MOLECULAR CHARACTERIZATION OF WILD POLIOVIRUS ISOLATES

THESIS SUBMITTED
UNIVERSITY OF MUMBAI

FOR THE DEGREE OF
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IN
MICROBIOLOGY

SUBMITTED BY
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UNDER THE GUIDANCE OF
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Zaeem Asif Siddiqui

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A

Thesis submitted to

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(Microbiology)

By

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STATEMENT BY THE CANDIDATE

As required by the university ordinances 770 and 771, I wish to state that the work embodied in this thesis titled " **Molecular characterization of wild poliovirus isolates**" forms my own contribution to the research work under the guidance of Dr. S V Gadre at the Haffkine Institute. This work has not been submitted for any other degree of this or any other university. Whenever references have been made to previous works of others, it has been clearly indicated as such as included in the "Bibliography".

Signature of the candidate
(ZAEEM ASIF SIDDIQUI)

Certified by

Signature of the Guide
(Dr S V Gadre)

Dedicated to my loving and caring parents

Acknowledgment

Today as the task of my research project is finally accomplished, I pause for a moment to look into the past to give my sincere thanks to all those who have helped me for fulfilling my goal. Although the words are good way of expressing one's feelings, but the best way of thanking the peoples are to pray for them to the Almighty from the bottom of the heart.

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CHAPTER 1

REVIEW OF LITERATURE

Review of literature

History of poliomyelitis

Within the first half of the 20th century poliomyelitis was a frightening epidemic infectious disease found throughout the world. Several paralytic afflictions of the central nervous system characterize the disease Poliomyelitis (polio=grey, myelos= marrow, spinal chord). The causative agent of the disease was identified as one of the smallest existing viruses, the poliomyelitis virus. The origin of poliomyelitis probably dates back to 3500 years. The oldest reference to poliomyelitis is an Egyptian engraving from about 1500 BC showing a crippled priest with shrunken and deformed leg supported by crutches (Paul, 1971). Characteristic descriptions of poliomyelitis like disease can be found in the literature of antiquity. Hippocrates described paralysis that afflicted patients predominantly in summer and autumn i.e. the period that has been considered as the polio season (Armstrong, 1950). Biblical reports of persons with paralyzed or crippled extremities may also reflect affliction by poliomyelitis. In archeological excavation in southern Greenland, 25 skeletons from the 15th century were discovered which showed bone deformities reminiscent of those typically associated with severe poliomyelitis.

Recognition of poliomyelitis as a distinct clinical entity dates back to the end of the 18th and to the beginning of the 19th century (Paul, 1971). The first description of poliomyelitis is generally accredited to Underwood (1789). He presented a general description of paralysis of the lower extremity, only some of which probably were poliomyelitis. The first exact publication that constituted poliomyelitis as a separate clinical disease and differentiated it from the other types of paralysis was published by Heine (1840).

Near the end of 19th century severe epidemics of increasing magnitude occurred in Europe and North America. The first recorded polio

epidemic occurred in 1889 in Stockholm, Sweden and afflicted 44 persons. Median (1891) described this epidemic that considered the disease to be an acute infection that breaks out in epidemic form.

A virus as the causative agent for poliomyelitis was established by Landsteiner and Popper (1909) who provided the first evidence of successful experimental transmission of the disease to monkeys by intra-cerebral inoculation of bacteria free filtrate from spinal chord material of a nine year old child who died of poliomyelitis. Subsequently Flexner and Lewis (1910) passed the viral agent from monkey to monkey.

In 1916, while the first world war was ravaging Europe, the worst polio epidemic known till date spread throughout the USA, afflicting more than 27,000 persons with 6000 fatal cases in New York city alone. The 1916 epidemic of poliomyelitis gave a great impetus to polio research particularly in the USA. In the twenties and thirties research focused on investigating therapeutic attempts to cure poliomyelitis. Many drugs and chemicals were tested in laboratories, but none proved to be of any therapeutic value (Lo-Grippo et al, 1949; Grulee et al, 1950). Finally it became clear that the only hopeful method to conquer the threat of poliomyelitis would be the development of a vaccine.

One important advance during that time was the elucidation of the actual route of infection the alimentary tract. A successful alimentary tract infection in sub-human primates was demonstrated (Kling et al, 1929; Sabin and ward 1941), and the regular presence of poliovirus in the stool of the infected human was discovered (Trask et al, 1938).

A collaborative effort of many investigators established that poliovirus belongs to only three distinct sero types; type1 (Brunhilde named after the female chimpanzee from which this virus was first isolated), type2; (Lansing name after the city in Michigan where the patient lived who was the host to this virus type), type3; Leon (named after the patient from California from whom this virus was isolated) (Bodian et al, 1949; Kessel and Pait, 1949).

In order to prepare an effective vaccine, it was necessary to have a non nervous but susceptible tissue in which large quantities of virus could be grown. Enders, Weller and Robbins (1949) showed that poliovirus could be readily propagated in cell cultures of non-neuronal human or monkey tissue. Within three years of this discovery Salk developed formalin-inactivated vaccine (Salk, 1953; Salk et al, 1954). Sabin, koprowski and Cox developed attenuated viruses in tissue culture for the preparation of live vaccine (Sabin 1955; Koprowski et al, 1956; Cox et al, 1959).

The introduction of invitro method to produce large amount of virus resulted in a numerous developments in animal virology in general and polio virology in particular. Of importance in this respect were the development of a plaque assay for infectivity by Dulbecco (Dulbecco, 1952; 1955; Dulbecco and Vogt, 1954), and the definition of the essential nutrient of cultured cells (Eagle 1955, Eagle and Hable, 1956). Other works in the fifties include analysis of the interaction of poliovirus with its antibody (Le Bouvier, 1959; Mendel, 1958; McBride 1959), isolation of the first temperature sensitive mutants (Dubes and Champin, 1958), the first X-ray crystallographic studies (Finch and Klug, 1959) and the first electron microscopic analysis of poliovirus-infected cells (Horne and Nagington, 1959).

The biochemical and genetic maps of the poliovirus genome were constructed (Wimmer, 1972; Rekosh, 1970; Saborio et al, 1974), the complete translation of the viral mRNA in vitro was achieved (Villa-Komaroff et al, 1975) the entire nucleotide sequence of the viral genome was determined (Kitamura et al, 1981; Racaniello and Baltimore, 1981; Toyada et al, 1984). Most recently Wimmer and colleagues have synthesized poliovirus from the Scrape (Jennifer, 2002)

Classification of poliovirus

Poliovirus is a member of family *Picornaviridae* and genus enterovirus. The genus enterovirus includes poliovirus (3 serotypes) coxsackieviruses A1 to 22 and A24, coxsackieviruses B 1 to 6, human echoviruses 1 to 9, 11 to 27, and 29 to 34, and human enteroviruses 68-71. Enteroviruses cause diseases ranging from mild to severe paralysis to aseptic meningitis, pleurodynia, hyper angina, myocarditis, skin rashes, hand foot and mouth disease, common cold and possibly diabetes (Melnick JL, 1982).

