

# A Fatal Outbreak of Parvovirus Infection: First Detection of Canine Parvovirus Type 2c in Israel with Secondary *Escherichia coli* Septicemia and Meningoencephalitis

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## ABSTRACT

A 10-week old female Italian Cane Corso puppy was presented with a history of mucoid diarrhea and vomiting, and a presumptive diagnosis of parvoviral infection. The dog presented with severe leukopenia and was hospitalized and treated intensively with intravenous fluids, electrolytes, glucose, antibiotics, human albumin and antiemetics. Clinical and hematological improvement was noted, and the white blood cell count normalized. However, on the fifth day, neurological signs and intractable hypoglycemia had occurred and the dog was euthanized. Cerebrospinal fluid (CSF) analysis and necropsy revealed bacterial meningoencephalitis due to a multi-resistant *Escherichia coli* strain. This same *E. coli* was isolated also from the lungs, liver and spleen, and likely spread systemically due to septicemia. Polymerase chain reaction analysis of blood identified the presence of DNA of the recently discovered canine parvovirus strain 2c (CPV-2c). Five of the puppy's six siblings exhibited clinical signs suggestive of parvovirus enteritis and died, despite conventional supportive treatment and hospitalization. Moreover, two littermates presented neurological abnormalities as well. The litter was vaccinated at the age of six weeks, and again two days prior to the appearance of clinical signs. Immunization failure due to interference by maternal antibodies was suspected to have occurred. Furthermore, the efficacy of maternally derived antibodies from bitches vaccinated with other CPV-2 strains to protect against the new strains is debatable, and might have put the puppies at risk of infection with CPV-2c. This is the first record of CPV-2c strain infection in dogs in Israel, as well as the first documentation of *E. coli* meningoencephalitis in a CPV-infected dog.

**Keywords:** Dog, Gastroenteritis, CPV-2c, Infection, Cerebrospinal fluid, Polymerase chain reaction

## INTRODUCTION

Canine parvovirus (CPV) is a small, non-enveloped, single-stranded DNA virus, which replicates in rapidly dividing cells (1,2). It is highly resistant to detergents and disinfectants, and survives in the environment for months (1-3). A hitherto unrecognized canine pathogen, it emerged in 1978 as a leading,

highly contagious, potentially fatal cause of infectious enteritis in puppies (4). It has been continuously evolving since its discovery, with the original, less virulent strain (CPV-2) being replaced by the now ubiquitous and more virulent CPV-2a and CPV-2b strains (1-3, 5). Another strain, CPV-2c, was discovered in Italy in the year 2000 (1,2).

Acute enteritis due to CPV is most often seen in young pups, aged 6 weeks to 6 months. Certain breeds appear to be at increased risk of developing severe clinical signs (1-3). CPV spreads rapidly through the fecal and oro-nasal exposure, first replicates in lymphoid tissues, and then disseminates to other rapidly dividing body tissues, notably intestinal crypts, lymphoid tissues, thymus and bone marrow (2). Viremia is detected one to five days post-infection. Intestinal crypt infection results in small intestinal epithelial villi destruction and collapse. Infection of myeloid and lymphoid cell precursors results in neutropenia and lymphopenia. The breach in the integrity of the intestinal epithelium with concurrent immunosuppression predisposes dogs to bacterial translocation, bacteremia and sepsis (1-3). Fecal shedding of viral particles occurs as early as three days post-infection, for as long as ten days (2).

Enteritis is the most common clinical syndrome of CPV infection. Clinical signs often include anorexia, abdominal pain, frequent vomiting, diarrhea, often bloody, and consequently severe dehydration and potentially hypovolemic shock (1-3, 5). Fever may be present. Lymphopenia and neutropenia are common, although leukopenia, often considered a hallmark of CPV infection, might occur in less than 50% of the dogs at presentation (6). Complications include hypovolemic shock, electrolytes imbalance, severe metabolic acidosis, sepsis, and disseminated intravascular coagulation (DIC) (1-3). CPV-2 – associated myocarditis occurs in younger pups, born to unvaccinated bitches, and might appear concurrently with the enteric form, or as a separate clinical entity, occasionally months after recuperation from the diarrhetic disease (2). Other, less common clinical manifestations and potential complications include acute respiratory distress syndrome, neurological symptoms and erythema multiforme (3, 7-10). Spread of bacteria (notably *Escherichia coli*) and endotoxemia are common sequelae to CPV infection (11, 12). Furthermore, a high incidence of bacteriuria and intravenous catheter contamination occurs in dogs with CPV, presumably from gastrointestinal contamination (13, 14).

