

INFECTIOUS DISEASE

BULLETIN

Evaluation of a new Fecal Antigen ELISA Test for the diagnosis of Canine Parvovirus Infection

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Since the late 1970s, viral enteritis has become recognized as one of the most common causes of infectious diarrhea in dogs younger than 6 months of age. Of the viral enteritides, parvoviral enteritis, caused by canine parvovirus type 2 (CPV-2), is probably the most common.^{1,2} Because of the highly contagious nature and serious portents of CPV-2 enteritis to susceptible dogs, rapid clinical diagnosis is essential in order to begin appropriate treatment as well as quarantine of the infected dog. A differential diagnosis can sometimes prove difficult in the early stages and milder forms of the enteritis. There are currently a variety of methods available for the detection of CPV-2 in feces. These include hemagglutination (HA), electron microscopy (EM), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA).³⁻⁷ Except for the ELISA, most of the methods are time consuming and require specific equipment. The ELISA methodology affords the opportunity to

provide quick, accurate in-hospital results.⁷

The purpose of the present study was to determine the performance characteristics of a newly developed fecal antigen

ELISA test as an in-hospital aid in diagnosing acute parvovirus-2 infection in young puppies. The manufacturer says that their newly developed fecal antigen ELISA-based detection assay, designated ASSURE®/PARVO (Synbiotics Corp, San Diego, CA), can detect CPV-2 antigen in either expelled fresh feces or feces collected directly from the rectum of infected dogs.

Objective

To evaluate the performance of a new fecal antigen ELISA test as an in-hospital aid in diagnosing acute canine parvovirus infection.

MATERIALS AND METHODS

Twenty-five 12 to 16 week old Beagle puppies obtained from a commercial source were used as test animals. At the supplier's location, puppies were not vaccinated and were given an ear tattoo. Test puppies were housed in individual cages according to AALAC space requirements. After 2 weeks of acclimation, puppies were given oronasally 10^5 TCID₅₀ of three virulent CPV fecally-derived isolates (CPV-2, CPV-2a, & CPV-2b). Following challenge exposure, all puppies were monitored daily for clinical signs of CPV-2 enteritis. Each puppy showing moderate-to-severe signs of enteritis were treated with 30 mg/kg of body weight of intravenously administered cephalosporin, intravenous lactated Ringer's solution supplemented with potassium chloride to correct blood volume and electrolyte deficits, and 0.3 mg/kg of body weight of prochlorperazine as needed to control vomiting.

Fecal samples were collected daily from each puppy using two sampling methods: expelled fresh feces collected from indi-

Study Protocol

Twenty-five puppies, 12-16 weeks of age from a well-recognized commercial source

Experimental design:

- Infected inoculum - CPV-2, CPV2a, and CPV-2b strains
- Oronasal dosage - 10^5 TCID₅₀ per ml
- Monitored daily the appetite, attitude, rectal temperature, body weight, vomiting, and stool character

vidual animal cages and feces collected directly from the rectum via use of a plastic sensing wand provided in the manufacturer's test kit. This wand is designed such that one end of the wand has a distinct, smooth bulbous enlargement. All fecal samples from both sampling methods were then evaluated according to the manufacturer's recommended protocol.

The format of the newly developed fecal antigen ELISA assay is as follows. The bulbous end of the plastic sensing wand is coated with antibody directed specifically against CPV-2 antigen. Procedurally, the bulbous end of the wand is lightly coated with expelled fresh feces or lightly coated with feces taken directly from the rectum of suspect dogs. The wand is then placed in a glass tube containing a second antibody, specific for CPV-2, which is conjugated to the enzyme horseradish peroxidase. After a short incubation period, if CPV-2 antigen is present it will be bound by both antibodies, thus forming an immuno-sandwich.

Fecal ELISA Testing Procedure

- Bulbous end of plastic wand is coated with antibody specific to CPV antigens.
- A second antibody also directed against CPV is conjugated to the enzyme horseradish peroxidase.
- Fecal sample is collected on the wand and the wand is added to a diluent containing the conjugated antibody.
- After short incubation, if antigen is present it will be bound by both antibodies, thus forming an immuno-sandwich.
- Unbound enzyme labeled antibody and feces are washed away and the wand is placed into a chromogenic substrate.
- Development of a distinct blue color in the solution indicates the presence of CPV antigen.

