Introduction
The Picornaviridae is a large family of icosahedral viruses that includes many important human pathogens. The name picornavirus comes from the fact that the viruses are small (pico) and the genome is RNA (rna). More than 230 picornaviruses have been described, and these are divided into five genera, Enterovirus, Rhinovirus, Heparnavirus, Aphthovirus and Cardiovirus. The enteroviruses include Poliovirus, the Coxsackie viruses and Echoviruses (ECVs). The enteroviruses other than polioviruses are referred to as nonpolio enteroviruses (NPEV) and these include:

**Echoviruses:** There are over 30 serotypes (1 to 7, 11 to 27, and 29 to 33) of the enteric cytopathic human orphan (ECHO) viruses and true to their name; there is still no clear association of all types with specific disease.

**Coxsackieviruses:** Coxsackievirus group A (CAV) has 23 serotypes 1 to 22 and 24 (CAV 23 is echovirus 9) and Coxsackievirus group B (CBV) has 6 serotypes 1 to 6, named after a town in New York State where the first member was isolated.

**Numbered Enteroviruses:** New virus isolates similar in all respects with enteroviruses are simply named as enterovirus and given the next available consecutive number (enterovirus 68-71).

Acute Flaccid Paralysis (AFP) is defined as acute onset of focal weakness or paralysis characterized as flaccid (reduced tone), without other obvious cause (e.g. trauma) in children < 15 years old. Transient weakness (e.g. postictal weakness) should not be reported. This case definition is based on national surveillance case definitions. The predominating sign of flaccid paralysis results from lower motor neuron damage. However, incoordination secondary to brain stem invasion and painful spasms of non-paralyzed muscles may also occur. The amount of damage and destruction varies from case to case. At times nonpolio enteroviruses have been associated with cases of polio like paralytic disease, but this has been uncommon. Coxsackie A7 has been associated with outbreaks of paralytic disease, and enterovirus 71 (Alexender et al.,
1994; Ho et al., 1999) has been involved in several outbreaks of CNS
diseases including polio like paralysis, with some fatal cases.

Polioviruses, the most pathogenic of all enteroviruses, include three
distinct serotypes, type 1, 2, and 3 on the basis of reactivity with
neutralizing antibodies. Polioviruses are the main causative agents of
poliomyelitis and are also associated with seasonal undifferentiated febrile
illness, particularly during summer outbreaks (Melnick, 1996a; Oberste et
al., 1999), and enteroviral meningitis (Berlin et al., 1993). Poliomyelitis, a
life-threatening acute paralytic disease, is being effectively controlled by the
oral poliovirus vaccine (OPV) (Sabin and Boulger 1973; Sabin 1985), which
mounts a long-lasting immune response and protects the individual from
future viral infections with wild-type poliovirus strains. However, rare
reversion of live OPV vaccine strains may occasionally cause vaccine-
associated paralytic poliomyelitis (Furione et al., 1993; Georgescu et al.,
1994; Guillot et al., 1994; Li et al., 1996). Detailed typing of all polioviruses
isolated from patients with poliomyelitis is therefore essential to public
health polio surveillance programs aiming to eradicate wild-type
polioviruses.

All Enteroviruses are non-enveloped with an icosahedral capsid
approximately 30 nm in diameter containing the single strand RNA
genome. The capsid is composed of four proteins, VP1, VP2, VP3 and
VP4, which occur in a complex containing one copy of each. Five such
complexes are located at each of the twelve capsid vertices, so there are
60 copies of each protein in the intact capsid. A cleft or canyon in VP1 is
the site used for recognition of picornaviruses by the virus receptor on the
host cell surface. The genome of EV is a single stranded RNA of positive
polarity, approximately 7.2 kb-8.4 kb in length depending on the particular
virus. All enterovirus genomes are organized similarly with capsid proteins
encoded near the 5' end of the RNA and RNA polymerase plus proteases
near the 3' end. Since the virus RNA must serve as an messenger RNA
(mRNA) immediately after it enters a host cell, it has some properties of an
mRNA including a poly-A tract at the 3' end. The 5' end does not contain an mRNA cap, but it does have an internal ribosome entry site (IRES), a series of stem-loop structures, that serves the same purpose as a cap. A small protein (Vpg) involved as a primer in RNA replication is bound covalently at the 5' end of picomavirus RNAs. The enteroviruses multiply in the cytoplasm, and their RNA acts as a messenger to synthesize viral macromolecules. Viral RNA replicates in complexes associated with cytoplasmic membranes via two distinct, partially double-stranded RNAs - the "replicative intermediates." One complex uses the sense RNA strand, and the other uses the antisense RNA strand as template.

