26 ++12 +++4(42)	+28 ++14 +++6(48)	+17 ++15 +++4(36)	+19 ++4 +++1(24)	+13 ++3 +++1(17)	+19 ++3 +++1(23)	+5 ++2(7)
Eosinophils	31	+7 ++4 +++2(13)	+6 ++9 +++2(17)	+10 ++4 +++3(17)	+1 ++1 (2)	+1	+5 ++2 +++1(8)	+1
Fibrosis	88	+42 ++16 +++5(63)	+43 ++17 +++8(68)	+39 ++23 +++7(69)	+30 ++10 +++1(41)	+20 ++5(25)	+33 ++5(38)	+17 ++2(19)
Atrophy	82	+16 ++9 +++1(26)	+23 ++14 +++9(46)	+19 ++15 +++27(61)	+12 ++2 +++2(16)	-	-	-
Bacteria Colonies	64	+19 ++8 +++5(32)	+9 ++14 +++6 (29)	+18 ++8 +++6(32)	+9 ++3 +++3(15)	+4 ++4 (8)	+16 ++8 +++5(29)	+2
Hemorrhage	55	+8 ++7 +++5(20)	+12 ++7 +++11 (30)	+12 ++4 +++17(33)	+10 ++14 +++19(43)	+3	+9 ++7 +++6(22)	+2 ++2 (4)
Affecting Crypts	88	+32 ++15 +++17(64)	+34 ++9 +++14 (57)	+50 ++12 +++12 (74)	+30 ++9 +++6 (45)	+10 ++4 +++4 (18)	+36 ++11 +++10(57)	+10 ++3 +++5 (18)
Depletion in Lymphoid Tissue	83	+3 ++3 +++3(9)	+7 ++3 +++4(14)	+21 ++32 +++21(74)	+7 ++6 +++1(14)	+6 ++3 (9)	+5 +++1(6)	+1 ++1(2)
Ulcer	9	+1	+1	+5	+1	-	++1	-
Inclusion Body	14	+2	+3	+3 ++1(4)	+1	+2	+1 ++2(3)	-

Note: Values within the brackets indicate the total number in each segment. Lesion severities have been explained in the materials and methods section. (Findings in bowel segments may be found at the same animal together or individually.)

ileum. Villous atrophy is found in 61 cases, necrosis in 19 cases and ulcer in 5 cases, inflammatory cell infiltrations in 96 cases, bleeding in 33 cases, affecting of crypts and depletion in lymphoid tissue in 74 cases (Figure 2C-2D). At ileocecal passage section, hemorrhages on propria are found in 25 cases, on submucosa and serosa in 18 cases as well as epithelial desquamation, bacteria clusters, and dilatation/necrosis in crypts (Figure 2E). It is determined that the lesions in the large intestine are milder compared to sections of small bowel and that they are intensified in the colon (Table 2). In caecum, acute inflammation is found in 4 of the dogs, and chronic inflammation is observed in 18 dogs. In the colon, an ulcer is seen in 1 case (Figure 2F). In histopathological diagnosis, acute colitis is detected in 5 dogs, chronic colitis in 10, diffuse colitis indicating diffuse cell infiltration spread all over the mucosa and intraepithelial cell passage in 6 dogs, and idiopathic mucosal colitis characterized by dense lymphocyte and plasma cells in mucosa in 20 cases in Table 3.

Distribution of IHC staining results according to organs, number of positive stained cases and staining degrees are presented in Table 4. In the study, CPV-2 positive reactions were determined in a total of 90 dogs, and stainings were found as brownish, small granules in superficial epithelial cells, crypt epithelia (Figure 3A), mononuclear cells, macrophage and lymphoid cells on Peyer's plaques also in erythrocytes within vein lumen. In CDV aspect, in the