Pathogenesis of Poliovirus

Poliomyelitis is an acute infection, which in its serious form affects the central nervous system (CNS). Mouth is the portal of entry of the virus and (Sabin and Ward 1941) becomes widely disseminated via the blood stream, but viremia is seldom detected in natural human infection because it occurs very early before significant illness is observed. The virus first multiplies in the tonsils; lymph node of the neck, peyer's patches and small intestine. The central nervous system may then be invaded. The anterior horn cells of the spinal chord are mostly involved, but in severe cases the intermediate gray ganglia and even the posterior horn and dorsal root ganglia are affected. As the chief site of action of poliovirus is the neuron, the changes that occur in the peripheral nerves and voluntary muscles are secondary to destruction of the nerve cells. In addition to pathological changes in the nervous system, hyperplasia and inflammatory lesion of lymph nodes and Peyer's patches and other lymphoid follicles in the intestinal tract are frequently observed (Melnick, 1990).

Immune response in poliovirus infection

Natural infection with poliovirus initiates a complex process that eventually results in humoral and mucosal immunity.

Humoral immunity:

IgM and IgG antibodies are detected in the serum as early as 1-3 days following natural infection (Ogra and Karzon, 1971). The level of IgM antibody is higher initially, but disappears after 2-3 months. The IgG level increases over the same period and is predominant long-lasting serum antibody. Serum IgA antibody is detected approximately 2-6 weeks following exposure, but remains at low level rising only slowly during the first 3 months after exposure.

Mucosal immunity:

Replication of poliovirus in the epithelium of the pharyngeal and intestinal mucosa produces local antibody and mucosal immunity to poliovirus. The predominant class of Immunoglobulins in alimentary tract secretion is IgA, which possesses neutralizing activity against poliovirus. Secretary IgA antibody can protect against the infection of the alimentary tract with poliovirus but the resulting local immunity is not absolute. Very large doses of poliovirus may result in replication of poliovirus in the oropharynx and intestine even in the presence of secretary IgA (Ogra and Karzon, 1969).

Transmission of poliovirus

Poliovirus infection usually spreads by faeco-oral route and close human contact appears to be the primary route of spread. Virus is present in the oropharynx from about 5 days before to 5 days after the onset of symptoms of the disease. Epidemiological evidence shows that this is the period of greatest infectivity. Fecal excretion commences a few days after the onset of symptoms, continues for several weeks and occasionally for

months (Trask et al, 1938). Transmission before onset of the symptoms is therefore likely to be due to droplet spreads from the pharynx, transmission more than a week after onset is most probably due to fecal virus. The association of endemic poliomyelitis with poor sanitation suggests that faeco-oral transmission between young children is chiefly responsible for the virus transmission (Sabin and Ward, 1941)

Diagnosis of poliomyelitis

The diagnosis of paralytic poliomyelitis depends upon the clinical course and virological investigations.

Clinical investigations:

A case of acute flaccid paralysis (AFP) includes, paralytic poliomyelitis, Guillain-Barre' syndrome and transverse myelitis. Less common etiologies are traumatic neuritis, tumors, encephalitis and meningitis. An AFP is most likely to be due to poliovirus infection if it shows fever at the onset, onset is sudden and paralysis reaches maximum within 48 to 72 hours. There is no sensory loss and the paralysis is descending. Because the anterior horn cells of the central nervous systems cannot be replaced or regenerated, the paralysis is permanent. The other symptoms include malaise, drowsiness, headache, nausea, vomiting, constipation, or sore throat in various combinations (Andrus J et al, 1997)

Virological investigations:

Laboratory confirmation of poliovirus etiology is critical for polio eradication, as the non-polio enteroviruses (NPEV) are also known to cause AFP. Poliovirus can be readily isolated from stool specimen and throat swabs but infrequently from cerebrospinal fluid (CSF), taken soon after the onset of the illness. Two stool specimens collected at the 24 hours interval soon after the paralysis increase the chances of poliovirus

isolation. The stool specimen is treated with chloroform and then inoculated into two cell lines of human or monkey origin. A mouse cell line (L20B) that has been genetically altered to express poliovirus receptor is relatively susceptible to poliovirus infection (Pipkin et al, 1993). The virus can identify and typed by microneutralization test using type specific antiserum (Melnick JL, 1973).

The wild or vaccine character of poliovirus can be determined by ELISA with polyclonal cross-absorbed anti-sera (Van Wezel and Hazendonk, 1979; Deshpande and Dave, 1991), type specific monoclonal antibody (Jarzbeck et al, 1992), Restriction fragment length polymorphism (RFLP) assay (Balanant et al, 1991) PCR (Yang et al, 1992) and RNA probe hybridization assay (De et al 1995). As the Sabin vaccine strain is well characterized at the antigenic and molecular level, all the strains, which differ from the Sabin vaccine strain, are classified as wild strain. The ELISA and monoclonal antibody assay differentiate the strains on the basis of antigenic differences, whereas the PCR, RFLP and probe hybridization differentiate the isolates on the basis of nucleotide differences at the genomic level. Nucleotide sequence analysis is considered as the gold standard method to define vaccine/wild origin of an isolate. The use of genomic sequencing, pioneered with polioviruses has given rise to a new discipline, molecular epidemiology of viruses, a hybrid discipline that combine the tools and concepts of classical epidemiology with those of microbiology, biochemistry, genetics and evolutionary biology (Kew et al, 1995).

Physico-Chemical properties of poliovirus

Poliovirus is a small, spherical particle, 28-30nm in diameter. The virion has a dry molecular weight of 8.5×10^6 Daltons, sediments at 150S-160S and has a buoyant density of 1.034 g/cm^3 in CsCl. Approximately 30% of the dry weight of virion is RNA of molecular weight 2.5×10^6 Daltons and the rest consist of proteins with a molecular weight of 5.8 x

10^6 Daltons (Schaffer and Fommhagen, 1965; Cooper et al, 1978). Purified poliovirus preparations do not contains detectable amounts of sugar and lipids (Drzeniek and Bilello, 1974).

Genomic structure and structural organization of poliovirus

The genome of poliovirus is fully characterized and the entire genome of all the three poliovirus serotypes of wild and vaccine strains have been fully sequenced (Kitamura et al, 1981; Racaniello and Baltimore, 1981; Nomato et al, 1982; Toyoda et al, 1984). The genome of poliovirus is a continuous, linear, single stranded molecule of positive messenger sense polarity). The isolated RNA has sedimentation coefficient of 35S and molecular weight of about 2.4-2.7x10^6 daltons (Granboulan et al, 1969; Tannock et al, 1970). The genome comprises of approximately 32% of the dry mass of the virion and each particle contains only one molecule of RNA.

The genome of poliovirus contains approximately 7500 nucleotides (Lee et al, 1979). The 5' end of the genome is linked to a small protein VPg, the 3' end to a stretch of poly A tract which is 40-100bp long. There are of more than 800 non-coding nucleotides located at the two ends of polio RNA; 742 untranslated bases at the 5' end and 70 untranslated bases preceding the poly A tract at the 3' end in poliovirus type 1.

