Aspects of the diagnosis, pathogenesis and epidemiology of canine parvovirus

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SUMMARY: Between 18 July 1980 and 2 January 1981, 188 samples (145 faeces and 43 intestinal contents) were submitted from dogs with suspected canine parvovirus (CPV) enteritis. CPV was demonstrated in 56 (30%) of these samples; the weekly rate of positive CPV identification was remarkably constant at ~30% even though clinical and often post-mortem findings strongly supported a diagnosis of CPV enteritis. The simplest, most sensitive and most rapid method for detection of virus was haemagglutination (HA) which was twice as sensitive as isolation of virus and 8 times as sensitive as electron microscopy (EM). Forty nine of 56 (88%) samples positive for CPV were from dogs < 1 year old and 44 (79%) CPV-positive samples were from pups < 6 months old; only one sample from a pup < 2 months old (pup was 7 weeks old) was positive. An additional 68 samples (53 faeces and 15 intestinal contents) were submitted from Beagle dogs that were part of a colony of ~ 1200 dogs. Epidemiological data pinpoints the entry of CPV into the colony in November 1978 at which time most dogs including pups < 6 months of age developed antibody to CPV without developing clinical disease. From these data an overview of some aspects of the pathogenesis and epidemiology of CPV is constructed.
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Introduction

The emergence of canine parvovirus (CPV) as a cause of pandemic disease about mid-1978 was a phenomenon in veterinary medicine seemingly without precedent. Three quite distinct disease syndromes have been attributed to the virus, namely CPV generalised neonatal disease (Lenghaus et al 1980; Lenghaus and Studdert 1982), CPV myocarditis both in an acute and a chronic form (Lenghaus et al 1980) and enteritis also recognised as 2 overlapping syndromes — namely enteritis with accompanying severe frequently fatal panleucopaenia and enteritis alone. This paper is concerned with the relative merits of 3 diagnostic procedures used at Colorado State University in diagnosis of CPV infection.

Materials and Methods

Origin of Samples

Samples of faeces (n=145) and intestinal contents (n=43) were submitted from dogs brought to the Veterinary Teaching Hospital, Colorado State University (n=61) or from dogs seen by practitioners primarily in Colorado (n=127). These 188 samples are referred to as random source. An additional set of samples (n=68; 53 faeces, 15 intestinal contents) were submitted from dogs with suspected CPV enteritis that were part of an ~1200 Beagle colony (Collaborative Radiological Health Laboratory (CRHL), Colorado State University). The colony occupies a 10 ha site. The pens are located as a central island separated by a zone of at least 60 m from a 2.5 m high, chain-wire mesh perimeter fence. The colony, one of 5 such facilities in the United States, was commenced in 1965 and is concerned with long term radiation studies. Nine of the 68 samples came from dogs that received a single injection of a killed FPV vaccine at 8 weeks of age and were relocated

one to 3 weeks later in one of 2 nearby locations (A and B) for non-CRHL related research projects. In one of the locations the total dog population was ~ 120. The other 59 samples were from Beagle dogs that remained in the CRHL colony and were either pups < 6 months of age (n=20) that were also vaccinated with a single injection of killed FPV vaccine or were older dogs (n=39) that developed diarrhoea.

Dogs in the CRHL colony were also a source of 182 serum samples. Thirty two serums were collected from dogs that died of miscellaneous causes or were otherwise removed from the colony between January 1977 and December 1978. On 12 December 1978, although the colony had experienced no clinical evidence of CPV infection, a decision was taken to vaccinate all dogs in the colony with 2 doses of a killed FPV vaccine. A sample of 50 dogs aged one to 11 years was bled just prior to vaccination (12 December 1978) and at 2 and 3 weeks after vaccination. The second dose of vaccine was administered 2 weeks after the first. Serums were examined for HI antibody to CPV.

Virus Isolation

Procedures for virus isolation were generally similar to those described elsewhere for FPV (Studdert and Petersen 1973; Lenghaus and Studdert 1980) except that a cell line derived from feline foetal kidney (CrFK) was used. Pilot studies showed that a blind passage in a 25cm² flask prior to inoculation of a Lab-Tek chamber slide increased the rate of virus isolation by ~ 20% so that this procedure was adopted routinely.