Diagnosis is based on the signalment, history, clinical signs, laboratory tests and pathologic findings (1-3). Specific tests for a definitive diagnosis include detection of viral fecal antigen using enzyme-linked immunosorbent assays (ELISA), detection of viral DNA through polymerase chain reaction (PCR), detection of viral particles through fecal and

tissue electron microscopy and immunohistochemistry of tissue sections (1-3).

Therapy is mostly supportive, aimed at restoring fluid, electrolyte and acid-base balance and preventing secondary bacterial infections (1-3). Accordingly, the cornerstones of treatment include intravenous (IV) fluids and electrolytes, glucose, antibiotics, anti-emetics and in cases with severe hypoproteinemia, colloids (plasma or synthetic colloids). Alternative treatments with variable efficacies have been investigated, attempting to curb virus replication, and avoiding the pernicious effects of endotoxemia (1-3, 15). The overall mortality rate in untreated dogs might reach 91%, in striking contrast to puppies receiving early proper supportive treatment, in which survival might reach 96% (1, 3).

Recovery from natural infection induces a long-lasting, possibly life-long, immunity from clinical disease (2, 3). Serum antibody titers and resistance to infection are correlated (2, 3). Effective prevention of CPV infection, especially unbridled outbreaks, can be achieved by vaccination (3). Most commercial, attenuated vaccines produce persistent protective antibody titers for two years at least. Immunization failure might result from interference of maternal antibodies (3). Consequently, the timing of vaccination is determined by the necessity to provide early protection against CPV infection through vaccination on the one hand, while minimizing maternal antibodies interference, on the other hand. A window of susceptibility is therefore unavoidable (16). Nonetheless, potentiated high-titer vaccines may overcome the effect of maternal antibodies interference when their levels are low (2).

## CASE HISTORY

A 10-week-old, 7 kg, female Italian Cane Corso dog was presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) with a history of mucoid diarrhea and vomiting. The dog was part of a seven-dog litter born to a vaccinated bitch. The entire litter was vaccinated twice, at six weeks (brand unknown, Pfizer) and at nine weeks (brand unknown, Schering-Plough) against canine distemper, adenovirus type-2, leptospirosis, parainfluenza, and parvovirus. Two days after the second vaccination, 6/7 littermates became lethargic. Within the next day, vomiting and diarrhea ensued. All six puppies were hospitalized and treated in a private veterinary practice. Three littermates died dur-

ing hospitalization, and the remaining three were referred to the HUVTH.

At presentation, this puppy was alert, responsive and with normal vital signs and hydration status, although mucoid diarrhea was noted. Complete blood count (CBC) and serum chemistry showed severe leukopenia, microcytic anemia (Table 1) and hypoalbuminemia (2.23 g/dL, reference interval [RI] 3-4.4g/dl). The dog was initially treated with IV fluids (Hartmann's solution, Teva-medical, Ashdod, Israel) at 7.5 ml/kg/h, with 5% dextrose supplementation (Cure medical, Emek-Hefer, Israel), Ampicillin (Penibrin, Sandoz GmbH, Kundl Austria, 25 mg/kg IV q8h), Gentamicin (Teva, Petach-Tikva, Israel, 6.6 mg/kg IV q24h), Maropitant (Cerenia, Pfizer, Sandwich, UK, 1 mg/kg, SC q24h), Metoclopramide (Pramin, Rafa, Jerusalem, Israel, 1.5 mg/kg/d IV at constant infusion rate), Famotidine (Baxter, Deerfield, Illinois, 1 mg/kg IV q24h), Ivermectin (Noromectin, Norbrook, Newry,

UK, 400 µg/kg, SC) and Praziquantel (VetBancid, CP-pharma, Burgdorf, Germany, 6 mg/kg, SC).