About 6700 nucleotides are used for coding approximately 2200 amino acids or a protein of about 2,50,000 daltons. A little more than one third of the coding capacity is used to code for capsid proteins VP4, VP2, VP3 and VP1 in that order. The rest is used to code for viral non-structural proteins and enzymes; NCVPs 1-10 (Non capsid viral proteins), which carry out important functions in recognition of cellular activities, processing of viral proteins, viral replication and virion assembly. Coding for all the poliovirus proteins falls into 3 major domains of poliovirus genome. These domains evidently are structurally and functionally distinct regions of the genomes reflected in the 3 primary products; NCVP1a (P-1 domain),

NCVP3a (P-2 domain) and NCVP1b (P-3 domain). The P-1 domain codes for the structural proteins, the P-3 domain for the replicative proteins and the functions of the P-2 region is yet to explore fully (Figure 1).

Genome-linked protein VPg:

At its 5' end the genomic RNA of poliovirus is covalently linked to a small viral coded protein called VPg (Lee et al, 1976; 1977). The length of the VPg protein is 22 amino acids and molecular weight 2400 Daltons (Kitamura et al, 1980, 1981). The coding region for VPg on the viral genome lies within the replicase region (Pallansch et al, 1980; Kitamura et al, 1980). The VPg is attached to the 5' end of all the newly synthesis plus and minus viral RNA strands and to all the nascent strands of the replicative intermediate (Nomoto et al, 1977). VPg is also attached to the double stranded replicative form RNA (Wu et al, 1978). This observation suggests that VPg might serve as the primer for the initiation of viral RNA synthesis (Kitamura et al, 1980). No significant change in the infectivity of RNA is observed when the RNA is not linked to VPg (Flanegan et al, 1977). VPg is not required during the early stages following the virus infection.

5'-End sequences:

Nearly 742 nucleotides at the 5' end of the poliovirus genome are untranslated. In the poliovirus type 1 the initiation codon AUG begins at the 743rd nucleotide, which is 9th AUG codon from the 5' terminus as confirmed by sequence analysis (Dorner et al, 1982). This region also contains 29 stop codons distributed among the 3 reading frames. The 3 poliovirus serotypes show 90% sequence homology within this region suggesting that 5' end terminus serves important functions in ribosome binding and initiation of protein synthesis. The importance of 5' terminal region is illustrated by the observation that cells transected with plasmid containing all but the first 115 bases of the poliovirus genome do not

produce virus in contrast to plasmid containing the complete copy of poliovirus genome (Racaniello and Baltimore, 1981).

3' End sequences:

About 60-70 nucleotides at the 3' end of the poliovirus genome is untranslated. The 3' end region of the poliovirus genome is short as compared to the eucaryotic mRNA (Hamlyn et al, 1978; Catterall et al, 1978). The function of this region is similar to the other untranslated regions i.e. to bind the virus polymerase complex during initiation of replication. The identical conserved sites are not present in the 3' end sequences of the plus and minus viral RNA strands so that the mechanism of replicase recognition must differ for plus and minus viral RNA strand.

Poly A tract:

Similar to many eucaryotic mRNAs poliovirus RNA has at its 3' end a terminal stretch of poly A tract approximately 40-90bp in length (Ahlquist and Kaesberg, 1979). The poly A tract is important for RNA activity, since RNA molecules with artificially shorted poly A tract of poliovirus have lower infectivity than with those of longer poly A tract (Spector and Baltimore, 1974).

Features of the coding region

The coding region of the poliovirus genome can be divided into 3 distinct regions, P-1, P-2 and P-3 region. The functions and the products of these regions are describe below,

P-1 region:

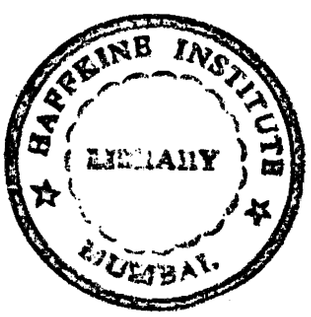
The P-1 region of the viral genome at the 5' end of viral RNA codes for the structural proteins VP4, VP2, VP3 and VP1 in that order.

P-2 region:

The P-2 region, the middle segment in the viral coding region encodes several non-structural polypeptides: 2A, 2BC, 2B and 2C. The 2A is a proteinase that cleaves at the VP1/2A junction (Toyada et al, 1986) and also the 3CD at the P-3 region into 3C' and 3D' (Wimmer et al, 1987). Very little is known about the functions of polypeptides 2BC and its cleavage product 2B and 2C. 2BC has been found to be associated with membrane bound replication complexes in infected cells (Bienz et al, 1990).

P-3 region:

The P-3 region of the viral genome codes for the non-structural proteins required for viral replication, assembly and release of the progeny virus. The gene products of the P3-region are cleaved into seven polypeptides: 3AB, 3A. 3B, 3C, 3D, 3C' and 3D'. The 3AB is a precursor of VPg protein, which in turn is covalently linked to 5' terminus of plus and minus strand of RNA (Nomoto et al, 1977). The 3AB is also found to be tightly associated with the membrane-associated complex in infected cells (Takegami et al, 1983). The functions of the cleaved 3A and 3B are not understood. Polypeptide 3CD processed at two alternate cleavage site; cleavage at glutamine/glycine peptide bond yield 3C and 3D; cleavage between tyrosine/glycine produces 3C' and 3D'. Biochemical evidence suggests 3CD and 3C are proteases which cleave the polyprotein at glutamine/glycine pairs in the P-1 capsid precursor (Ypma-Wong et al, 1988) and P-2, P-3 precursors respectively (Hanecak et al, 1982). The 3D is the viral RNA dependent RNA polymerase (van Dyke and Flanegan, 1980), which copies the viral genome. The functions of 3C' and 3D' are not known.



Architecture of poliovirus

Experimental studies on the structure of poliovirus were made possible by the development of methods for the purification and crystallization of the viruses (Schaffer and Schwerdt; 1955, 1959, Steere and Schaffer, 1958). With the availability of such crystals, excellent X-ray diffraction patterns of crystallized poliovirus were obtained at the resolution of 2.9A^0 (Hogle et al, 1985). Based on the theoretical grounds and from the X-ray crystallography data, it was concluded that the virion capsid is made up of 60 (or 60xN) identical asymmetrical structure units with a diameter of 6.0 to 6.5nm each, arranged in an icosahedral lattice with 5:3:2 fold symmetry. The fundamental structural unit of capsid protein is termed as protomer. Each protomer in the native virion consists of a single copy of each of four individual proteins, VP1, VP2, VP3, VP4 named in order of decreasing molecular weight and derived from a common precursor protein (NCVP1a). Five protomers are located in a pentameric arrangement around the five-fold symmetry axis. Twelve pentamer build up the complete virus capsid. At the interface between the pentamers, twofold and threefold symmetry axes are located. The smallest protein, VP4 is located on the inner surface of the virion and is relatively disordered.