Unbounded enzyme-labeled antibody and feces are then washed away from the wand with copious amounts of distilled water. The washed wand is then placed into a second glass tube containing chromogenic substrate tetramethyl benzidine which detects the chromatophore specific to horseradish peroxidase. The development of a distinct blue color in this solution indicates the presence of CPV-2 antigen.

Interpretation of the fecal antigen ELISA results for this study were read as changes in color intensity as visualized by the assay operator and by determining the optical density on the same visually read sample with an ELISA plate reader. The results of the color change by visual reading were recorded according to the following detection system scheme:

- (-) **Negative.** No color development at all; clear solution.
- (Wk+) **Weak positive.** Faint but distinct blue color development.
- (+) **Positive.** Definite blue color development at the end of the substrate incubation.
- (++) **Strong positive.** Distinctly blue within one minute of substrate incubation and deep blue at the end of the substrate incubation.

The results of the color change as read by optical density were recorded according to the following detection system scheme:

- (-) **Negative.** Reading of ≤ 0.01
- (Wk+) **Weak positive.** Reading of 0.051
- (+) **Positive.** Reading of ≥ 0.101

Note: Visual detection for the blue color by most humans is at an optical density reading of ≥ 0.02 .

Three weeks after challenge exposure, all surviving puppies were sacrificed and necropsied. Puppies whose quality of life deteriorated beyond effective medical management were euthanized for humane reasons prior to conclusion of the study. Gross and microscopic histopathologic evaluations were performed for all puppies by a board-certified veterinary pathologist.

Fecal Sample Collections

Fecal samples were collected daily from each puppy after oronasal CPV administration.

Fecal samples were collected by two methods for CPV antigen detection:

- Expelled feces.
- Direct sampling.

RESULTS

Fecal antigen ELISAs were negative for the first 3 days after CPV-2 challenge exposure for all puppies by both fecal sampling and reading methods.

Results

- ▶ Fecal ELISAs were negative during the first 3 days after oronasal inoculation.
- ▶ 4/25 puppies were ELISA positive on expelled feces by day 4.
- ▶ By day 5 after oronasal inoculation, all puppies had clinical signs of acute CPV infection.
- ▶ Combined sensitivities for expelled feces for days 5-7 was 91.8% by direct visualization.
- ▶ Combined sensitivities for direct rectal sampling for days 5-7 was 97.3% by direct visualization.

(96%), 25/25 (100%), and 24/25 (96%) puppies, respectively. At the same time, optical density readings on expelled feces were weak or strong ELISA positive in 22/25 (88%), 23/24 (96%), and 23/23 (100%), and 24/25 (96%) puppies, and by direct rectal sampling was weak or strong ELISA positive in 23/25 (92%), 25/25 (100%), and 24/25 (96%) puppies, respectively. Figures 1 and 2 show the differential in the visual versus optical density reading methods for each fecal sampling method used.

Number of Positive Test Results in Infected Puppies for Days 5-12 by Sampling Method

Avg. Days (+)	Median Days (+)	Range
Expelled: 4.2	3.5	1-7 days
Direct: 6.2	7.0	3-8 days

The combined sensitivities for days 5-7 was highest for feces collected directly from the rectum using either visual (97%) or optical density readings (96%). The combined sensitivities on expelled feces were 92% and 94% by direct visualization and optical density, respectively. In terms of actual test sensitivity, it was 100%. All infected puppies tested ELISA positive on at least one

day during the CPV-2 infection by both fecal sampling methods.

In addition, the number of weak or strong ELISA positive results for days 5-12 after challenge exposure for expelled feces ranged from 1-7 days with an average of 4.2 days, whereas fecal samples taken directly from the rectum ranged from 3-8 days with an average of 6.2 days. These results also demonstrate the variability of fecal antigen shedding of CPV-2 in an acutely infected dog as well as the potentially narrow window in which an infected dog may test fecal antigen ELISA positive. Furthermore, the window appears to stay open longer when collecting a fecal sample directly from the rectum.

Positive Results by Visual Detection

	Day 5	Day 6	Day 7
Expelled:	22/25 (88%)	23/24 (96%)	22/24 (92%)
Direct:	24/25 (96%)	25/25 (100%)	24/25 (96%)

Gross microscopic histopathologic evaluations for all test puppies were compatible with CPV-2 infection as determined by the board-certified veterinary pathologist (Taylor). Interestingly, mild intestinal wall lesions were still apparent microscopically 3 weeks after challenge exposure when all surviving puppies were looking good clinically, gaining body weight, and passing well-formed stools.

