Korean J Vet Res(1999) 39(4) : 825~831

Studies on the etiological agents in pups with the concurrent intestinal and respiratory tract disorders

Hee-myung Park, Tae-ho Oh, Sin-keun Youn*, Hong-ryul Han

College of Veterinary Medicine, Seoul National University
*Pet Animal Hospital**
(Received May 25, 1999)

Abstract : This study was performed to determine the etiological agents in concurrent disorders in gastrointestinal and respiratory tract. Most of dogs had clinical signs including nasal and ocular discharge, coughing, vomiting, and diarrhea. Of the 22 dogs, seropositive rates of each virus were 54.5% (12/22) against canine distemper virus, 90.9% (20/22) against canine adenovirus 1, 36.4% (8/22) against canine adenovirus 2, 18.2% (4/22) against canine parvovirus, 81.8% (18/22) against canine hepatitis virus and 59.1% (13/22) against canine coronavirus.

Canine distemper virus and canine parvovirus infection were 54.6% (12/22) in histopathological examination. In addition, mixed infections of canine distemper virus and adenovirus 2 were 9.1% (2/22). While simple infection of canine adenovirus 2 were 9.1% (2/22). *E coli* and *Staphylococcus* spp were isolated in feces as a rate of 72.7% (16/22) and 40.9% (9/22), respectively.

Conclusionally, it is also estimated that environmental stress might be one of the causative factors.

Key words : dogs, gastrointestinal disorder, respiratory tract disorder.

Introduction

Most of pups after/before the age of 8 weeks have been dead due to a variety of infectious agents with clinical signs of intestinal and respiratory tract problems. It has well known that these infectious agents were canine parvovirus (CPV)[1,2], canine distemper virus (CDV)[3], canine herpesvirus (CHV)[4,5], canine adenovirus 1 (CAV1)[6], canine adenovirus 2 (CAV2)[7,8], and canine coronavirus (CCV)[9]. Change of diet, stress, environment temperature, and no vaccination were also closely associated with high mortality of pups.

Viral infections are typical diseases of the young animal. In the young animal, it is the developing immune system, because of its immaturity, that is simply not capable of mounting a defensive response to the invading virus[14]. Mat-

Address reprint requests to Dr. Hee-myung Park, College of Veterinary Medicine, Seoul National University, Seoul 441-744, Republic of Korea.

ernally derived antibody plays an important role in the protection of neonates against disease[10].

The objective of this study was to determine the causative agents of acute death of pups with intestinal and respiratory signs simultaneously. Additionally, I hope that these basic data would be useful for veterinarians, not only to monitor health of pups, but also as an aid to evaluate pups' health in clinical veterinary practices.

Materials and Methods

Selection of pet stores and pups ; Twenty-two dogs with concurrent infections in respiratory and intestinal tracts were selected. The respiratory symptoms were mainly cough, nasal discharge, and moistured lung sound. The symptoms of intestinal diseases were mainly diarrhea, vomiting, fever, and anorexia. Age, sex and body weight distributions were as follows ; age ($<$ 8 weeks ; 27%, 8 weeks ; 45%, $>$ 8 weeks ; 27%), sex (female; 41%, male ; 59%), body weight ($>$ 800g; 55%, $\leq$800g ; 45%). Nonvaccinated and vaccinated dogs were 95% and 0.5% respectively. Breed distribution were as follows ; Maltese (18%), Dobermann Pinscher (5%), Mixed breed (18%), Jindo (18%), Yorkshire terrier (23%), Poodle (9%), other purebreds (9%).

Data collection : Data related to puppies were collected by the questionnaire filled out by each owner. Also, physical examination was performed by the standard method of veterinary medical teaching hospital in Seoul National University.

Definition of disease : Definitions were based on the history taking, clinical signs and/or laboratory test results. CDV infection was defined by the lesions characterized by intracytoplasmic inclusions in stomach, kidney and cerebellum. Mixed infection (CDV+CAV2) included the pathognomic lesions characterized by eosinophilic intracytoplasmic and basophilic intranuclear inclusion body in lung, respectively. CPV infection was defined by diarrhea and vomiting, fever and dehydration, the atrophy and destruction of crypt cells of villi.

Clinical examination : During 1 month, 22 dogs were evaluated. General condition of the dog was recorded. Data included breeds, body weight, age, sex, vaccination history, environment housed, diet, body temperature and clinical signs.

