

Studies on Canine Viral Enteritis

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OBJECTIVES :

1. To identify and describe the etiologic agent producing a severe, bloody, often fatal diarrheal viral disease of canines in the military working dogs at the Royal Thai Army National War Dog Center in Pak Chong and the Royal Thai Navy Military Dog units at Sattaheep.

2. To produce Canine Viral Enteritis in susceptible, weanling dogs.

BACKGROUND : Parvoviruses are the smallest known viruses isolated from humans or animals. They have an affinity for rapidly replicating cells such as intestinal epithelial crypt cells and bone marrow. A parvovirus is the known etiologic agent of feline panleukopenia, a severe, often fatal disease of cats in which the epithelium of the intestinal tract in general and the crypt cells in particular are destroyed, producing fatal hemorrhagic diarrhea. Binn *et al.*, first isolated and characterized viral agents from the feces of asymptomatic dogs in 1970 (1). These agents, referred to as Minute Virus of Canine measured 20 to 21 nm in diameter by electron microscopy, were present in the nuclei of infected cells where they sometimes produced intranuclear inclusions, were resistant to ether, chloroform, and heat treatment, were inhibited in their growth by 5-iodo-2-deoxyuridine, and caused hemagglutination of rhesus red blood cells at 5°C. These properties are consistent with membership in the parvovirus or picornavirus group.

The first report of parvoviruses being associated with diarrhea in young dogs was published in 1977 (2). Parvoviruses have been associated with enteric disease in several species including cats, rabbits, rodents, and calves (3, 4, 5, 6, 7). During 1978, several outbreaks of a severe, hemorrhagic diarrheal syndrome were reported from dogs (8, 9). In each incidence a parvovirus or parvo-like viral agent was demonstrated by electronmicroscopy.

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During 1979, a severe, often fatal, hemorrhagic diarrhea began to appear in dogs in Thailand. The disease was particularly severe in areas where there were large numbers of dogs in close contact, i.e., the larger cities, veterinary hospitals, commercial kennels, and military and police dog training centers. The Royal Thai Army National War Dog Center in Pak Chong, Nakorn Ratchasima, experienced a heavy death loss in their military working dogs due to enteritis from January to June, 1979. (Table I). Of the total deaths occurring during this period, 49% were due to some form of enteritis. Thirty-five of the deaths were the result of the same clinical syndrome of vomiting, rapid dehydration, and hemorrhagic diarrhea. No treatment appeared to alter the course of the disease. Simultaneously, several dogs stationed with the Royal Thai Navy at Sattaheep were stricken with an identical illness. In addition, numerous reports of a fatal diarrheal disease in privately-owned dogs were being received from private practitioners in Bangkok and from the School of Veterinary Medicine, Chulalongkorn University.

METHODS : In June, 1979, a team of AFRIMS investigators visited the Royal Thai Army National War Dog Center in Pak Chong for the purpose of obtaining specimens of blood and stool from acutely ill dogs in an attempt to isolate and identify the etiologic agent responsible for this fatal diarrheal syndrome. Serum and stool samples were obtained from dogs on five separate trips to Pak Chong and three trips to the Naval Station at Sattaheep (Table II). In addition, several serum and stool specimens were collected from dogs presented to the small animal hospital at the School of Veterinary Medicine, Chulalongkorn University, Bangkok, Thailand, and from private veterinary clinics in the city of Bangkok.

The second phase of this study involved attempts to reproduce canine viral enteritis in susceptible weanlings puppies.

Source of Parvovirus

One of the stool specimens from one of the dogs (#9717) at the Royal Thai Army National War Dog Center was positive for parvo-like virus by electron microscopy and served as our source of parvovirus. A 2% suspension (Vol/vol) of this virus containing stool was buffered with 1% phosphate buffer solution (pH 7.2) containing 1% Bovine Serum Albumin and filtered through a 0.45 U millipore filter. This served as the inoculum used to infect the susceptible weanling dogs. A second stool from a dog (#4521) positive for both a parvo-like virus and an adenovirus was prepared in an identical manner and given to a second group of susceptible weanling puppies. One puppy from each group was given only phosphate buffered saline and served as a control. The infected stool suspension was given orally in each instance.