In the native virion the three major capsid proteins VP1, VP2 and VP3 all occupy discrete domains in space and they all penetrate the width of capsid backbone. Certain stretches of VP1 seem to be particularly exposed on the external surface (Figure 2). Each of the capsid protein can also be cross-linked to viral RNA by UV irradiation. VP3 seems to be least involved in interaction with RNA. VP4 in contrast is located internally in close proximity to the RNA and can be cross-linked externally to RNA by UV irradiation.

Antigenicity of poliovirus

Neutralization antigenic sites on the surface of virion have been identified with neutralizing monoclonal antibodies that selected neutralization "escape mutants" among the virus population (Emini et al, 1982; Evans et al, 1983; Crainic et al, 1983; Ferguson et al; 1984). Three distinct antigenic sites have been identified which are designated as site 1, 2 and 3. The site1 made up of 12 amino acids of VP1 from 89-100 and is strongly immunodominent for type 1, 2 and type 3 poliovirus. Site 2 is a complex site composed of residues 220-222 from VP1 (site2a) and residues including 169 and 170 of VP2 (site2b). Both site 2a and 2b have been detected in poliovirus type1 but only site 2b is detected in poliovirus type3. Site 3 is again a complex site, which includes residues from 286-290 from VP1 (site 3a) with residues including 58 and 59 of VP3 (site3b). Both the site 3a and 3b have been detected in poliovirus type3 while only 3b has been detected in poliovirus type1 (Emini et al, 1983; Minor PD et al, 1985; Minor PD et al, 1986).

Figure 1: Structural organization of poliovirus genome.

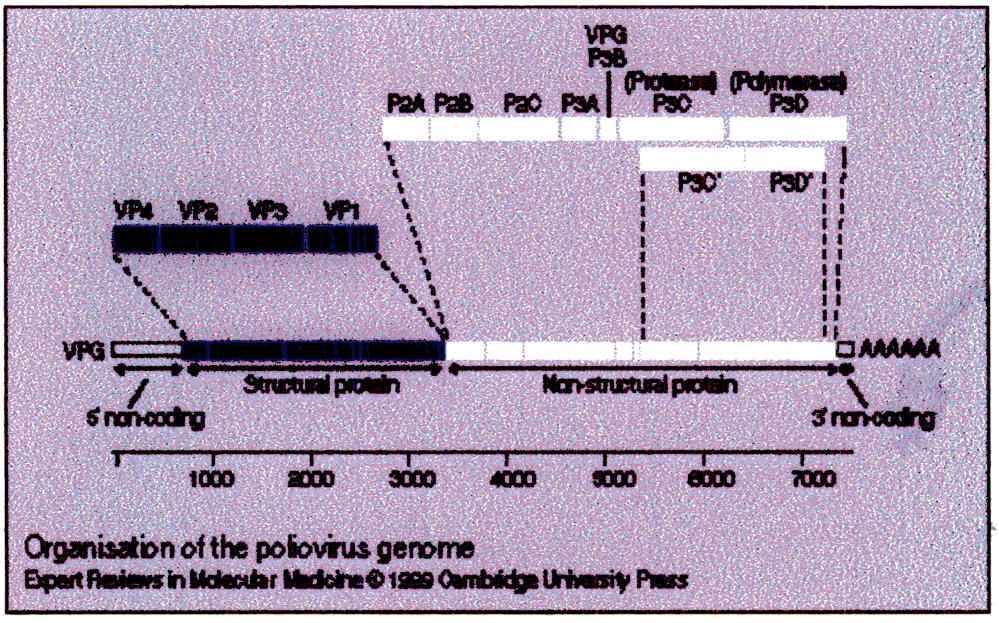
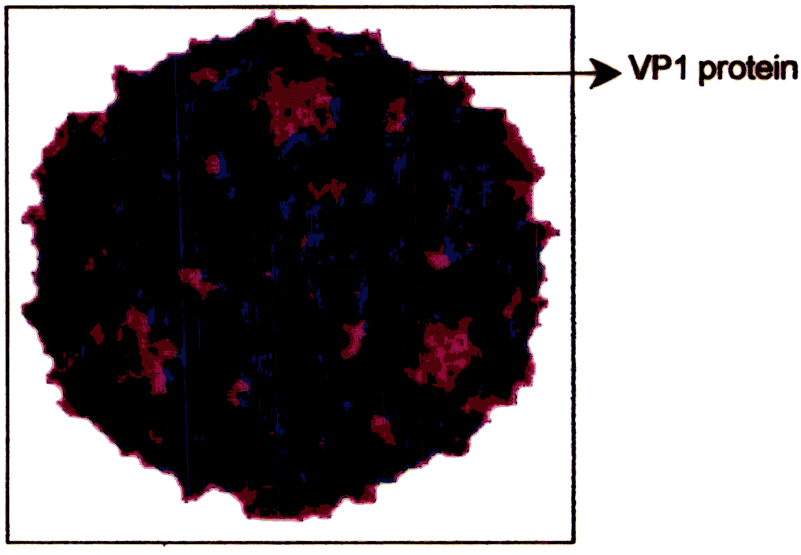


Figure 2: Three-dimensional structure of poliovirus.



Epidemiology of poliovirus

The epidemiology of polioviruses has changed substantially during the last century from endemic transmission to periodic epidemic form. This shift was observed especially in some temperate climate countries such as United States, United Kingdom, Finland etc, in the beginning of the 20th century with economic development, improved sanitation and household hygiene. In developing countries, particularly in tropical areas an endemic epidemiological pattern predominated until recently. The annual incidence rate of the paralytic poliomyelitis in the US before the introduction of polio vaccine was 36 per 100,000 populations. After the introduction of the two very effective polio vaccines i.e. the IPV in 1954 (Salk, 1955) and trivalent OPV in 1963 (Sabin, 1985) the average incidence rate gradually fell to 0.01 to 0.1 per, 100,000 populations (Gracchev, 1984). Similar decrease in the incidence of poliomyelitis was observed in the former USSR, European countries, Australia, New Zealand etc. It was estimated that approximately 350,000 cases of paralytic poliomyelitis occurred worldwide until 1988.

Poliomyelitis has been an endemic disease in India. The first epidemic was reported at Car Nicobar in the Andaman groups of Island of the Indian union (Pandit, 1955). Gharpure and Dave conducted the first field trial of OPV in India in the year 1961 after the outbreak of poliomyelitis in the state of Andhra Pradesh (Gharpure and Dave; 1962). Although OPV was available in India since 1965, immunization coverage was very low. In early 1980s it was estimated that 199,000 to 448,000 children developed paralytic poliomyelitis each year (Basu and Sokhey; 1982). In order to control the disease, the government of India included vaccination with OPV in the Expanded program of immunization (EPI) in the year 1978. Decline trends of poliomyelitis have been reported at the national, state and district levels. The poliomyelitis incidence fall from a reported figure of 28,350 cases in 1987 to less than 10,000 in 1990 and 5669 in 1991. In Bombay, the incidence has declined to 4.2 in 1990 and 2 per 100,000 populations in 1991 (Sokhey, 1992).