Haemagglutination (HA) Haemagglutination-inhibition (HI)

Procedures used for haemagglutination (HA) and haemagglutination-inhibition (HI) have been described by Carmichael et al (1980) and Appel et al (1980).

Electron Microscopy (EM)

For EM a 1:10 suspension of 1 to 3 mL of faeces or intestinal contents in PBS was clarified by centrifugation at
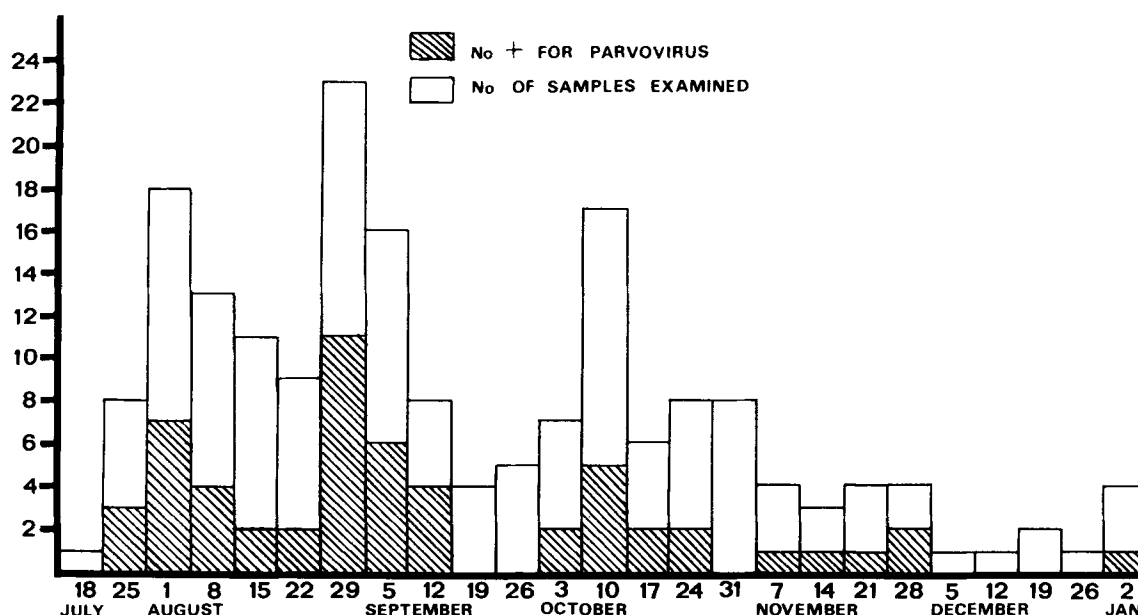

Figure 1. Histogram showing the weekly rate of submission of samples from dogs with suspected canine parvovirus enteritis and the rate of CPV in these samples.

3000 g for 15 min; the supernatant fluid was then pelleted at 100,000 g for one h. The pellet was resuspended in 2% phosphotungstic acid and examined for virus.

Results

Diagnosis

CPV was demonstrated in 56 of 188 (30%) samples; 48 of 145 (34%) faeces and 8 of 43 (19%) intestinal contents were positive. The weekly pattern of the samples between 18 July 1980 and 2 January 1981 is shown in Figure 1.Not all samples were examined by all 3 diagnostic procedures (EM, virus isolation, HA). Of 155 samples examined by EM 17 (11%) were shown to contain parvovirus. Thirty-four of 142 (24%) samples were positive based on the demonstration of typical parvovirus inclusion bodies in Lab-Tek slide cultures stained with haematoxylin and eosin. In several instances the identity of CPV was confirmed by fluorescent antibody staining of infected slide cultures with an FPV antibody conjugate. By HA, 33 of 112 (30%) samples tested gave positive reactions; 11 of 112 (10%) samples showed non-specific HA activity, that is the HA titre of the samples was the same in the presence of FCS as the titre in the presence of CPV antiserum. Ten of the 11 non-specific reactions were <80; one showed non-specific titre of 20,480. Non-specific HA was more commonly observed in faecal samples from dogs > 2 years of age. Data summarised in Tables 1 and 2 correlates the relative sensitivities of EM, isolation of virus and HA for diagnosis of CPV infection. It was concluded that HA was twice as sensitive as isolation of virus (Table 2) and 8 times as sensitive as EM (Table 1) for the detection of CPV.