Hematology and serum chemistry : Blood samples were collected from either cephalic or jugular vein. The complete blood counts (IDEXX), serum alanine aminotransferase (Deutschen Gesellschaft method), calcium (O.C.P.C method), glucose (GOD-PAP method), inorganic phosphate (Fiske-Subbarrow method), albumin (B.C.G method), lipase (Cherry-Crandall method), chloride (Schales-Schales method), and blood urea nitrogen (Urease-Indophenol method) were determined. Sodium and potassium were determined by electrolyte analyzer (Nova biomedical). In addition, HCO_3^- was measured by blood gas analyzer (Ciba Corning).

Serological examination : Sera were referred to the diagnostic laboratories and tested for antibody titers against each virus. Antibody titers of CDV, CHV, CCV, CPV and CAV1 were tested in the diagnostic laboratories of Cornell University and CAV2 was tested in the Diagnostic Laboratories of Washinton State University. Hemagglutination inhibition test was performed by the method of Macartney *et al*[11]. The serum was examined for the presence of antiviral antibody in a routine tissue culture system employing primary cultures of canine kidney under the conditions recommended for this system. Neutralization tests for CDV serum antibody were done using the method of Appel and Robson[12]. Sera samples were tested for neutralizing antibodies against CAV1 and CAV2 as described by Bass *et al*[6], by Binn *et al*[13] for CCV, and by Carmichael *et al*[5] for CHV.

Histopathological examination : Moribund dogs were euthanized and were immediately necropsied. Tissues were fixed in 10% buffered formalin and examined by stand histopathological techniques with H & E stain.

Bacteriological examination : Fifty μl of diluted feces (1 : 100) of each sample was plated immediately on 5% bovine blood agar (Difco Laboratories, USA) after sampling in the pet store. The blood agar plates were incubated at 37℃ for 48 hours. For further isolation of bacteria, bacteria were identified by the methods of Bergey's manual of determinative bacteriology (1987).

Parasitological examination : Flotation technique and

direct smear method[14] were used to detect the larvae and eggs of parasites.

Results

Clinical examination : Coughing (27.3%), nasal discharge (67.5%) and moistured lung sound (49.5%) were detected. Vomiting and diarrhea were detected in all case group (Table 1). Author observed "chewing gum" convulsions in 20% of the suspected CDV infection, and the clinical signs of fever, mucopurulent nasal and ocular discharge, anorexia, and diarrhea.

Table 1. Frequency of clinical signs in 22 cases of the concurrent intestinal and respiratory tract disorders of puppies

Clinical findings	No. evaluated	Percent(%)
Diarrhea	22/22	100
Emesis	22/22	100
Fever	2/22	9.1
Anorexia	22/22	100
Cough	6/22	27.3
Nasal discharge	15/22	68.2
Moistured lung sound	11/22	50.0

Diarrhea; included hemorrhagic, yellowish, mucoid and watery diarrhea, Emesis; included yellowish and watery vomiting, Anorexia; included the decreased appetite, Fever; ≥39.4℃, Nasal discharge; included mucopurulent, mucoid, and watery nasal discharge, Cough; included slight, mild, and severe coughing, Moistured lung soud included moisture rale.

Hematology and serum chemistry : PCV and total protein were significantly lower than those of control group ($p < 0.05$). Fibrinogen was, however, significantly higher than that of control group ($p < 0.001$). In differential counts, band neutrophil showed a significant increase ($p < 0.001$). Monocyte and eosinophil values appeared significantly decreased ($p < 0.001$), and lymphocyte ($p < 0.001$), as well (Table 2). Leukopenia was found in 13.6% of dogs (Table 2).

Concentrations of calcium ($p < 0.01$), glucose ($p < 0.01$), and albumin ($p < 0.001$) were lower than those of control group. But concentrations of inorganic phosphate ($p < 0.001$) and blood urea nitrogen ($p < 0.05$) were significantly higher

Table 2. Complete blood count values in puppies with the concurrent intestinal and respiratory tract disorders

Complete blood counts	Reference range (Mean±SD)	Case range (Mean±SD)
WBC ($10^3/\mu l$)	15.0±2.0	19.2±11.6
RBC ($10^6/\mu l$)	5.5±0.5	5.6±1.6
PCV (%)	37.2±2.9	30.5±5.6*
Total protein (g/dl)	7.0±0.7	5.0±0.8*
Fibrinogen (mg/dl)	200±80	505±326***
Monocyte (%)	6.7±2.7	3.2±2.1***
Segmented neutrophil (%)	56.4±7.8	46.8±18.8
Band neutrophil (%)	0.6±1.1	22.9±12.7***
Lymphocyte (%)	33.5±8.1	16.7±9.0***
Eosinophil (%)	2.3±1.6	0.7±0.8***
Basophil (%)	0±0	0±0

Entries in a row with shared superscripts stand for *; $p < 0.05$, ***$p < 0.001$.