Polio Eradication

In 1988, the World Health Assembly (WHA) committed World Health Organization (WHO) and its member states to the goal of poliomyelitis eradication by the year 2000 (WHA, 1988). The goal was defined as follows:

1. No more cases of paralytic poliomyelitis due to wild poliovirus.
2. Absence of wild poliovirus from the human and the environment.

The polio eradication efforts being led by partnership of four agencies WHO, the world summit for children convened by UNICEF, the Unite States Centers for Disease Control and Prevention (CDC) and a private group Rotary international, also declared support for the global eradication goal. All the agencies with member states set the stage for global polio eradication initiative (PEI), which is making dramatic progress towards the complete global eradication of poliomyelitis. Success of PEI would mean eliminating a second viral disease of mankind.

The strategies for global polio eradication evolved from the work of Chumakov (Gracchev, 1984) and other scientists who work with Sabin to pioneer the use of mass vaccination campaign for the control of polio endemic in Hungary (Domok, 1984) and the Soviet Union. The strategies were applied in Cuba (Cruz, 1984) and Brazil (Risi, 1984) and then applied on hemispheric basis by the Pan American Health Organization. The WHO recommended the following four major strategies for the polio eradication (Wright et al, 1991):

1. High routine immunization coverage with at least three doses of OPV.
2. Supplemental immunizations provided in the form of National Immunization Days.
3. Acute flaccid paralysis (AFP) surveillance system.
4. Mopping up campaigns in high-risk areas.

Progress toward polio eradication

Significant progress has been made worldwide towards the global eradication of the poliomyelitis. At the start of the program 125 countries on five continents were polio infected, while by end of the year 1999 only 30 countries on the two continents remained infected. Poliovirus transmission is now limited to South Asia and Western and Central Africa (Hull, 2001).

Wild poliovirus type 2 has been eradicated from the globe in the year 1999. The last cases of wild type 2 etiology were detected in the Northern states of India namely Utter Pradesh and Bihar (CDC, 1999).

The last case of polio caused by wild type poliovirus in South America was that of a two year old boy from Peru in August 1991. In the year 1994 America was certified polio free by the International Commission for Certification of Poliomyelitis Eradication (ICCPE) (deQuadros et al, 1997), the first region to be certified as polio free. In October 2000 the commission declared Western Pacific region to be free of polio (WHO, 2000). On 21st June 2002 the Regional Commission for the certification of poliomyelitis eradication in the European region certified WHO European region as free of indigenous wild poliovirus transmission, the third out of 6 WHO region to be certified as polio free (WHO, 2002).

In the Eastern Mediterranean region, polio is reported at extremely low levels by the end of the year 2000 in North Africa, the Middle East and the Arabian Peninsula. In 2001 wild poliovirus type 1 and 3 were detected in Pakistan, type 1 in Afghanistan, Egypt and Sudan and only type 3 in Somalia (WHO, 2001).

Between the year 1999 and 2000, in WHO's South east Asian region the number of wild virus confirmed cases decreased from 1161 to 272 of which 265 reported from India. The number of cases reported from Bangladesh decreased from 29 confirmed cases to 1 case in 2000. In year 2000 two confirmed cases were detected in Myanmar along the

Bangladesh border and four cases in Nepal along he border with India (WHO, 2001).

The first nationwide NIDs started in the year 1995 immunizing the children below 5 years of age (Banerjee et al, 2000). In the year 1997 active AFP surveillance was started in India. Due to the action taken under the eradication program the overall number of reported cases decreased in India from 1934 confirmed cases in the year 1998 to 265 confirmed cases in 2000 (WHO, 2001a).

Benefits of polio eradication

The most obvious benefit of polio eradication is a decrease of burden of the disease and its subsequent disability. WHO estimates that at least 500,000 cases of paralytic polio will be prevented each year, reducing health care expenditure for hospitalization and rehabilitation of the victims of the disease. The major financial benefit comes from stopping polio immunization after eradication is certified. The recent cost benefit analysis projected that the direct financial benefit could be as high as $1.7 billion annually (Bart et al, 1996). Additional indirect benefits of eradication are the strengthening of primary health care system and revitalization of immunization program (Hull et al, 1997).

Challenges to polio eradication

Although tremendous progress has been made toward eradication of polio the target is still not archived. There are a number of obstacles, which comes in the way of polio eradication. One obstacle is inadequate development of surveillance system and insufficient immunization campaigns in the countries with armed conflicts.

The inadequate political and financial support for polio eradication is also the issue. WHO estimates that at least $300 million will be needed to support eradication in developing countries between 2000 and 2005, the target date for the global certification (Aylward et al, 2000).

The major challenge to the polio eradication program is the circulating Vaccine Derive Polioviruses (VDPVs). The major adverse event associated with these circulating VDPVs is the Vaccine Associated Paralytic Poliomyelitis (VAPP). The molecular characterization of these VDPVs often points to the back mutation with the nucleotide change precisely those found in the parental neurovirulent strain. For instance analysis of an outbreak of more than 16 case of paralytic poliomyelitis in Hispania (the island shared by the Dominican republic of Haiti) has revealed that the virus responsible was a vaccine derived type 1 poliovirus, whose genetic divergent from the parental vaccine strain indicates that it had been circulated for more than 2 years before causing illness (CDC, 2001). A retrospective study of poliovirus isolates from Egypt has also revealed that more than 32 cases of paralysis that occurred from 1988 through 1992 were associated with the type 2 vaccine derived isolates (CDC, 2001a). Similar studies identified three cases of poliomyelitis due to circulating type 1 VDPVs in Philippines in the year 2001 (CDC, 2001b).

The most important issue is to safeguard the population, so that when and how to stop the administration of poliovirus vaccine may be the most critical and potentially far reaching decision of the entire eradication initiatives. Given the rare but the real risk of VAPP, the current OPV strains may need to stop soon after the certification of eradication. The ongoing research will determine which, if any vaccine may be needed in the post eradication era (Dove and Racaniello, 1997).

The next challenge for the eradication program will be the containment of the laboratory stocks of polioviruses before the global certification of polio. Many of the world's laboratories store samples that are known to contain wild type polioviruses. The risk of inadvertent transmission of pathogens from the laboratory to the community is small, but not non-existent. For example, the last case of smallpox that occurred in Birmingham, England in 1978 (one year after the global eradication of

the smallpox) was the result of laboratory containment failure (Fenner et al, 1988). To date 11 countries have completed the activities of pre-eradication phase and submitted national inventories for review (CDC, 2001c).

Laboratory network for polio-eradication

One of the key strategies for polio-eradication is establishing sensitive surveillance. To ensure that specimens from AFP cases undergo appropriate investigations for poliovirus isolation, WHO has established a three tiered global laboratory network. At the first level is the Global Specialized Laboratories (GSL), which provide advanced tests such as genomic sequencing, training, research and production of tests reagents for poliovirus isolation and identification. At the seconed level is the Regional Reference Laboratories (RRL), which perform intratypic differentiation of poliovirus isolates as wild or vaccine, besides doing the isolation and identification of poliovirus serotypes. At the third level the network consists of National Polio Laboratories (NPL). The NPLs carryout primary isolation and identification of poliovirus serotypes from the stool specimen of AFP cases (Aylward et al, 2000). In India the laboratory network consists of one GSL, two RRL and seven NPL.