Coronavirus-like particles were observed in 12 of the 151 samples (8%) examined by EM. Six of these 12 samples were also positive for parvovirus. Rotaviruses or other viruses were not detected.

When it was determined that direct faecal HA would be used as a standard diagnostic test clinicians were requested to submit a serum sample as well as faeces. When the HA test was negative, HI serum antibody to CPV was measured. Of 46 such serums tested for CPV antibody all reacted, usually at high titre (> 1,280). In 6 instances in which faeces contained CPV, the serums were found to contain HI antibody to CPV; such titres were usually low (< 320) and were interpreted as rising antibody or non-specific reactions.

TABLE 1

Correlation between electron microscopy (EM) and virus isolation (VI) and/or haemagglutination (HA) for detection of canine parvovirus

	EM VI and/or HA	EM VI and/or HA	EM VI and/or HA	EM VI and/or HA
	+ +	- +	+ -	- -
No. of Samples	12	35	4	95

TABLE 2

Correlation between virus isolation (VI) and haemagglutination (HA) for the detection of canine parvovirus

	Method							
Observation	VI	HA	VI	HA	VI	HA	VI	HA
	+	+	-	+	+	-	-	-
No. of samples	9		9		0		35	

TABLE 3

Age distribution of faeces or intestinal contents yielding canine parvovirus

	Age in months						
Observation	<2	2-3	4-6	7-12	13-18	19-24	> 24
No. positive	1	24	19	5	2	1	3
No. examined	8	60	31	19	5	8	34

Aspects of Pathogenesis

Table 3 shows the age distribution of dogs from which samples of faeces or intestinal contents were received. In only 3 instances were samples of myocardium submitted from pups with suspected CPV myocarditis. CPV was confirmed by isolation of virus from only one of these tissues and histopathology also confirmed the diagnosis of CPV myocarditis in this pup.

Observations on the CRHL Colony

HI antibody to CPV could not be demonstrated in 30 of 32 serums collected from dogs in the 2-year period January 1977 to December 1978. The 2 positive serums had titres of 5120 and > 10,240 and were collected on the 28 and 29 November 1978. Thirty four of 50 serums collected immediately prior to a decision to vaccinate (on 12 and 13 December 1978) reacted usually at high titre in HI tests (>640; 19 of the 34 serums had titre >5,120). These data indicate the CPV gained entry into the colony in late November 1978 and spread rapidly throughout the colony. Clinical disease caused by CPV infection was not observed at this time, indeed with the exception of a single case of CPV myocarditis diagnosed histopathologically in July 1979, overt CPV disease was not confirmed in the colony until October 1980 despite the fact that in the years 1978 to 1980 some 963 pups were born in the colony. In October 1980 30 of about 120 beagles relocated to site A developed enteritis and 11 pups died. At site B there were 20 relocated pups 11 of which developed severe diarrhoea and 5 died. About 2 weeks after the occurrence of CPV enteritis in pups relocated at sites A and B, CPV enteritis was confirmed in CRHL pups. About 20 pups were affected and 11 died. CPV was demonstrated by HA in samples from 5 CRHL pups.

Discussion

Diagnosis

HA was the quickest, simplest and most sensitive of the 3 procedures used to diagnose CPV infection. Non-specific HA was generally not a serious difficulty. It was encountered to low titre in 11 of 112 (10%) samples from random sources and in 13 of 65 (20%) samples from CRHL beagle dogs. Eighteen of these 24 (75%) non-specific HA's were from dogs > 6 months of age. Non-specific HA was more common and present in higher titre in older dogs. It was considered important to verify the specificity of HA by parallel titration of each sample with a known CPV immune serum.