DISCUSSION

The sequential pathogenesis of CPV-2 enteritis in young dogs is as follows. CPV-2 spreads rapidly from dog to dog via oronasal exposure to contaminated feces. Virus replication begins in lymphoid tissue of the oropharynx, mesenteric lymph nodes, and thymus and is disseminated to the intestinal crypts of the small intestine by means of viremia. Marked plasma viremia is observed 1-5 days after infection. Subsequent to the viremia, CPV-2 localizes predominantly in the epithelium lining the tongue, oral cavity, esophagus, and small intestine and the lymphoid tissue (e.g., thymus and lymph nodes) and bone marrow. It may also be isolated from the lungs, spleen, liver, kidney, and myocardium.⁸

Our study indicates that detectable fecal shedding of CPV-2 antigen begins on the fourth day after challenge exposure, before any clinical signs of CPV-2 enteritis appear. Fecal antigen is then shed sporadically thereafter for up to 7-12 days. The preferred detection time with this in-hospital ELISA-based test being 5-7 days after challenge exposure. In addition, direct fecal sampling from the rectum of a suspected dog is more sensitive for detecting CPV-2 antigen than is sampling expelled feces alone.

Conclusions

- All puppies tested positive on at least one day during their acute CPV infection by either fecal sampling method.
- The fecal antigen ELISA test is highly sensitive and easily performed as an in-hospital procedure.
- Direct rectal sampling is more sensitive for detecting acute CPV infection than sampling expelled feces.
- CPV antigen shedding in the feces is limited to a certain window in time and is somewhat cyclical.
- Which says: – there is significant variability of fecal antigen shedding as well as the potentially narrow window in which a positive animal tests positive in the fecal ELISA test.
- The window appears to stay open longer when using the direct sampling method.

With this pathogenetic understanding and considering the difficulty of detecting CPV-2 antigen in feces, results of our study indicate that this newly developed fecal antigen ELISA-based assay is a highly sensitive and easily performed in-hospital test and can serve as an effective aid in diagnosing acute CPV-2 infection in young dogs.

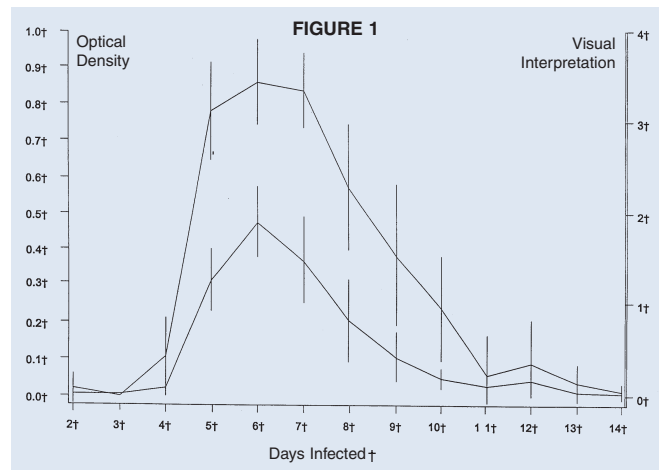


Figure 1. Comparison of the mean fecal antigen ELISA results as read by visual detection (top line) versus optical density (bottom line) on expelled fresh feces from CPV-2 infected dogs.

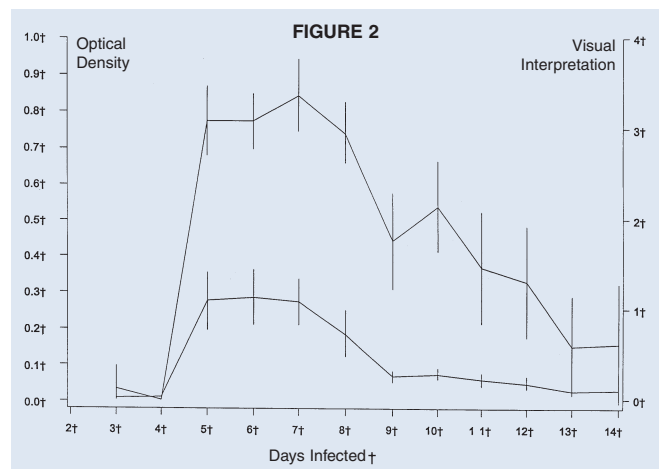


Figure 2. Comparison of the mean fecal antigen ELISA results as read by visual detection (top line) versus optical density (bottom line) on feces collected directly from the rectum of CPV-2 infected dogs.

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