Molecular epidemiology

Molecular epidemiology provides the tools that have predictive significance and that epidemiologist can use to better define the etiology of specific diseases, and to work towards their control (Thompson et al, 1998). Molecular epidemiology is now regarded as an integral component of most epidemiological studies outbreak investigations, and surveillance may soon be considered incomplete without the application of appropriate molecular tools (Hall, 1996). This is because molecular epidemiology added dimensions to studies of disease etiology and provides information that classical epidemiology cannot.

Molecular epidemiology in Polio eradication

Molecular epidemiological approaches have been widely used within the Poliomyelitis Eradication Initiatives (PEI) to:

- Determine the sources of imported viruses.
- To follow the pathway of virus transmission.
- Monitor the progress of control activities.
- Identify the reservoirs sustaining wild poliovirus transmission.
- To develop molecular reagents for the rapid detection of wild polioviruses in clinical and environmental samples.
- Provide critical evidence that poliovirus eradication has been achieved.

The ultimate goal of PEI is to break all chains of wild poliovirus transmission. In response to effluent surveillance system PEI require increasingly powerful molecular methods like PCR, RFLP, nucleic acid probe hybridization and partial genomic sequencing for tracking wild poliovirus. Insight gained from the molecular epidemiological investigations has contributed to the formulation of improved strategies for poliomyelitis control.

The identification of poliovirus by serotype and whether they are vaccine like or wild has important epidemiological and programmatic implications (Kew OM et al 1993). Although the conventional virological techniques are adequate enough to characterize a clinical isolate as vaccine derived or wild type, they are nor suitable for establishing relationship among strains or to pinpoint the source of a virus. Oligonucleotide finger printing was used extensively in the molecular studies of poliovirus epidemiology in the 1980s (Nottay et al, 1981; Minor et al, 1982; Kew and Nottay, 1984). Fingerprinting however, is highly sensitive to small mutational changes in viral genome, quantitative estimates of relatedness are most reliable when RNA molecule shares >95% base sequence homology (Aaronson et al 1982). Examination of serial isolates taken during epidemics revealed an average rate of fixation

of mutations over the entire genome of about 1 or 2 nucleotide substitutions per week (Nottay et al, 1981; Kew and Nottay, 1984). Fingerprinting thus was helpful to recognizing relationship between the isolates separated from the ancestral infection no more than 3-5 years.

To extend the epidemiological range of molecular methods for genomic analysis, nucleotide sequence of a 150 bp fragment encompassing the VP1/2A junction region was used by Rico-Hesse et at (1987). This segmented consist of 90 nucleotides from the carboxy terminal residue of the capsid protein VP1 and 60 nucleotides encoding the amino terminal of protease 2A. The power and precision of sequencing as a surveillance tool is derived from the exceptionally rapid rate at which poliovirus RNA genome evolve (Nottay et al, 1981). Because mutations accumulate stepwise, pathways of virus transmission can be reconstructed from genomic evolution. Genetic similarity of 98% or more is considered to imply a direct epidemiological link. Lineages are defined as the group of viruses showing >95% sequence similarity in this region. The genotype is defined as group of viruses showing at least 85% sequence similarity in this region (Rico-Hesse et al, 1987). In addition to the VP1/2A region, analysis of larger regions of the genome e.g. the VP1 capsid region (900 to 906 bp long) may give additional information on the molecular evolution of the virus during an epidemic e.g. the clonal spread of the virus versus multiple lineages and antigenic drift (Huovilainen et al, 1988), and on transmission mechanism within an epidemic (Kew et al, 1993).

Indigenous strains isolated in different geographical regions usually exhibit a significant genetic diversity. Many independent genotypes of wild polioviruses existed worldwide (Rico-Hesse et al 1987). On the other hand a high degree of similarity has been observed among strains isolated during any given epidemic. The geographically genetic clustering of polioviruses has important implication for poliomyelitis control because it implies that local conditions (such as immunization coverage or pattern of human migration and interaction), rather than the specific properties of

each virus (such as antigenicity), largely determine the extent of endamicity of the disease. Although some polioviruses have wider range than others, no pandemic genotypes have been recognized. When virus importations do occur, they usually originate from adjacent areas and do not establish endamicity in well-immunized populations (Kew et al; 1993).

Global distribution of wild poliovirus genotypes:

In the pre vaccine era all the three-poliovirus sero types had worldwide endamicity (Paul, 1971). Immunization has dramatically altered the pattern of poliovirus distribution. Most countries have made good progress in immunization against poliomyelitis and the picture in many endemic areas is improving rapidly. While OPV derived strains are now ubiquitous, wild poliovirus is endemic to parts of the eastern hemisphere. Poliovirus endamicity is most intense in tropical latitude, in areas with high population density and poor sanitation (de Quadros et al, 1994; Melnick, 1990; Nathanson et al, 1993). Eradication of wild genotypes has so far been most readily achieved in areas with climate, which have the shortest season of poliovirus transmission, including tropical highlands (Nathanson et al, 1979; Nathanson et al, 1993). However all poliovirus genotypes are eradicated through comprehensive NIDs. Several genotypes are believed to have been recently eradicated by PEI, in Americas three type 1, one type 2 and four type 3 (Kew et al, 1993), in South Africa one type 1 (Rico-Hess et al, 1987), in china four type 1 and one type 3 (Zheng et al, 1993) and in Philippines one type 1 and one type 3 (Kew et al, 1995).

Identification of zones of endamicity:

Molecular epidemiological analysis has revealed zones in which continued endemic circulation of related polioviruses spans several adjacent countries. For e.g. sequence analysis has shown that cases in the southern Mekong delta region of Vietnam are very closely linked to

cases in Cambodia, and that type 1 poliovirus spreads bi-directionally across the border (Kew et al, 1995).

The extent of the epidemiological zones differs by sero type. Type 1 epidemiological zones usually have wider geographical ranges than type 3 zones, an observation consistent with the much greater frequency of large endemics associated with type 1 genotypes. The boundaries of epidemiological zones may overlay. In the year 1990 three different genotype of poliovirus type 1 were indigenous to the areas of Central Asia Republics, North Pakistan and western China (Ricco-Hesse et al, 1987; Zheng et al, 1993; Mulders et al, 1995; Huvilainen et al, 1995). Several highly populous countries continued to be major reservoir of wild poliovirus. In highly endemic areas, poliovirus circulates via multiple chains of transmission proceeding from many independent endemic reservoirs. Multi chain transmission is indicated virologically by a high degree of sequence heterogeneity among contemporary isolates of the same genotype within a region. In some endemic countries such as Pakistan and Central Asia more than one type 1 or type 3 genotype co-circulated (Kew et al, 1995).