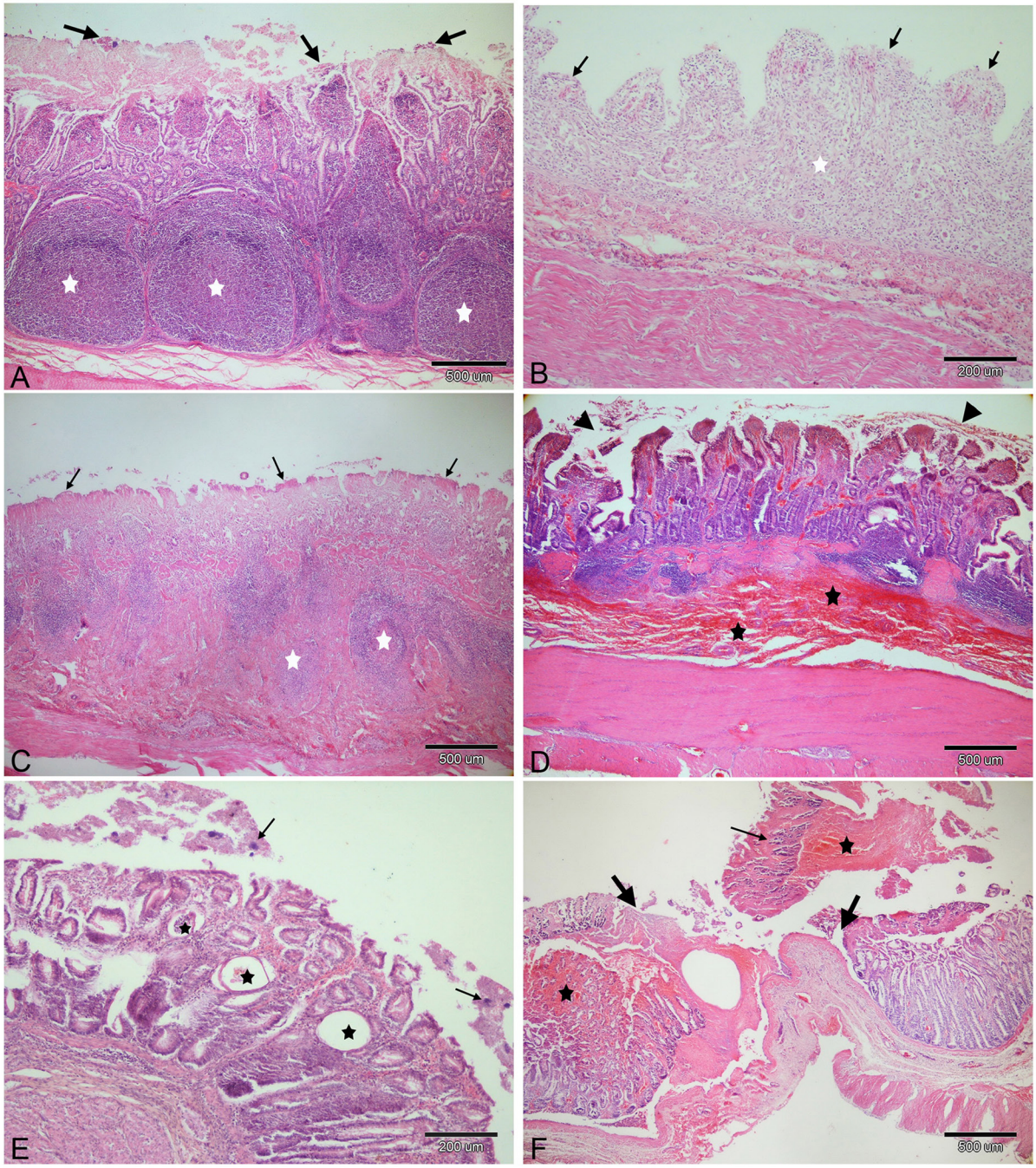

Figure 2. A) Jejunum. Desquamation, necrosis and bacteria colonies in epithelia (arrows), hyperplasia in lymphoid tissue (stars). HE. B) Villous atrophy (arrows), mononuclear cell infiltration and increased connective tissue in propria (star). HE. C) Ileum. Severe villous atrophy and necrosis on surface (arrows), mononuclear cell infiltration on propria, depletion in lymphoid tissue (stars). HE. D) Ileum. Degeneration and desquamation on lamina epithelialis (arrow heads) and severe bleeding on submucosa (stars). HE. E) Ileocecal valve. Desquamated cells and bacteria colonies on surface (arrows), dilatation and necrosis in crypts (stars). HE, and F) Colon. Ulcer area (between thick arrows), bleedings (stars), detached necrotic mass and bacteria colonies (thin arrow). HE

intestines of 26 dogs; positive staining is detected on nucleus and cytoplasm of surface epithelia, crypt epithelial and desquamated epithelial cells within lumen, mononuclear cells, macrophages and lymphoid cells as yellowish-brownish granules (Figure 3B). 24 of these dogs are also CPV-2 positive. For distemper disease, IHC was also implemented on the sections of cerebellum, and CDV positive reactions were determined in a total of 6 animals. While 5 of them had positive reactions in their alimentary tract organs too, a positive reaction was found in cerebellum individually in only 1 dog.

