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## Detection of CPV-2 in puppies by PCR with sequencing confirmation

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**Statement of the Problem:** Parvoviral enteritis is a major cause of mortality in puppies, caused by canine parovirus-2 (CPV-2). Vaccination is the major prevention tool; however the emergence of new antigenic variants (CPV-2a, 2b, 2c) has raised a great challenge on the success of vaccination. The present study documents screening of fecal samples from suspected puppies for CPV-2 and sequencing of some of the positive samples to find out the locally prevalent antigenic types.

**Methodology:** Fecal samples were collected from puppies showing typical symptoms of parvoviral enteritis, presented at Veterinary Polyclinic, Indian Veterinary Research Institute, India and viral DNA was isolated. Polymerase chain reaction was done using newly designed primers and epidemiological analysis was carried out with respect to age, sex and breed. Oligonucleotide sequencing was done using the amplified product and phylogenetic analysis was performed with other sequences reported from India and abroad using Neighbor joining method based on p-distance model with the bootstrap test based on 2,000 resamplings in MEGA 6 software.

**Results:** Out of 44 samples, 23 were found to be positive for CPV-2 which was confirmed by 564 bp products on 1% agarose gel (Figure 1). Epidemiological analysis revealed that the disease was found to be more common in Labrador pups and 3-6 months male puppies were mostly affected. The positive cases represented 17.4% vaccinated puppies. Sequencing and BLAST analysis of 15 random positive samples (Gen bank accession numbers: KJ-364526, KM-003870 to KM003883) indicated 99% identity with CPV-2a. However, there were mutations observed at positions 297 (Ser 297 Ala) and 440 (Thr 440 Ala) amino acid residue, and hence typed as new antigenic variant of CPV-2a. Moreover, another mutation at position 264 (Gly 264 Val) was present only in 10 sequenced samples (10/15) which was not reported previously from India. Also there was a mutation at nucleotide position 3978 in two sequences in which cytosine was replaced by thymine. Phylogenetic analysis indicated that the genetic changes between the Indian sequences were much lesser when compared with foreign sequences. All the isolates in the present study showed more similarity with sequences from China.

**Conclusion & Significance:** CPV-2 is a major threat to puppies despite of regular vaccination, as the present study has shown that there is a significant percentage of infection in vaccinated puppies. The emergence of new antigenic variants makes the vaccine efficacy questionable. Further investigation is needed to determine the variation in the prevalence of antigenic types and evaluation the efficacy of current vaccines against new types of CPV-2.

### Biography

Jobin Thomas has completed his master of veterinary science in Veterinary Immunology from Indian Veterinary Research Institute, India. He investigated on the disease canine parvoviral enteritis. His research mainly includes antigenic identification, cell culture, recombinant protein expression, serological diagnosis using LAT and Dot blot of CPV-2. Currently he is doing his PhD in Bovine Tuberculosis at IREC, SaBio, UCLM, Spain.

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