It seems significant that of 188 random source samples CPV was demonstrated in ~30% even though with few exceptions clinical and sometimes findings at gross postmortem and histopathologic examination supported a diagnosis

of CPV enteritis. It is suggested that the rapid onset of an immune response and the variable time in onset of clinical signs after infection account for the fact that 70% of submitted faecal samples and 91% of intestinal samples were negative. The latter is a surprising figure because for many of these samples, tissues that were available for histopathology strongly supported a diagnosis of CPV enteritis. The significance of the figures is further underlined by its consistency, on a weekly basis, throughout the study period (Figure 1).

Pathogenesis

Like feline panleucopaenia virus the pathogenesis of CPV is complex. Where the environment and management of dogs is good the usual consequence of CPV infection, even for pups, is subclinical. This is well supported by observations on CRHL beagle colony dogs. An attempt to provide an overview of the pathogenesis of CPV enteritis and CPV enteritis with panleucopaenia is provided in Figure 2. Experimentally, as for FPV, it has proved extremely difficult to reproduce these syndromes; when pups that have no demonstrable antibody to CPV are experimentally infected with large doses of CPV virus they develop antibody to CPV but usually show little evidence of disease (Lenghaus and Studdert 1980; Appel et al 1980).

Parvoviruses are among the most potent of antigens and as indicated in Figure 2 the onset of an immune response is rapid (4 to 5 days after infection) and antibody titres after infection are usually high. To a certain extent, the immune response and the onset and course of clinical disease appeared independent. During the study we requested that faecal samples from suspected cases of CPV enteritis be accompanied by a serum sample. When CPV could not be demonstrated in faeces, as was the case for 70% of random samples, then the accompanying serum was tested for HI antibody; with few exceptions antibody to CPV was present at high titre (>1,280). Not uncommonly such dogs died.

The remarkably sharp delineation of the 3 syndromes of CPV infection underlines the significance of age in determining the pathogenesis of this disease. Data in Table 3 emphasises the fact that CPV enteritis/panleucopaenia is a disease of pups < 6 months of age; 99 of 165 (60%) samples, for which the age was known, were from pups < 6 months
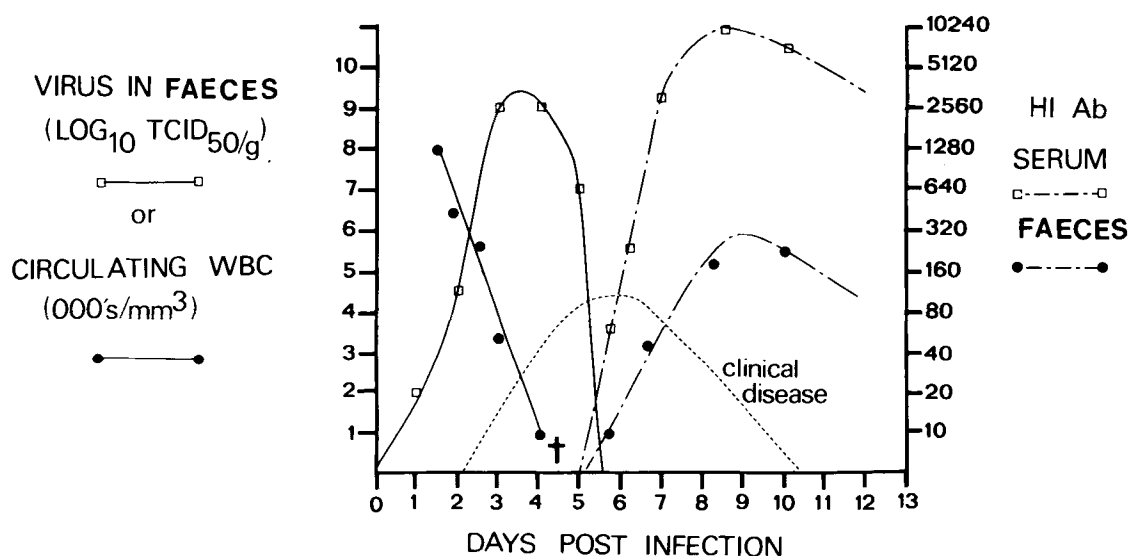