Detection of imported viruses:

The distinction between indigenous and imported genotypes becomes arbitrary in view of the frequency of the introduction of the wild poliovirus into the other areas. If the imported virus is able to maintain circulation after introduction then it becomes the indigenous genotype after some time and in some cases even displaces the genotype(s) previously recognized as indigenous (Kew et al, 1995). Importation may be long-range introduction across region or local cross transmission. Imported genotypes are most easily recognized if they have low sequence similarity with the recognized indigenous genotype, or if they are known to be indigenous to a distant reservoir. Importation is most evident when wild polioviruses are found in polio free zone. For example, wild polioviruses

from Mexico were reportedly introduced into United States through the 1970s and were occasionally associated with sporadic cases. Molecular analysis of isolates from cases revealed close sequence similarities to polioviruses indigenous to specific regions of Mexico (Ricco-Hesse et al, 1987; Kew et al, 1995). Sequence analysis of the cases isolated from The Netherlands, Canada and US provided independent evidence that Turkey was the endemic reservoir for the type 1 virus of the 1978-79 outbreak in those countries (Nottay et al; 1981, Ricco-Hess et al, 1987). The type 3 virus of the 1984 epidemic in Finland appears to have originated from the Eastern Mediterranean (Kew et al, 1990; Poyry et al, 1990). The Eastern Mediterranean also appears to have been the source of type 1 poliovirus associated with 1982-84 cases in Spain (Bernal et al, 1987, Kew et al, 1990). The 1992-93 outbreak of poliovirus type 3 in unvaccinated population of The Netherlands was due to the importation of wild poliovirus from India as the outbreak strain showed 97% sequence similarity with the Indian isolate on the year 1991 indicating the importation of wild poliovirus type 3 from India (Mulder et al, 1995a). Two outbreaks in 1980s due to wild type 1 poliovirus occurred in South Africa. Partial sequence analysis of the 1982 outbreak in Gazankulu in the North East part of the country (Johnson et al, 1984; Chezzi et al, 1997) shows that the virus genotype was indigenous to the SA. The 1988 outbreak in Kwazulu-Natal was due to imported genotype introduced in SA in 1985 from the countries north of the border. This genotype displaced the three genotypes previously in circulation and circulated till the last confirmed case of poliomyelitis associated with wild type was documented (Chezzi et al, 1997). The genomic analysis of the 1993-94 poliovirus type 1 which caused the paralysis in the Central African Republic (CAR) in the city of Zangui shows that two distinct genotypes were co-circulated in 1993, while in 1993 only the third genotype was observed (Morvan et al, 1997).

The outbreak due to wild type 1 poliovirus from Albania in the year 1996 showed 99 to 100% sequence similarity within the epidemic strains

and 95% related to isolates from Pakistan in the year 1995 (Fiore et al, 1998). A massive outbreak of poliomyelitis due to wild type 3 poliovirus occurred in Angola in the year 1999. The sequence data shows that the virus is not related to any of the previously circulated polioviruses from Africa. Wild poliovirus has not been isolated from Angola previously and hence indicates that the virus was either was circulating silently or imported (Valente et al, 2000). The only case of wild type 1 etiology in China is related to the isolated from the Indian sub-continent (CDC, 2000). In March 2001 a 13-month-old unvaccinated girl from Bourgas (Bulgaria) developed paralysis and type 1 poliovirus isolated from the case is related to the isolate from Utter Pradesh from India isolated in the month of July 2000 (WHO, 2001b).

The role of molecular epidemiology in infectious diseases

Controlling the infectious diseases depends upon the ability to rapid identification and characterization of the etiological agents and the establishment of adequate surveillance system that monitors the effects of control programs and warns outbreaks, so that appropriate strategies can be implemented. . The availability of the molecular tools such as PCR, RFLP, oligoneucleotide mapping, genomic sequencing etc has had an enormous impact on the genetic characterization, taxonomy and diagnosis of infectious diseases. Molecular epidemiology is also very useful tool not only for the diagnosis but also for the monitoring of the effect of treatment (Lipuma, 1998).

The molecular epidemiological techniques shown that in South Africa there are two phylogenetically quite distinct rabies virus lineages; one is related to the European stocks and persisting in candid population, whereas the other probably more ancient introduction of rabies circulating in viverrids such as mongoose (Smith et al, 1993).

RFLP typing investigations have been extremely useful in understanding transmission dynamics of Tuberculosis. There are multiple

studies from different countries investigating the Tuberculosis outbreaks on the basis of molecular epidemiology and have provided the insight not available through conventional epidemiology and provided the scientific basis for improving the diseases control (Otal et al, 1991).

The molecular epidemiology has provided important information about the global distribution and the spreading of the Human Immunodeficiency Virus (HIV) and the newly seeded epidemics. For example molecular epidemiology have provided a very important information about the virtually and simultaneous introduction and spread of two HIV-1 strains in the Thailand of which each appears to be largely confined to a different population groups (Ou, et al 1993).

Genomic analysis of the hemagglutinin (H) protein and or the nucleoprotein of measles virus can support the surveillance activities; monitor the control program of measles, which is the next target in viral disease eradication. The molecular epidemiological data documented 3 genotypes of measles in Japan (Katayama et al, 1997). The single genotype detected in US in 1996 linked to importation from Philippines (Rota et al, 1998). The 4 genotypes of measles detected in United Kingdom in 1990 include the main genotype of Europe and the genotypes, which had widespread circulation in Japan (Jin et al, 1997). The 1993 outbreak of measles in island of Palau linked to a Japanese tourist, which arrived on the island with rash and fever. The genomic analysis shows that the genotype was same as circulated in Japan.

Six genotypes of Hepatitis B virus (HBV) were documented with the sequence analysis. Genotype A is most common in northern Europe and North America, genotype B predominates Asia, genotype C is found in Asia as well as in Pacific region. genotype D is found in Mediterranean countries, North America, while E is from Africa. Genotype F is the most divergent of all and is found in South Africa (Norder et al, 1992; Blitz et al, 1998).

There are many other instances where the molecular characterization and phylogenetic analysis have revealed the important evolutionary relationship among the infectious agents and the data is useful in monitoring the impact of the controlled strategies.

Molecular Phylogenetics

Molecular Phylogenetics is the study of evolutionary relationship among organisms or genes by the combination of molecular biology and statistical techniques. It is also commonly called molecular systematic, if the relationship of organisms are the concern.