During the following four days, the dog was alert with normal vital signs, although inappetence and bloody diarrhea persisted. Since follow-up blood tests showed severely decreased total plasma protein (TPP, 3.2 g/dL, RI 5.5-7.5 g/dL) on the second day of hospitalization, human albumin was consequently administered (Zenalb 20, Bioproducts laboratory, Elstree, UK, 1gr/kg/d, IV). On the fourth day of hospitalization, the white blood cell (WBC) count increased to  $12.87 \times 10^3/\mu\text{L}$ .

On the fifth day, the puppy deteriorated mentally, and vocalization and cervical hyperesthesia were noted. Neurological signs exacerbated within the following hours, and vertical nystagmus and seizures were observed. In spite of continuous administration of dextrose infusion (0.2 g/kg/h), serum glucose levels decreased (40 mg/dL, RI 64-123 mg/dL), and a bolus of dextrose (0.5 g/kg IV) was also administered. Since neurological signs persisted, the owner opted to euthanize the puppy, and the dog was later sent for necropsy.

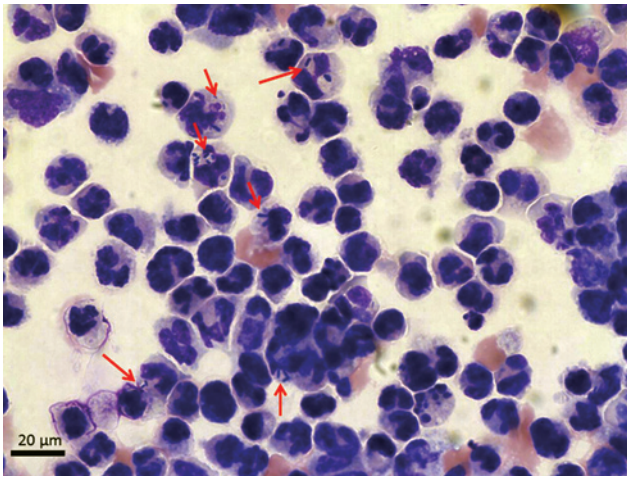
Cerebrospinal fluid (CSF) was collected immediately following euthanasia. Cytological evaluation revealed numerous degenerate neutrophils, many of which contained intracellular bacterial rods, indicating the presence of central nervous system (CNS) bacterial infection, in corroboration with the neurological signs the puppy had exhibited (Figure 1).

Gross post mortem findings included intestinal edema and multi-focal mucosal hemorrhages, pulmonary congestion and edema, with bloody bronchial exudate and thymus atrophy. A pus-like exudate was present along the ventral aspect of the cranium, pons, optic chiasma, medulla oblongata and within the lateral ventricles (Figures 2 and 3). Small intestinal microscopic lesions included severe villous atrophy and fusion and necrosis of epithelial cells within most crypts. Some crypts showed occasional hyperplasia and others were filled with cellular debris. The mucosa was moderately infiltrated by macrophages, lymphocytes and neutrophils with submucosal multifocal extension of the infiltrate. Peyer's patches were atrophic. Viral inclusion bodies could not be detected. In the brain, diffuse pyogranulomatous leptomeningitis was present (Figure 4). Extensive white matter necrosis and to a lesser extent gray matter necrosis as well as occasional microabscesses were diffusely present throughout the brain. Moderate vasculitis and mononuclear perivascular

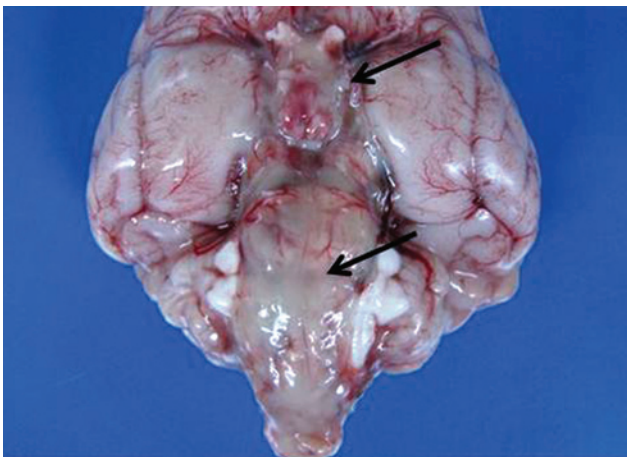
**Table 1:** Complete blood count measures from a puppy with parvovirus enteritis and bacterial meningoencephalitis at presentation and at day four of hospitalization