Table 3. Profiles of serum chemistry in puppies with the concurrent intstinal and respiratory tract disorders

Serum chemistry	Reference range (Mean±SD)	Case range (Mean±SD)
Ca (mg/dl)	9.0±0.7	8.0±2.8**
P (mg/dl)	4.0±1.2	7.1±2.4***
Glu (mg/dl)	90±15	40.1±31.4***
BUN (mg/dl)	15.0±5.1	40.4±37.1*
Alb (mg/dl)	3.5±1.0	2.0±0.39***
ALT (U/L)	40.1±20	35.3±20
Lipase (U/L)	1.0±0.7	0.8±0.6
Na (mmol/L)	140±3.5	138.7±10.4
K (mmol/L)	4.0±1.5	5.1±3.1
Cl (mmol/L)	100±9.0	105.5±16
HCO_3^-(mmol/L)	1.0±0.7	1.4±0.35

Entries in a row with shared superscripts stand for *; $p < 0.05$, ***$p < 0.001$. Ca; calcium, P; inorganic phosphorous, Glu; glucose, BUN; blood urea nitorgen, Alb; albumin, ALT; alanine aminotransferase, Na; sodium, K; potassium, Cl; chloride, HCO_3^-; sodium bicarbonate.

than those of control group (Table 3).

Bacteriological examination ; Of 22 clinically sick dogs, *E coli* and *Staphylococcus* spp were isolated in feces as a rate of 72.7% and 40.9%, respectively. In addition, *Klebsiella* spp and *Proteus* spp were found in 27.3% and 13.6% respectively. *Salmonella* spp or other possible anaerobic enteric pathogens were not isolated (Table 4).

Table 4. Isolation and identification of bacteria in feces of puppies with the concurrent intestinal and respiratory tract disorders

Bacteria isolated	No. of dogs tested	Percent(%)
E coli	16/22	72.7
Staphylococcus spp	9/22	40.9
Klebsiella spp	6/22	27.3
Proteus spp	3/22	13.6
Serratia spp	1/22	4.5
Streptococcus spp	1/22	4.5

Serological examination : Of 22 clinically ill dogs 8 dogs were seropositive to canine adenovirus 2 (CAV2). The positive rates of CHV and CCV were 81.8% and 59.1%, respectively. In addition, the positive rates of CAV1 and CDV were 90.9% and 54.5% (Table 5). Seropositive rate of CDV (54.5%) was higher than those of CPV (18.2%).

Table 5. Prevalence of antibodies to each virus in 22 pups with the concurrent disorders in intestinal and respiratory tract

Virus	Test	No. positive	Percent(%)
Canine parvovirus	HI	4	18.2
Canine distemper virus	SN	12	54.5
Canine adenovirus 1	SN	20	90.9
Canine adenovirus 2	SN	8	36.4
Canine herpesvirus	SN	18	81.8
Canine coronavirus	SN	13	59.1

HI; Hemagglutination inhibition test, SN; Serum neutralization test. Definitions of seropositivity; Canine parvovirus; ≥1 : 64, Canine destemper virus; ≥1 : 8, Canine adenovirus 1; ≥1 : 4, Canine adenovirus 2; ≥1 : 10, Canine herpesvirus; ≥1 : 4, Canine coronavirus; ≥1 : 4, these tests were performed in Cornell University and Washington State University.

Pathological examination : Rate of CDV and CPV infection were 27.3%, respectively (Table 6). In addition, mixed infection of CDV and CAV2 were 9.1% and infection of CAV2 alone was 9.1%.

Table 6. Positive rate by pathological findings in puppies with the concurrent disorders in intestinal and respiratory tract

Diagnosis	No. of sample	Percent(%)
CPV	6	27.3
Unknown*	6	27.3
CDV	6	27.3
CDV+CAV2	2	9.1
CAV2	2	9.1

* Unknown; when there were no pathognomic lesions in tissue. CDV; canine distemper virus, CAV2: canine adenovirus 2. CPV: canine parvovirus.

Parasitological examination : Two kinds of parasites were detected. Infection rates of *Isospora* spp and *Toxocara* spp were 18.2% and 9.1%, respectively.