In the result of routine microbiological culture performed from the bowel segments with lesions observed macroscopically and from the particularly final section of jejunum and ileum of intestines; 7 different bacteria types

Table 3. Histopathological evaluation and number of dogs

Histopathological Diagnosis	Small Intestines	Large Intestines
Acute enteritis	29	5
Chronic enteritis	51	10
Lymphocytic and plasmacytic enteritis	15	-
Eosinophilic gastroenteritis	1	-
Diffuse colitis	-	6
Idiopathic mucosal colitis	-	20

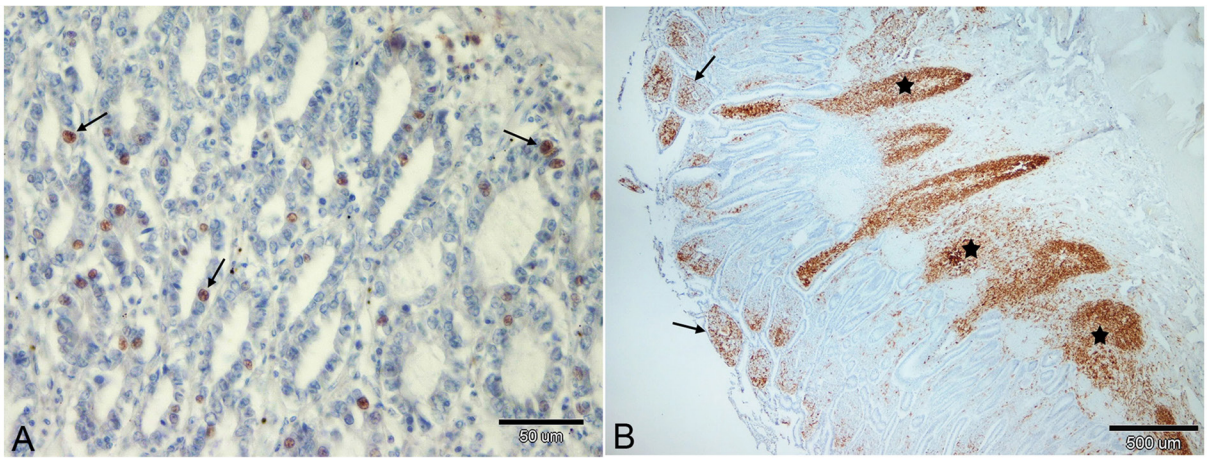

Figure 3. Immunohistochemistry stainings (Streptavidin-biotin peroxidase). A) Ileocecal valve. Canine parvovirus positive staining on crypt epithelia (arrows), and B) Ileum. Dense canine distemper virus positive staining on mononuclear cells (arrows) and Peyer's plaques (stars).

Table 4. Number of positive cases in IHC staining and distribution of staining density according to organs

Organs	Parvovirus (CPV-2)		Distemper (CDV)	
	Number of Positive (90)	Staining Density	Number of Positive (27)	Staining Density
Duodenum	72	+66 ++2 +++4	25	+12 ++2 +++11
Jejunum	67	+64 ++1 +++2	25	+14 ++2 +++9
Ileum	78	+72 ++3 +++3	24	+13 ++2 +++9
Ileocecal Valve	76	+74 ++1 +++1	24	+15 ++2 +++7
Caecum	60	+59 ++1	24	+17 ++1 +++6
Colon	72	+71 ++1	24	+13 ++4 +++7
Rectum	66	+64 ++1 +++1	24	+15 ++3 +++6
Cerebellum	-	-	6	+1 ++1 +++4

Table 5. Parasites determined in intestines of cases and existing segments

N=33/100	*Toxocara canis*	*Toxoscaris leonina*	*Dipylidium caninum*	*Joyeuxiella pasqualei*	*Taenia spp.*	*Giardia spp.*	Total*
Cases	18	1	5	1	10	1	36
Duodenum	*10*	*1*	*2*	*-*	*4*	*1*	*17*
Jejunum	*11*	*1*	*4*	*1*	*6*	*-*	*23*
Ileum	*5*	*-*	*1*	*-*	*5*	*-*	*11*
Colon	*2*	*-*	*1*	*-*	*1*	*-*	*4*

*: There is disparity between total number of parasites (36), cases and number of dog (33) due to the existence of multiple parasites or multiple affected segments in the same animal

were found in 58 of the dogs (7 *Campylobacter* spp., 47 *E. coli*, 24 *Enterococcus* spp., 10 *E. faecium*, 5 *E. faecalis*, 2 *E. avium* and 1 *Streptococcus* spp. =total of 96 isolations) and while there was a single agent in 23 cases, multiple bacteria were isolated from the same animal in 35 cases. During macroscopic examination of intestines, 6 different parasite types and total of 36 parasites were determined in 33 dogs (33/100). The names, numbers and laying bowel sections of parasites are indicated in Table 5.