Figure 2. Hypothetical overview of the pathogenesis of canine parovirus (CPV) enteritis/panleucopaenia. With the possible exception of the immune response, the figures as plotted are nominal and each is quite variable. Most infections of dogs with CPV may be subclinical. The time between infection and the onset of clinical sign is variable, and may be delayed until 4 to 7 days post infection by which time an antibody response, which rises very rapidly to high titre, is underway and virus can no longer be detected in faeces. Systemic signs, including profound panleucopaenia, may or may not occur. They are more likely to occur in pups 2 to 6 months of age that completely lack maternal antibody and are maintained under stressful conditions (early weaning, unhygenic (pet shop, shelter), poor diet etc). Panleucopaenia to the points of death in association with severe enteritis is more likely to be observed under such conditions.

of age. The significance of age can be overidden. The 3 dogs older than 24 months (Table 3) that yielded CPV are of interest. Two were 10-year-old Beagles located within CRHL colony and were part of a long term radiation study. The third dog from a random source was 9 years old and had lymphosarcoma. In older dogs with confirmed CPV enteritis one should look for intercurrent disease particularly of a type likely to be immunosuppressive.

Epidemiology

Close contact between dogs, as illustrated by the introduction of CPV into the CRHL beagle colony about November 1978, appears not to be neccessary for the spread of CPV. A barrier of 60 m that separated the dogs from a 2.5 m high perimeter fence was inadequate to exclude CPV. Rodents, feral cats or more likely migratory starlings or wind may have been responsible for the initial introduction of CPV into the colony.

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Equine onchocerciasis in Queensland and the Northern Territory of Australia

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SUMMARY: Investigations were conducted on the taxonomy, distribution in the carcase, pathology and transmission of *Onchocerca* spp. in equids from Queensland and the Northern Territory. Examination of small groups of horses and ponies revealed high infection rates with *O. cervicalis*, while lesser numbers were infected with *O. gutturosa*. *O. reticulata* was not found. Neither of the Australian species is likely to be of economic importance to the horsemeat industry. The findings support the belief that *O. cervicalis* is a pre-disposing factor in the aetiology of equine nuchal disease, most commonly seen clinically as fistulous withers. *O. gutturosa* is virtually non-pathogenic. *Forcipomyia (Lasiohelea) townsvillensis, Austrosimulium pestilens* and *Culicoides victoriae* are suggested as potential vectors, and it is unlikely that *C. brevitarsis* is involved.
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Introduction

This investigation was undertaken to define the distribution of the adult worms of *Onchocerca* spp. in the carcases of Australian horses and to determine the species present. The former aspect may become important in the event of expansion of the export trade in slaughter horses and in horse meat for human consumption. Furthermore, the pathology associated with the adult worms, and the transmission of equine onchocerciasis in Australia has never been investigated in any detail.

Materials and Methods

In addition to the horse carcases referred to in Ottley and Moorhouse (1978), 32 animals showing nuchal disease lesions (as defined by Steward 1935) and 14 Northern Territory Timor ponies were examined. Gross pathology and histopathology were also studied in 43 nuchal ligaments. Twenty-two of these were removed from animals destroyed at the Veterinary School, University of Queensland, the other 21 came from a Brisbane knackery. The mean age of the first group was 6.9 years. Ages in the knackery group were unknown but were obviously much greater than those in the first group.

Examination for infection with *O. reticulata* was carried out by serially slicing suspensory ligaments and flexor tendons obliquely along their entire lengths to form 0.5 cm sections. This method was recommended by Dr O. Bain (personal communication), whose experience shows it to be a more reliable technique for finding this species than palpation as described by Pader (1901). Each cut surface was examined under a dissecting microscope. Eighty ligaments were selected at random from horse limbs at a south-eastern Queensland knackery, along with those from 14 Timor ponies, comprising 12 aged stallions and mares and 2 yearling colts. These were destroyed near Smiths Point on the Cobourg Pensinsula of the Northern Territory.