Parameter	Day 1	Day 4	Reference interval
White blood cells ( $X10^3/\mu\text{L}$ )	1.45	12.87	5.2 – 13.9
Red blood cells ( $X10^6/\mu\text{L}$ )	5.59	4.90	5.7 – 8.8
Hemoglobin (g/dL)	11.3	9.6	12.9 – 18.4
Hematocrit (%)	30.3	28.2	37.1 – 57.0
Mean corpuscular volume (fL)	54.2	57.6	58.8 – 71.2
MCHC (g/dL)	37.2	34.1	31.0 – 36.2
Red blood cell distribution width (%)	14.3	13.3	11.9 – 14.5
Platelets ( $X10^3/\mu\text{L}$ )	381	509	143 – 400
Mean platelet volume (fL)	13.5	9.5	7-11
Neutrophils ( $X10^3/\mu\text{L}$ )	0.03	0.27	3.9 – 8.0
Lymphocytes ( $X10^3/\mu\text{L}$ )	1.32	3.79	1.3 – 4.1
Monocytes ( $X10^3/\mu\text{L}$ )	0.03	8.56	0.2 – 1.1
Eosinophils ( $X10^3/\mu\text{L}$ )	0.05	0.01	0.0 – 0.6
Basophils ( $X10^3/\mu\text{L}$ )	0.01	0.09	0.0 – 0.1

MCHC, mean corpuscular hemoglobin concentration Values highlighted by bold characters deviate from reference interval



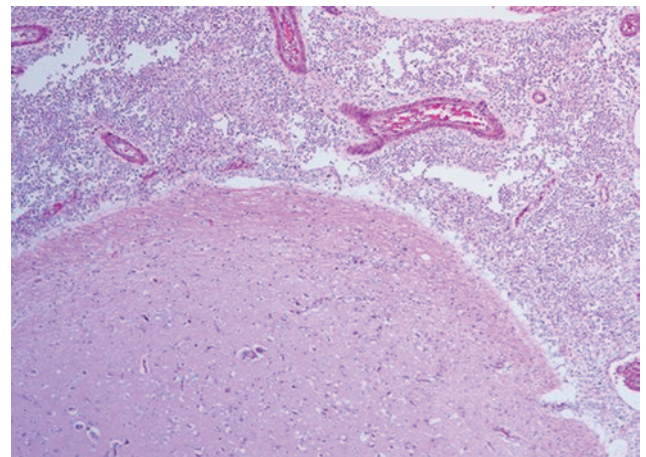
**Figure 1:** Cytology of cerebrospinal fluid, collected immediately from the cisterna magna after euthanasia from a puppy with parvovirus enteritis and *Escherichia coli* meningoencephalitis. Numerous neutrophils, some of which contain intracellular rod-shaped bacteria (red arrows) can be observed (Modified Wright's stain, X100, Bar = 20  $\mu$ m)



**Figure 2:** Gross view of the brain of a puppy with parvovirus enteritis and *Escherichia coli* meningoencephalitis. Pus-like exudate (arrows) can be seen at the ventral aspect of the brain



**Figure 3:** Gross view of the brain of a puppy with parvovirus enteritis and *Escherichia coli* meningoencephalitis. Pus-like exudate (arrows) in the lateral ventricles



**Figure 4:** Microscopic view of a fixed, stained tissue section of brain from a puppy with parvovirus enteritis and *Escherichia coli* meningoencephalitis. Severe neutrophil, and to a lesser degree mononuclear cell, infiltration of the arachnoid and pia matter (H&E, original magnification X100)

cuffing were present throughout the white and gray matter of the brain. Immunohistochemistry of brain tissue for canine distemper virus was negative. The myocardium was mildly to moderately infiltrated with macrophages, and to a lesser degree, with neutrophils. The pulmonary parenchyma showed diffuse alveolar edema, and numerous bacterial colonies were observed diffusely, surrounded by neutrophils. Both renal cortices showed foci of microabscessation.