Susceptible Weanling Dogs :

Twelve, weaned, susceptible puppies approximately 2 months old were obtained from the municipal dog pound, Din Daeng Road, Bangkok. Six of the puppies were given anthelmintics to remove intestinal nematodes while six puppies were left unwormed. Reports in the literature indicate that intestinal parasites and pathogenic bacteria affect the severity of the disease so one group was left unconditioned (9). Complete blood counts and pre-infection blood and stool

specimens were collected for baseline data on all dogs. Serum was checked for the presence of anti-parvovirus antibodies. Stools were checked for pathogenic bacteria or protozoa. Stools and blood for antibody titer and virus isolation were collected starting at day - 4 and periodically during the study according to Table IV. Other parameters such body temperature, body weight, vomiting, diarrhea, and appetite were also followed during the course of the study according to the schedule in Table IV.

A complete necropsy was performed on all animals that died during the study. Specimens from the ileum, jejunum, liver, lung, kidney, spleen and stool were frozen at -70 C and saved for virus isolation. Likewise specimens from the ileum, jejunum, lung, liver, kidney, spleen were placed in 2.5% glutaraldehyde, processed in the usual manner and examined for the presence of parvo-like virus particles by the electron microscope. Tissues from all organ systems were placed in 10% buffered formalin and processed in the usual manner for light microscopy. Dogs still alive at day +42 of the study were euthanized and their tissues processed as stated above.

RESULTS : Two of fourteen stools collected from military working dogs at the Pak Chong War Dog Center were positive for parvo-like virus particles by electron microscopy (#9717 and #4521). The stool from dog #4521 also was positive for adenovirus particles.

One hundred and fifty seven serum specimens from seventy five dogs and twenty four stool specimens from twenty four dogs were shipped to Dr. L.N. Binn. Results of the examination of these specimens for the presence of antibody or virus are not yet available.

Results of the study designed to experimentally reproduce Canine Viral Enteritis in susceptible puppies are pending the analysis of the data.

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Table I

Month	Jan	Feb	Mar	Apr	May	Jun	Total
No Deaths	5	32	14	5	7	22	85
No Enteritis	0	15	10	1	4	12	42

Table II. Summary of Trips Made and Types of Specimens Collected

Trip Number	Date 1979	Location	Specimen
1	28 June	Pak Chong, National War Dog Center	Serum and stool
2	7 July	Pak Chong, National War Dog Center	Serum and stool
3	26 July	Pak Chong, National War Dog Center	
4	7 August	Pak Chong, National War Dog Center	Necropsy
5	9 August	Pak Chong, National War Dog Center	Serum
6	30 July	Naval Station, Sattaheep	
7	2 August	Naval Station, Sattaheep	Serum and stool
8	14 August	Naval Station, Sattaheep	Serum

Table III. Summary of Specimens Collected

Study Location	Number of Dogs Sampled	Number of Samples		
		Paired Serum	Stool	Necropsy
NWD-P	27	26	8	1
NS-S	50	36	12	0
CU-VH	38	5	5	0
PC-B	8	3	5	1

NWD - National War Dog Center, Pak Chong

NS-S - Naval Station, Sattaheep

CU-VH - Chulalongkorn University, Veterinary Hospital

- Private Clinic, Bangkok

Table IV. Schedule of Laboratory and Clinical Observations

1979 Day/ Month	Day	Clinical Observations					Laboratory Work					Remarks
		Temp.	Wt.	Eat	Vomition	Diarrhea	** CBC	Serum	Stool Virus Isolation	Stool Culture	Fecal Exam.	
6-8	-4	X	X				X	X	X	X	X	
7-8	-3	X	X				X		X			
8-8	-2	X					X		X	X	X	
9-8	-1	X					X	X	X			
10-8	0	X					X		X	X	X	
11-8	+1	X					X		X			
12-8	+2	X					X		X			
13-8	+3	X	X				X		X			
14-8	+4	X					X		X			
15-8	+5	X					X		X			
16-8	+6	X					X		X			
17-8	+7	X					X	X	X			
18-8	+8	X					X		X			
19-8	+9	X					X		X			
20-8	+10	X	X				X		X			
21-8	+11	X					X		X			
22-8	+12	X					X		X			
23-8	+13	X					X		X			
24-8	+14	X					X	X	X			
27-8	+17		X									
31-8	+21							X				
3-9	+24		X									
7-9	+28							X				
10-9	+31		X									
14-9	+35							X				
17-9	+38		X									
21-9	+42							X				*

* Sacrifice & Necropsy, Terminate the project.

** Nct, WBC, Differential Count.