Enteroviruses replicate in the gastrointestinal tract and are transmitted by the fecal-oral route. Although enteroviruses replicate in the gastrointestinal tract, they do not cause severe enteric disease. More serious disease results when the virus spreads to the central nervous system or other sites. Enteroviruses cause a wide range of diseases (Grist et al., 1978), although a high frequency of subclinical infections is characteristics for most serotypes (Kogon et al., 1969). The clinical manifestations include undifferentiated febrile illnesses, upper and lower respiratory tract infections, gastrointestinal disturbances, conjunctivitis, skin and mucous membrane lesions, and diseases of the central nervous system, muscles, heart, and liver. Less commonly, EVs are associated with generalized neonatal infections, diabetes mellitus, pancreatitis, orchitis, and occasionally hemolytic-uremic syndrome and intrauterine infections. Paralytic poliomyelitis can occur without antecedent minor illnesses. A patient may suffer from aseptic meningitis with pain in the back and neck muscles for several days without progressing to paralytic poliomyelitis. The incubation period is about 3 to 5 days for minor illness and 1 to 2 weeks for central nervous system involvement, with a range of 3 to 35 days between ingestion of virus and onset of symptoms.

Humans are the only natural host for these agents. Most infection caused by coxsackieviruses are inapparent or mild. Illnesses include acute
nonspecific febrile disease and common cold-like or influenza like-respiratory diseases, pharyngitis, croup, and pneumonia. Rashes and vesicular lesions are most commonly caused by group A viruses. Herpangina presents as small, scattered oral vesicles with red areolae in the posterior oropharynx, tonsils, tongue and palate, which progress to shallow ulcers and heal within a week. Coxsackieviruses also cause exanthematous diseases that may be mistaken for rubella and aseptic meningitis that is clinically indistinguishable from meningitis caused by polioviruses and a list of other viruses. Occasionally, they cause paralytic and encephalitic diseases or other cerebral dysfunction. The most important cause of viral pericarditis and myocarditis in children and adults is CBV. Patient develops fever, tachycardia, dyspnea, precordial pain, and occasionally pericardial friction rub. Electrocardiography and radiography are helpful in confirming the diagnosis. The prognosis of uncomplicated pericarditis is good, but when myocarditis is also present the situation is serious.

Coxsackievirus A24 (CA24V) variant is the first human EV known to cause a disease, which has the eyes as the primary site of clinical manifestations. Since its discovery in Singapore in 1970, CA24V continues to give rise to sporadic cases and epidemics of acute hemorrhagic conjunctivitis (AHC) world over. Clinically, it is not possible to distinguish conjunctivitis caused by CA24V from conjunctivitis caused by Enterovirus 70. Headaches, respiratory and gastrointestinal complaints may accompany conjunctivitis. The conventional serotyping methods could not help in identification of these variant viruses, as the neutralization epitopes are located in highly variable VP1 region (Mateu, 1995). So, for development of molecular typing scheme, the ideal target should be a relatively more conserved region like 5'UTR (Arola et al., 1996). Echoviruses (ECVs), like coxsackieviruses, are associated with various disorders including respiratory illnesses, febrile illnesses with or without
rash, Boston exanthema, aseptic meningitis, paralytic diseases, and occasional conjunctivitis.