Bacterial culture of samples obtained from lung, liver, spleen and brain yielded pure growth of a multi-resistant

*Escherichia coli*. It was resistant to ampicillin, gentamicin, fluoroquinolones and sulfamethoxazole/trimethoprim, moderately susceptible to amoxicillin/clavulonic acid and second generation cephalosporins, and susceptible to amikacin and first and third-generation cephalosporins.

PCR detection of viral DNA was done using the Canine Parvo PCR detection kit (Karnieli Ltd, Kiryat-Tivon, Israel) as described in the user manual. Briefly, DNA was isolated from 5  $\mu$ L of whole blood by addition of 100  $\mu$ L of the rapid DNA blood isolation buffer (Karnieli Ltd, Israel) and the

sample incubated for 15 minutes. Five  $\mu\text{L}$  of the extracted DNA was placed in a PCR tube with 10  $\mu\text{L}$  of activation buffer and 5  $\mu\text{L}$  of PCR mix. These were placed in a thermocycler, visualized on a 1.5% agarose gel, resulting in a 358 bp band. Once identified, the positive DNA sample was amplified again using the primers described by Kapil et al., 2007 (17), and isolated from the gel using the PureLink quick gel extraction kit (Invitrogen, CA, USA). The isolated DNA fragment was sent for sequencing at HyLabs (Rehovot, Israel) with the forward primer 5-CAGGAAGATATCCAGAAGGA-3. The mutation found was identified as a single mismatch mutation at the critical codon position 426 at which T was replaced by A, characteristic of the CPV-2c strain, as previously described (17). CSF PCR analysis for presence of CPV DNA tested negative

The other two siblings presented to the HUVTH also exhibited neurological signs. The first was presented in a comatose state, with absent menace response, did not respond to treatment, and was euthanized 12 hours later at the owner's request. The second sibling was treated similarly as described for the above puppy, however, neurological signs (vocalization and mental dullness) were observed during the fourth day of hospitalization, and 12 hours later this puppy was found dead in the cage. Necropsies of these two puppies were not performed. The seventh littermate did not show any signs of disease and seemed to be unaffected.

## DISCUSSION

This is the first record of CPV-2c infection in dogs in Israel. Since the discovery of CPV-2c, in 2000, it has reached worldwide distribution and has superseded the older strains as a leading causative agent of parvovirus-enteritis in several areas (18–20). It is characterized by a substitution of residue 426 in the capsid protein VP2 by glutamate, in lieu of asparagine or aspartate. This substitution represents a major antigenic change, distinguishable from older strains by monoclonal antibodies (18). Consequently, it might also negatively affect vaccination efficacy (21–23). The present puppies were vaccinated twice, however, the second vaccination was administered only two days prior to the onset of clinical signs, so they were most likely already incubating the virus then, and thus, this vaccination was probably ineffective at best, or might have induced additional immunosuppression in face of an ongoing infection, at worse (24). The first vaccination was

administered at the age of six weeks, and might have been ineffective, due to interference of maternally derived antibodies. Successful immunization rates against CPV at six weeks of age might reach 50% at best, and are proportional to the antibody titer of the bitch as well as passive (colostral) transfer efficacy (2, 16, 25). Since this bitch was regularly vaccinated, the levels of maternal antibodies in the colostrum were presumably adequate. For the above mentioned reasons, the puppies might not have been properly immunized against CPV at the time of infection.

Immunization failure, which may have occurred in the present case, has also been proposed as the cause of a CPV-2c outbreak among vaccinated adult dogs in Italy (22). On the other hand, classical, live attenuated vaccines have been shown to be efficacious against infection with the CPV-2c strain (26). This discrepancy between the two reports might be in part a result of exposure to two different CPV-2c variants, of disparate antigenicity and virulence. One salient difference between these studies is that in the latter, dogs were exposed to CPV-2c four weeks after immunization, when antibody levels are at their peak (22). The effect of a longer interval from vaccination to challenge with this strain, when antibody titers are lower, has not been investigated. Most of the currently used vaccines are based on the original CPV-2 strain. Although their protection efficacy against circulating strains has been validated in several studies, variations in the antisera neutralizing activity against CPV-2 and its variants displayed differences in protection against heterologous strains (23, 27). In dogs immunized with CPV-2, the serum neutralizing titers to the antigenic strains CPV-2a, CPV-2b, and CPV-2c were significantly lower than the homologous titers (raised to the original virus strain) (27). A certain maternal antibody titer might be sufficient for protection against one strain, but not against another. The differences in neutralization tests among the different strains might be of particular clinical importance in young puppies with passively acquired antibodies at their lower protective titers (21).