Discussion

Hird *et al*[15] reported a frequency of kennel cough of approximately 40% among pups from a California pet store. Despite differences in the approach to estimate the frequency in our study vs. Hird *et al*'s study[15], the 27% incidence of cough in our data were lower than that of Hird *et al*'s study. In addition, CAV2, CDV and mixed infection with CDV and CAV2 were involved in the incidence of respiratory tract disorders as the causative agents in the pathological examinatioin. Fortunately, however, simple CAV2 infection generally is self-limiting and rarely results in life-threatening complications. The high incidence of respiratory tract disorders among pups from pet stores were not surprising because these pups have been stressed by weaning and handling, and then gathered in the broker's facility and in the pet store, enhancing both the risks of exposure and infection.

Results from hematology and serum chemistry showed that the lower state of PCV, glucose and albumin were due

to severe dehydration and anorexia. Whereas, the levels of electrolytes were normal due to the supplement of electrolytes intravenously as soon as the clinical signs appeared in pet stores.

As was true for respiratory tract disease, irrespective of the source, pups of 10 to 12 weeks old have the highest risk of intestinal tract disease. Firstly, the high incidence of intestinal tract disease among pups from pet stores reflects increased risk of exposure associated with aggregating pups from various backgrounds. In addition, because the neonatal immune system is not mature until the second or third week of age, primary targets are puppies with compromised or poorly developed immune responses. Younger puppies are the most susceptible, but all stressed dogs (boarding kennels, dogs in shows) are also at risk. Secondly, the opportunity for exposure coinciding with loss of maternal antibodies probably explains this high-risk period. These passively absorbed antibodies are beneficial in preventing diseases in the newborn. Differences in levels of maternal antibodies among pups possibly reflected differences in nursing and differences in levels of maternal antibodies in colostrum. In the present serologic study, the prevalence of mixed infections was higher than that of simple infection. Theses results show consitency with the reports[2] on the role of concurrent infections, including CPV and CCV. The stress of concurrent disease has been demonstrated to play a role in the activation of latent infections.

Bacterial culture was performed to find the bacterial overgrowth. However, pathogenic bacteria were not detected. But it is estimated that the considerable damage to the mucosa caused by canine viral infection may allow access of gut bacterial flora to the circulation. In bacterial involvement, it is thought that further studies on anaerobic bacteria were essential.

The low prevalence of parasitism certainly reflects the effect of preventive health programs in pet stores. For this reason, most animals acquire infection soon after birth from a contaminated environment and rapidly become immune to clinical disease. Even young ($\langle$ 6 months) animals normally acquire immunity within a few weeks after exposure to low numbers of oocysts. It seems likely that clinical disease may result if massive numbers of oocysts are ingested by non-immune animals. In this study, the infection rate of *Isospora* spp was the highest. Whereas, some studies reported that *Isospora* spp has an effect of stress to intestinal defense mechanisms.

Most of the dogs were unvaccinated in this study, and thus were susceptible to a variety of agents at the time of declined maternal immunity. Also pet store owners had no knowledge of optimal vaccination time to protect viral infections fatal to puppies.

Overally, present study shows that various stress to pup and lack of maternal antibody against various viral infections were causative factors. Additionally, nonvaccination, age and crowded housing of pups were most important factors in pups' death.

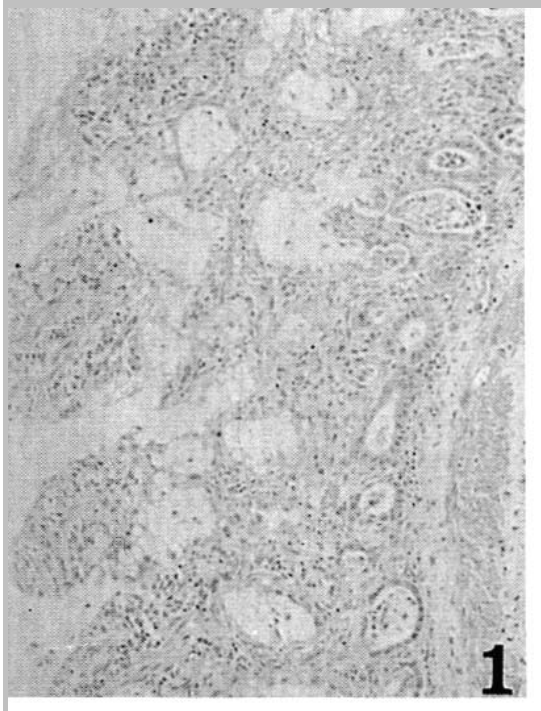
Legends for figures

Fig 1. Destruction and atrophy of villi is shown in this figure diagnosed as canine parvoviral enteritis. H & E stain. × 100.

Fig 2. Eosinophilic intracytoplasmic and intranuclear viral inclusions of gastric parietal cells are shown in this figure diagnosed as canine distemper viral infection. H & E stain, × 400.