Enterovirus types 68 and 69 cause respiratory illnesses in infants and children. Enterovirus type 70 gives rise to epidemic and pandemic outbreaks of acute hemorrhagic conjunctivitis that is clinically similar to that caused by coxsackievirus A24 variant. Enterovirus type 71(EV71) causes meningitis, encephalitis and hand-foot-mouth disease with or without encephalitis.

The laboratory diagnosis is traditionally based on isolation of virus in cell culture, which can be applied to all types of clinical specimens, is relatively sensitive, and yields an isolate that can be further serotyped for clinical or epidemiological purposes. However, it takes about 3-7 days, it is expensive and not readily available. The recent introduction of PCR-based methods has markedly improved the speed and sensitivity of clinical EV detection. Most of the reported experience is limited to testing CSF specimens where the sensitivity of PCR in confirmed or suspected cases of enteroviral meningitis ranges from 66% to greater than 90%, compared with the 35-40% yield of generally achievable with cell culture. Experience with clinical specimens other than CSF is limited.

The poliovirus-serotyping procedure recommended by World Health Organization allows only intertypic differentiation but not intratypic differentiation of clinical poliovirus isolates (WHO, 1997; NIPHE, 1998). Recent advances in molecular virology by highly efficient PCR amplification methods have provided new alternatives to poliovirus detection and typing (Van der Avoort., 1995; Muir et al., 1998). Thus, PCR genotyping of polioviruses includes serotype-specific PCR primers (Kilpatrick et al., 1998), genotype Sabin-specific PCR primers (Yang et al., 1991), and restriction fragment length polymorphism (RFLP) analysis (Balanant et al., 1991; Furione et al., 1993), which may potentially allow their inter- and intratypic differentiation.
As enteroviruses include 66 serotypes, it is clearly impractical to perform neutralization of virus isolate using 66 different antisera. Hence alternative methods were developed, that uses intersecting antiserum pools (Lim and Benyesh-Melnick 1960). But the problem of "untypeable" EVs (UTEV) is frequently encountered in using intersecting antiserum pools. These UTEV may represent mixture of EVs (Kok et al., 1992), or may belong to a serotype for which antiserum is not included in the pools (Enterovirus 68-71 and CAV 3, 11 etc). These untypeable isolates may also be the so-called prime strains that are antigenic variants of recognized serotypes (Melnick, 1996b) or may be a new or previously unrecognized serotype. As poliovirus is on verge of eradication, if any NPEVs isolated from acute flaccid paralysis children remain untypeable, its correct identification is very important (Melnick et al, 1984). Identification of such isolates is also necessary to recognize an antigenic variant of existing serotype or a new EV serotype.

Enterovirus characterization and typing thus require an integrated technological approach, using both immunological and molecular methods. Typing of NPEV infection is traditionally based on a serum neutralization assay. However, this method is time consuming, labor-intensive, expensive, and may fail to identify antigenic variation. The final identification and confirmation requires the help of a number of different molecular approaches, including reverse transcription (RT)-PCR, restriction fragment length polymorphism (RFLP) analysis, and nucleotide sequence analysis of amplicons from various regions of the genome. These methods may be useful to identify all NPEV serotypes prevalent in India and to assess the possible impact of circulating NPEV populations, as we enter the final stage of poliomyelitis eradication.

Keeping this in view, the present study was undertaken to identify the NPEV serotypes prevalent in northern part of India from children having acute flaccid paralysis and carry out their molecular characterization. As the evolution rate of EV71 is very high, the conventional techniques could
not ensure very sensitive detection of EV71 among Indian isolates. The molecular makeup of EV71 isolate circulating in India is also not known. Hence, as an alternative to the antiserum neutralization, attempts were made to design an non-radioactively labeled DNA probe complementary to highly conserved, 5'UTR of EV71 prototype-BrCr strain and use for screening of untypeable isolates for the presence of EV71.