Data regarding the virulence and mortality rates amongst CPV-2c – infected puppies are lacking. In one report, there was no association between CPV strain and the outcome (19). In the present affected litter, the mortality was unusually high (100%), despite conventional intensive, supportive therapy while hospitalized. Virulence of the CPV variant, coinfections and genetic susceptibility amongst the littermates

might have contributed to the presently observed unusually high mortality rate. Notwithstanding, in the puppy presently described, neurological signs, intractable hypoglycemia, most likely due to sepsis, and terminal deterioration have all developed in face of an increase in WBC, which was within RI. Specifically, a marked absolute monocytosis was noted, with a concurrent moderate increase in neutrophil count which was still well below RI. This concurrent increase in the white blood cell count with monocytosis characteristically occurs in dogs rebounding from neutropenia, and can be explained by the shorter maturation time of monocytes, relative to neutrophils, from their common precursor cell (28). The neutropenia that was still present in this puppy might have rendered the dog susceptible to bacterial infection.

This chain of events and the widespread colonization of various organs with *E. coli* strongly suggest that septicemia, secondary to CPV-induced neutropenia and immunosuppression, appeared to have constituted the cause of death rather than CPV infection per se. Dogs with CPV infection have an increased risk of secondary bacterial infections due to neutropenia and immunosuppression. Previous studies have reported a high incidence of urinary tract infections, IV catheter site contamination and bacterial translocation from the gastrointestinal tract in parvovirus enteritis (12-14). Any of these sites might have been the present source of infection.

Unfortunately, the isolated *E. coli* strain was resistant to both ampicillin and gentamicin that were presently used. These antibiotics have been the standard treatment of CPV enteritis at the HUVTH for a long time, with good results. Resistance to either fluoroquinolones or gentamicin, such as was observed here, is rare amongst strains of *E. coli* isolated from dogs where the incidence of multi-resistant strains is considered even lower (29, 30). Multi-resistant *E. coli* strains have been detected more commonly in kennel dogs than in individually-owned dogs (30). Alternatively, this *E. coli* strain might have been acquired as a nosocomial infection in the puppy.

In the present case, use of a combination of a first- or second-generation cephalosporin with amikacin might have led to a better outcome. Third-generation cephalosporins have an advantage over first-generation cephalosporins in meningoencephalitis due to their ability to cross the blood-brain-barrier and reach the CNS (31).

The three littermates presented to the HUVTH

showed neurological abnormalities during hospitalization. Neurological signs, albeit uncommon, might occur in CPV enteritis due to vascular CNS accidents (i.e., resulting from disseminated intravascular coagulation), hypoglycemia, sepsis and acid-base or electrolyte imbalances. Only rarely are they a direct result of CPV infection (2). Nonetheless, CPV can cause a primary CNS disease. Previous molecular studies have demonstrated presence of CPV DNA in brain tissues of two dogs with cerebellar hypoplasia and from a dog with vasculitis and encephalomalacia (7, 9). Diffuse leukoencephalomalacia was documented in another puppy with severe necrotizing myocarditis secondary to CPV-2 infection, although viral antigens and inclusion bodies could not be detected in the brain (8). In the present case, secondary bacterial meningoencephalitis due to *E. coli* septicemia accounted for the neurological disease in this puppy, and CPV most likely did not play a part in the neurologic disease, since the CSF tested negative for the presence of CPV DNA by PCR analysis. It should be pointed out, however, that the underlying cause of the neurological signs exhibited by the other two siblings was not investigated.

In conclusion, this is the first record of CPV-2c infection in dogs in Israel. It was complicated by septicemia due to an unusually resistant *E. coli* strain, which has resulted in bacterial CNS infection and a fatal outcome. To the best of our knowledge, this is the first description of such an infection in dogs with confirmed CPV enteritis.

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