Discussion

In this study, all pathological changes, microbiological and parasitic results in the small and large intestines of dogs are researched in accompany with immunohistochemical findings relating to parvoviral enteritis and distemper diseases. During literature reviews, some field studies and specific case reports relating to one disease are found. However, no comprehensive research relating to pathology covering all bowels has drawn attention. The study presented has the nature of first pathologic scanning research on dogs in this aspect.

Dogs may frequently swallow foreign objects due to reasons such as behavioral disorders like curiosity, hypersensitivity-hyperactivity, nervous system diseases such as rabies and distemper as well as vitamin-mineral deficiencies or liver diseases (Brown et al 2007, Merola and Giussani 2010). In total, foreign objects have been seen in the stomachs of five dogs (5%), reported in an article (Kanat and Ortatatli 2011), of which two of them have foreign objects in their large bowels in this study as well. It is considered, since these objects are circular and blunt, that they have passed through the ileocecal valve and could be carried to this point, where they would probably be discharged through anus without causing any damage. Although the formation of invagination in dogs is not known exactly, it is notified that various factors affecting the intestine peristaltic (such as foreign objects, severe parasitic conditions, enteritis) are effective and that they can cause digestive disorders and sometimes death (Carlton and McGavin 1995, Brown et al 2007). Acute or chronic enteritis is seen in all 14 dogs observed with invagination in the study conducted, and parasites are seen in the intestines of 3 dogs too. No definite reason other than infection was determined in the invagination cases observed in this study. According to some researchers, it is notified that severe morphological degradation is formed as a result of 3 hours of ischemia in the small intestines and that all mucosa layers become necrotized within 6 hours starting from the top of villus (Pablo et al 1983, Schoenberg et al 1985, Ceylan 2001, Ceylan et al 2005). In the study conducted, deep necroses have been seen starting from the lamina epithelia extending to villus depths, even to lamina muscularis in the small intestines of 19 cases. It has been considered that 9 of these necroses are formed as a result of ischemia and the other 10 are due to autolysis.

Although reason of lymphocytic and plasmacytic enteritis (LPE) is not exactly known, parasitic conditions, immune system disorders, specific enterobacteria, mucosal barrier damage, and dietary reasons are suggested as an important hypothesis. Microscopically, it is noted that the most significant changes are dense lymphocyte and plasma cells on propria, also neutrophil and eosinophil infiltrations and that there is a varying degree of atrophy in the villi (Haziroglu et al 1995, McTavish 2002, Garcia-Sancho et al 2005, Rousseau 2005, Ohno et al 2006, Brown et al 2007, Kobayashi et al 2007). Kleinschmidt et al (2006) reported that there is lymph vein dilatation and mild mucosal oedema in the small intestines in all cases with LPE. In our study, LPE has been determined in 15 dogs, from 2 months to 6 years, and enterobacteria has been isolated from 8 of them, and it is considered that these bacteria might be the cause of LPE findings in these animals. Although *E. coli* is found in 1 of other 7 dogs, no significant factor is found in the remaining 6 dogs, and it has been evaluated that other reasons suggested as hypotheses in the etiology of the disease might have played a role. It is known that idiopathic mucosal (lymphocytic and plasmacytic) colitis is the most common form of dog colitis (Brown et al 2007) and similarly in this study, it has been noticed that idiopathic mucosal colitis is the most commonly observed colitis with 20 cases. Researchers have notified that young dogs are more sensitive to parvoviral enteritis, and segmental or common bleeding is seen on the entire intestine mucosa, that Peyer's plaques can be easily distinguished from the serosa and in a dark red colour, and that similar changes might occur on the colon (Macartney et

