Electron Microscopic Detection of Canine Parvovirus in the Faeces of Dogs

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ABSTRACT

Parvoviruses (Parvoviridae) are small, non-enveloped, single-stranded DNA viruses that are known to cause disease in a variety of mammalian species, although most parvoviruses are species specific. In the present study, a total of 98 dogs were presented with hemorrhagic gastroenteritis to Small Animal Medicine Unit of TVCC, RIVER. Faecal samples collected in PBS were subjected for PCR assay and five samples were randomly selected for electron microscopic study. All the samples subjected for electron microscopic study showed parvo like viruses.

Introduction

Canine parvovirus enteritis (CPE) has become an important problem to dog population worldwide and is diagnosed usually in unvaccinated and seldom in vaccinated puppies. Inadequate immunization to parvovirus during the first year of life is an important risk factor for the disease. Unvaccinated dogs are about 13 times more likely to become infected than vaccinated dogs. Susceptibility of puppies to viral infection increases as the maternally acquired antibody titer declines to non-protective levels.

The age, breed, sex, history and clinical signs and haematological findings of dogs are important in ranking a viral etiology as a likely cause of diarrhoea. Commonly, canine parvovirus (CPV) infects 4-12 week old pups that are prone to acquire the virus in concomitance with the wane of Maternal Derived Antibodies (MDA). Eight weeks is the age when large numbers of puppies succumb to CPV (Marulappa and Sanjay, 2009).

Clinical diagnosis is indecisive and several other viral pathogens may cause diarrhea in dogs, such as corona viruses, adenoviruses, morbilliviruses, rotaviruses and reoviruses, (Greene and Decaro, 2012). Thus, a suspect clinical case should always be confirmed by laboratory tests. The most frequent diagnostic methods for titration of viruses in feces are haemagglutination tests (HA) and enzyme-linked immuno sorbent assays (ELISA). CPV
can be diagnosed by isolation from feces, Polymerase Chain Reaction (PCR), detection by electron microscopy and identification by immune electron microscopy (Dow, 1996; Sherding, 1992).

**Identification by electron microscope**

Karasaki (1966), electron microscope and immunofluorescent microscope studies of CPV-infected DKSV (dog kidney SV40 transformed) cells provide strong confirmation that this agent is a parvovirus. Negatively stained particles from purified preparations were 23 to 26 nm in diameter, which appears to be the characteristic size range of paroviruses.

Burtonboy et al., (1979) reported that during acute illness, paroviral virions are readily demonstrated in faeces by negative staining and use of electron microscopy.

**Materials and Methods**

In the present study, a total of 98 dogs were presented with hemorrhagic gastroenteritis to Small Animal Medicine Unit of TVCC, RIVER. Faecal samples collected in PBS were subjected for PCR assay.

A total of 53 dogs were diagnosed for canine parvovirus infection by PCR and 5 samples were randomly selected for the electron microscopic study.

The primer pair was H\textsubscript{for} / H\textsubscript{rev} that amplify a large fragment of the capsid protein-encoding gene (VP2) of CPV-2 (Buonavoglia et al., 2001). Primer H\textsubscript{for} (5’ CAG GTG ATG AAT TTG CTA CA3’) and H\textsubscript{rev} (5’ CAT TTG GAT AAA CTG GTG GT 3’), located at nucleotide position 3556-3575 and 4166-4185 of the CPV genome respectively yield 630bp product (Plate -1).

**Plate.1** Agarose gel electrophoresis of PCR product of canine parvovirus

![Agarose gel electrophoresis of PCR product of canine parvovirus](image)

Lane 1: negative control  
Lane 2: Positive control  
Lane 3: 100 bp ladder  
Lane 4-10: positive samples
Plate.2 Electron micrograph of CPV like virions. Bar marker represents 100 nm

Processing of sample for electron microscopic study was done using the procedure described by Alicia et al., (1999), where 1 ml of each fecal sample was homogenized with 10 ml of phosphate buffered saline (PBS) pH 7.2, and centrifuged at 8,000 rpm during 15 minutes at 4°C. The sediment was discarded and a second centrifugation at 45,000 rpm. for two hours at 4°C was performed. The sediment was re-suspended 1:1 with PBS, and one drop was placed on a cooper grid, and allowed to stand one minute. The excess was removed with a piece of filter paper. Two grids were negatively stained with 2% phosphotungstic acid, pH 7 during 1 minute, and examined with scanning electron microscope (Plate -2).

**Results and Discussion**

In the present study all the 5 samples which were subjected for scanning electron microscope showed canine parvo virus like particles under 100nm size (Plate -2). Burtonboy et al., (1979) also reported that during acute illness, parvovirus like virions are readily demonstrated in faeces by negative staining with use of electron microscopy.

The viral detection in faecal suspensions by electron microscopic examination directly confirms the presence of the virus like particles (Mc Adaragh et al., 1979) but this method also presents some drawbacks because the virus may also be detected in the faeces of healthy animals (Azetaka et al., 1981; Danner et al., 1982).

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