Prevalence of canine parvovirus in dogs in Ludhiana, Punjab
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Abstract: In the present study a total of 100 faecal samples from dogs suspected of CPV were screened using PCR and nested PCR. The prevalence using PCR was 11% and by nested PCR 50%. Nested PCR was found to be more sensitive than conventional PCR.

Key words: Canine Parvovirus, dogs, prevalence, PCR, nested-PCR

Introduction
Canine Parvovirus (CPV) caused by CPV-2 is a highly contagious disease causing severe enteritis and systemic disease in dogs (Hoelzer and Parrish, 2010). The evolutionary changes in CPV-2 within few years globally replaced it from its other antigenic variants viz. CPV-2a and CPV-2b (Ozkul et al., 2002, Filipov et al., 2011). The virus spreads rapidly in the domestic as well as in the wild population of canines. The virus replicates in the villi of the small intestine in the rapidly dividing epithelial cells and sheds in the feces four to seven days post infection (Hoelzer et al., 2008). Parvovirus is very stable in the environment, kennel and veterinary clinics and spread directly or indirectly among dog population. CPV-2 infection is reported in all sexes, ages and breeds of dogs (Castro et al., 2007, Gombac et al., 2008) but is more severe in young puppies (Appel et al., 1979, Jacob et al., 1980). The genome of CPV is linear, negative sense single stranded DNA and VP2 is the major capsid protein that plays an important role in the determination of antigenicity and host range of CPV (Phromnoi et al., 2010). Molecular diagnostic techniques such as Polymerase chain reaction (PCR) are the most reliable techniques with greater diagnostic sensitivity and specificity (Schunck et al., 1995, Kumar et al., 2011). Thus, in the present study prevalence of CPV-2 in dogs was estimated using PCR and Nested-PCR.

Materials and Methods
Collection of sample: The rectal swabs (n=100) from dogs suspected of CPV were collected in phosphate buffered saline (pH=7.2) from the small animal veterinary clinics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab from January, 2013 till January, 2014.

Extraction of DNA from the samples: Rectal swabs were squeezed in the collection tube and the liquid was boiled in a water bath for 10 minutes. The supernatant was collected by centrifugation at 10,000 x g for 10 minutes and was stored at -20°C to be used as a template DNA in the subsequent PCR reactions.

Extraction of DNA from a known positive control: For control, DNA from a commercially available vaccine viz., Nobivac DHPPi was extracted using DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA) following manufacturer’s instructions with slight modification.

Polymerase Chain Reaction (PCR) to detect CPV: The primers for PCR were as per Mizak and Rzezutka (1999). The PCR reaction was set up by adding 15µl of the template DNA, 5.0 µl of 10X PCR buffer (with 15 mM MgCl2), 1.0 µl of forward and reverse primer (25 pm/µl) each, 1.0 µl of dNTPs mix (10 mM each), 0.5 µl of MgCl2 (50mM), 1 U Taq DNA polymerase and the reaction was made up to 50µl using nuclease free water.

Nested PCR (NPCR) to detect CPV: The primers used for NPCR were as per Mizak and Rzezutka (1999). NPCR reaction was set up by adding 5µl of the PCR product (from above reaction), 2.5 µl of 10X PCR buffer (with 15 mM MgCl2), 1.0 µl each of forward and reverse primer (25 pm/µl), 1.0 µl of dNTPs (10 mM each), 0.5 µl MgCl2 (50mM), 1 U Taq DNA polymerase and the final volume was made up to 25µl by adding nuclease free water.

In both the PCR and NPCR rectal swab from a healthy dog was used as a negative control and a DNA from a vaccine was used as a positive control. Both PCR and NPCR reaction was put in a thermocycler (Veriti®, Life Technologies, USA) with similar cycling conditions 35 cycles of denaturation at 94°C for 60s, annealing at 55°C for 60s, elongation at 72°C for 150s and a final elongation at 72°C for 10 min.

Visualization of PCR and NPCR products: PCR and NPCR products (10 µl) were run using 1% agarose with ethidium bromide (10mg/ml) @ 8µl/100ml at 5 volts/cm with Gene Ruler ladder plus 100bp (New England Biolabs, USA). The gel was visualized and photographed using Gel documentation system (Alphalmager, USA).

Results and Discussion
In the present study, out of 100 samples screened from dogs suspected of CPV 11 were positive by PCR yielding a product size of 548bp and 50 samples were positive with nested-PCR yielding a product size of 548bp (Fig. 1). CPV-2 is mainly responsible for hemorrhagic gastroenteritis and myocarditis in dogs and spreads rapidly in the domestic as well as in the wild population of canines. The disease is widely prevalent in Asia (Khadiilkar et al. 1994). In India disease was first reported in Madras (Balu and Thangaraj, 1981) and ever since isolation of CPV-2 was done for the first time in India by Ramadass and Khader (1982) several occurrence of disease have been reported from different parts of the country both from vaccinated and non-vaccinated animals (Biswas et al., 2006, Deepa and Saseendranath 2000, Phukan et al., 2004). Various methods have been used by earlier workers for its diagnosis that...
Balu, P.A. and Thangaraj, T.M.: Canine viral gastroenteritis - A clinical
screened 100 faecal samples from dogs with signs of gastroenteritis
and specificity (Schunck to molecular assays and thus, PCR and nested PCR has been
CPV was 50% using nested PCR in Ludhiana, Punjab and nested
positive for canine parvovirus by PCR and Singh
from Pondicherry reported 53.12% dogs as positive for CPV using
CPV-2 similar to the earlier finding of Mochizuki et al.1993, Hirasawa et
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Fig. 1: PCR and Nested PCR for Canine Parvovirus. Lane M- Gene
includes virus isolation in cell culture, HA, HI, Electron Microscopy,
IFT, ELISA and PCR (Mochizuki et al., 1993). However, sensitivity of these traditional diagnostic methods has been proven to be inferior
to molecular assays and thus, PCR and nested PCR has been
used for the detection of CPV-2 in faecal samples with high sensitivity and specificity (Schunck et al., 1995). In our study we have found
nested PCR to be more sensitive than PCR and the results are
similar to the earlier finding of Mochizuki et al.1993, Hirasawa et al.1994, Sakulwira et al., 1999, and Schmitz et al., 2009.
Hirasawa et al. (1996) detected prevalence of CPV-2 using
polymerase chain reaction and out of a total of 74 samples tested
diarrhoeal dogs 54.1 per cent were CPV-2 and were involved
in acute infectious diarrhoea. Kim and Jang (1997) reported that
nested PCR offers a better test as it is rapid, sensitive and specific
method for detecting canine parvovirus. Similarly, Sakulwira et al.
(2001) detected 34 out of 55 specimens (61.8 per cent) from dogs
with enteritis to be CPV positive. In India, Parthiban et al., 2001 and Schmitz et al., 2009
It can be concluded from the study that the prevalence of
CPV was 50% using nested PCR in Ludhiana, Punjab and nested
PCR is more sensitive than conventional PCR.

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