al 1984, Brown et al 2007). It is realised in the study that ages of 90 dogs with parvoviral enteritis are expanded in wide range from a few weeks to 10 years. Hemorrhages especially in the ileocecal valve and in the various intestine sections, bloody or fibrinous exudate on mucosa and expansion in Peyer's plaques have been seen, as in the literature. Villous atrophy in varying degrees, hyperaemia, severe mononuclear cell infiltrations and fibrosis, affecting in the crypts have been observed. It is seen that in peyer's plaques on submucosa, there is enlargement along with remaining only reticular structure as a result of severe depletion in their centres and also hemorrhages in ileocecal valve, and it has drawn attention that all these findings observed are similar to those of other researchers (Agungpriyono et al 1999, Tunca and Toplu 2007, Haligur et al 2009). Specific inclusion bodies are reported to be observed in intestine crypt epithelia, myocytes and vein endothelium in certain studies (Agungpriyono et al 1999, Tunca and Toplu 2007), and they have been encountered in 14 out of 90 dogs in the epithelium and crypts of intestines. They were not observed in the myocytes and vein endothelium in this study.

During immunohistochemical examination, it has drawn attention that CPV-2 antigen is stained as small brownish granules in varying densities in the nucleus and cytoplasm of epithelial cells on entire intestine sections, in inflammatory cells, and in Peyer's plaques and that they are similar to the findings of researchers (Svara et al 2003, Tunca and Toplu 2007, Haligur et al 2009). In the study, CPV-2 positivity was found in 90 out of 100 dogs, and it is realised that this rate is higher than the expected rate. In fact, it is noted that there are less cases indicating specific findings relating to this disease during histopathological examinations. Also, CPV-2 and CDV are found positive together in the intestines of 24 cases and this condition is interpreted as that each viral infection suppresses the immune system and could facilitate the emergence of the other co-infection or other viral and bacterial infections and predispose the animals. Canine distemper virus, which has negative effects on nervous, respiratory, urinary and immune systems and on skin, causes moderate congestion and bleeding in the gastrointestinal tract as well as severe dehydration (Lan et al 2006, Liang et al 2007, Rodriguez-Tovar et al 2007). Microscopically, it is recorded that eosinophilic cytoplasmic and nuclear inclusion bodies are observed in the epithelial cells of intestine crypts (Lan et al 2009). In the study conducted, the inclusions in the stomach mentioned in previous article (Kanat and Ortatatli 2011) were more significant and denser than the inclusions seen in the intestine. Immune definition allowing absolute diagnosis of the disease has been determined as yellowish-brownish granules in epithelial and mononuclear cells in the study, and these findings are analogous to those of other authors (Lan et al 2006, Hammer et al 2007, Liang et al 2007). In this study, CDV antigen was detected in a total of 27 dogs (27%), and positive staining was also observed on cerebellum in 6 of these animals. While there is staining in the alimentary tract in 26 of the cases, it has drawn attention that staining on cerebellum exists in only 1 dog, and this suggests the idea that the digestive form of the disease is more common.

In the current study, 7 different bacteria were isolated in 58 dogs, and it was discovered that most of them (35 cases) have two or three coexisting factors on the same animal. Also, various parasites were observed in the 33 dogs, and 20 of them tested positive in bacterial isolation. In some cases (32 cases), bacteria colonies have been histopathologically observed on the epithelium surface and depths of propria, but this condition is not assessed as a specific lesion that may set forth the type or etiology of the infection. It has drawn attention from the results of a study that eosinophil granulocytes known to be observed more in parasitic and allergic incidents have no significant connection with the cases containing parasites. On the other hand, since parvovirus is observed in 90 dogs in the study and also 24 of them have distemper disease, it has been interpreted that possible bacterial and parasitic specific lesions may be masked or changed accordingly.

Conclusion

In conclusion, it has been seen that alimentary tract diseases and lesions are the most common causes of death in dogs brought mostly from animal shelters to the Department of Pathology as deceased for the purpose of necropsy. It is considered that Parvoviral enteritis among them (with a determination rate of 90%) has a special place and that this condition may be caused by the lack of vaccination and maternal antibody in stray dogs. It has been foreseen that this disease prepares suitable ground for secondary infections along with distemper, and the mortality rate is increased in this way. It is also seen that it is difficult to make a diagnosis with clinical and pathological findings in mix infections forming the majority of the cases, and it is understood that it is necessary to use techniques such as immunohistochemistry and PCR to emphasize the presence of agents in the tissues. It has been concluded as a final result that this study may shed light on similar further studies and that it will be useful to vaccinate with combined vaccines containing especially parvovirus and distemper by holding the ownerless/collected dogs, which have been brought to newly, in a separate section before placing them in mass living units such as animal shelters.

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Conflict of Interest

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Ethical Approval

Ethics approval was obtained from Selçuk University Veterinary Faculty Ethics Committee for the study (number of decisions: 2007/018).

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