Fig 3. Inflammatory exudates and amphophilic large intranuclear viral inclusions of bronchial epithelia and macrophage are shown in this figure diagnosed as canine adenovirus 2 infection. H & E stain, × 400.

Fig 4. Eosinophilic intracytoplasmic and basophilic intranuclear inclusions of bronchial epithelia are shown in this figure diagnosed as mixed infection of canine distemper and canine adenovirus 2. H & E stain, × 400.



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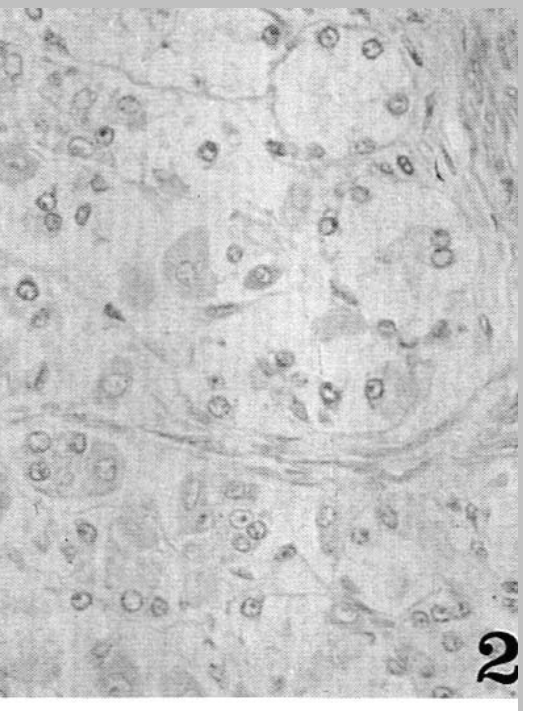

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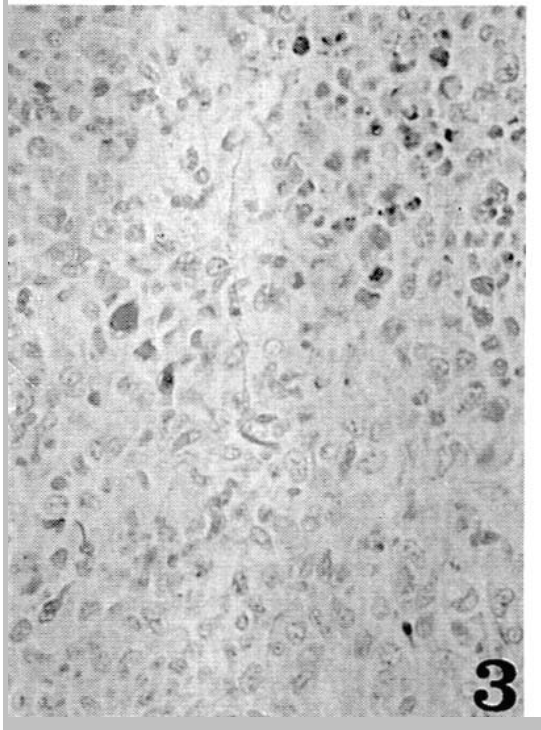

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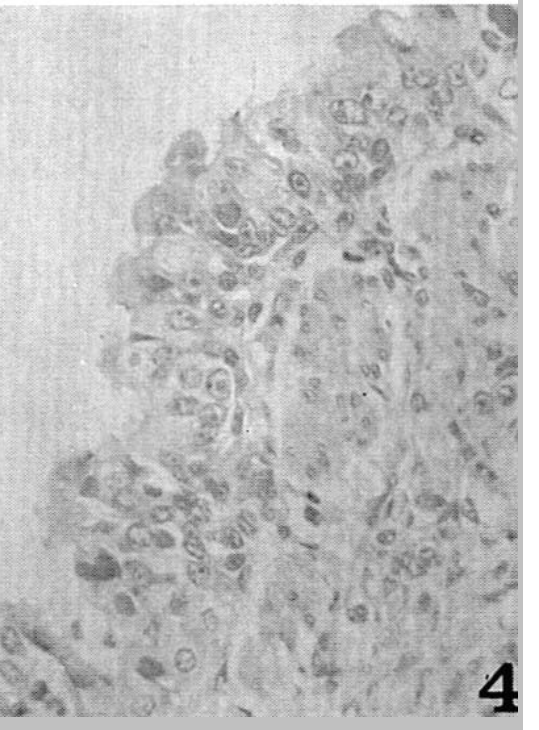

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