Phylogenetic trees:

All life forms are related by descent to one another. Closely related organisms are descendent from more recent common ancestor then are the distantly related ones. In phylogenetic studies, the evolutionary relationships among a group of organisms are illustrated by means of phylogenetic tree. A phylogenetic tree is composed of nodes and branches in which only one branch connects any two adjacent nodes. The nodes represent the taxonomic units and the branches define the relationship among the units in terms of descent and ancestry. The branch length usually represents the number of changes that have occurred in that branch (Felsenstein, 1993). Phylogenetic trees can be rooted i.e. the direction of evolution is known. If the direction is not known than the tree is called "unrooted". A phylogenetic tree, which is based on a gene, is called gene tree, while a tree representing evolutionary pathways of a group of species is called an species tree (Li and Graur, 1991)

Estimating the rater of nucleotide substitution:

To detect nucleotide substitutions in DNA sequences, one usually relies on comparison of sequences that have been derived from a common ancestral sequence. The number of nucleotide substitutions

between two sequences is important because it computes the rate of evolution, estimate the divergence time, and reconstructs the phylogenetic trees by many methods. To study the dynamics of nucleotide substitution, one must make assumption regarding the probability of substitution of one nucleotide by another. Many such mathematical schemes have been proposed in literature some of which are quite complicated.

Kimura two-parameter method:

In this method the differences between the two sequences are classified into transition and transversion (Kimura, 1980). Let P and Q be the proportion of transition and transversion differences between the two sequences, respectively, then the number of nucleotide substitution per site between the two sequences is estimated by the following formula;

$K = \frac{1}{2} \ln (a) + \frac{1}{4} \ln (b)$.

Where a = 1/1-2P-Q and b = 1/ 1-2Q

Methods for tree construction:

Numerous tree making methods have been proposed (Sneath and Sokal, 1973; Nei, 1987; Felssentein 1988), because no single method performs well under all circumstances. The method can be classified into four types; distance matrix methods, maximum parsimony methods, maximum likelihood methods, and methods of invariant. In the distance matrix method evolutionary distances (Usually number of nucleotide or amino acid substitution between the sequences) are computed for all pairs of taxa, and phylogenetic tree is constructed by using an algorithm based on some functional relation relationship among the distance value. There are five distance matrix methods by which tree can be constructed, they are;

- Unweighted pair group method with arithmetic mean (UPGMA).
- Transformed distance method.
- Neighbor-Relation method.

- Neighbor-Joining method.
- Minimum-Evolution method.

The Neighbor-Joining method (Saitou and Nai, 1987) which is employed in this thesis to construct trees is based on the principle of finding the neighbors sequentially that may minimize the total length of the tree. Computer simulations have shown that the Neighbor-Joining method is the most efficient distance method recovering the correct tree topology (Kumar et al, 1993).

The bootstrap:

In statistics, the bootstrap is a computational technique for estimating statistics or parameters when the distribution is difficult to derive (Effron, 1982). Since its introduction into phylogenetic by Felsenstein (1985), the bootstrap has been used as means to estimate the confidence level of phylogenetic hypotheses

DNA Sequencing

In the year 1975, Fredrick Sanger and his colleagues (Sanger et al, 1977), and Alan Maxam and Walter Gilbert (Maxam and Gilbert; 1977) developed two different methods for determining base sequence of a defined piece of DNA. This spectacular breakthrough revolutionarized molecular biology.

Sanger's method known as chain termination method involves the in vitro synthesis of DNA strand by a DNA polymerase using a single stranded DNA template. Synthesis is initiated at only site where an oligonucleotide primer annealed to the template. The synthesis reaction is terminated by the incorporation of a nucleotide analog that will not support continued DNA elongation hence the (chain-termination). The chain-terminating nucleotide analogs are the 2', 3'-dideoxynucleotide 5'-triphosphate (ddNTPs). These lack the 3'-OH group necessary for DNA

chain elongation. When proper proportion of dNTPs and one of the four ddNTPs are used, enzyme catalyzed polymerization will be terminated in a fraction of populations of chains at each site where the ddNTP was incorporated. Four separate reactions, each with a different ddNTP give complete sequence information. A radioactively labelled nucleotide is included in the synthesis, so the labelled chain of various lengths can be visualized by autoradiography after high-resolution electrophoresis in polyacrylamide gels.

The Maxam-Gilbert method follows a different strategy. This method starts with a labeled DNA. The strand is then broken at specific bases using base specific reagent (Table 1). The labeled fragments are then run on high resolution PAGE and then detected by autoradiography, this procedure orders the fragment by size and therefore tells us the sequence of DNA.

Table no. 1: Reagents and chemicals used for the cleavage of bases at specific sites in Maxam-Gilbert method.

Ragent	Cleavage site
10%Dimethylsulfoxide	Cleaved at G
1M piperdine formate pH 2.0	Cleave purine (A+G)
95% Hydrazine	Cleave pyrimidine (C+T)
95% Hydrazine+ 5M NaCl	Cleaved at C
1.2N NaOH + 1mM EDTA	Cleaves at A and C

The concept of DNA amplification by PCR given by Mullis in the year 1983 changed the course of molecular biology (Saiki et al, 1985). PCR is an invirto method for method for enzymatically synthesizing defined segments of DNA. The reaction uses two oligonucleotide primers that hybridize two opposite strands and flank the target DNA to be amplified. Taq DNA Polymerase, a heat stable polymerase, catalyzes the elongation of the DNA. This enzyme was isolated from eubacterium, *Thermus aquaticus*. A repetitive series of cycles involving template

denaturation, primer annealing and extension of the annealed primers by Taq DNA polymerase results in an exponential accumulation of newly synthesized DNA. The 5' ends of the primer define the ends of the fragments. Because the primer extension products synthesized in a given cycle can serve as a template in the next cycle, the number of target DNA copies approximately doubled every cycle.

Sensitive methods for detection and analysis of RNA molecule are important aspect of molecular biology. Since RNA cannot serve as template for PCR, reverse transcription was combined with PCR to make RNA into a complimentary DNA (cDNA) suitable for PCR. The combination of both the techniques is known as Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Three different enzymes with the reverse transcription activity viz, mesophilic viral reverse transcriptase from Avian myoblastosis virus (AMV). Molony murine leukemia virus (M-MuLV) and the heat stable DNA polymerase derived from Thermus thermophillus can be used to convert RNA to cDNA (Skalaka and Goff, 1993).

The process of PCR and RT-PCR has proved to be useful for detecting gene expression (Kawasaki et al 1988, Roppolee et al1988), for amplifying RNA sequences prior to sub-cloning and analysis (Todd et al, 1987), for diagnosis of infectious agents or genetic disease and for direct sequencing of the defined fragment.

Sequencing the PCR product without cloning is a powerful technique. It enables rapid generation of sequence data even when the sample is available in a small quantity. The sequencing techniques of Sanger or the Maxam and Gilbert are sensitive but relatively slow if a large number of samples are to be analyzed. In automated sequencing method each of the 4 ddNTPs are labeled with four different fluorescent dyes (Table 2). The radiolabel is not required. The cycle sequencing reaction is performed in a single tube. The sample is then run in PAGE (or capillary). The separated four dyes labeled newly synthesized DNA fragments are

then detected by an excitation by laser beam and the four different colored emissions identify the nucleotides (Figure 3).

Table 2: Dye-terminators used to label the DNA in automated DNA sequencing reaction.

Dye-terminator	Acceptor dye	Color on electrophorogram
A	dR6G	Green
C	dR0X	Blue
G	dR110	Black
T	dTAMRA	Red

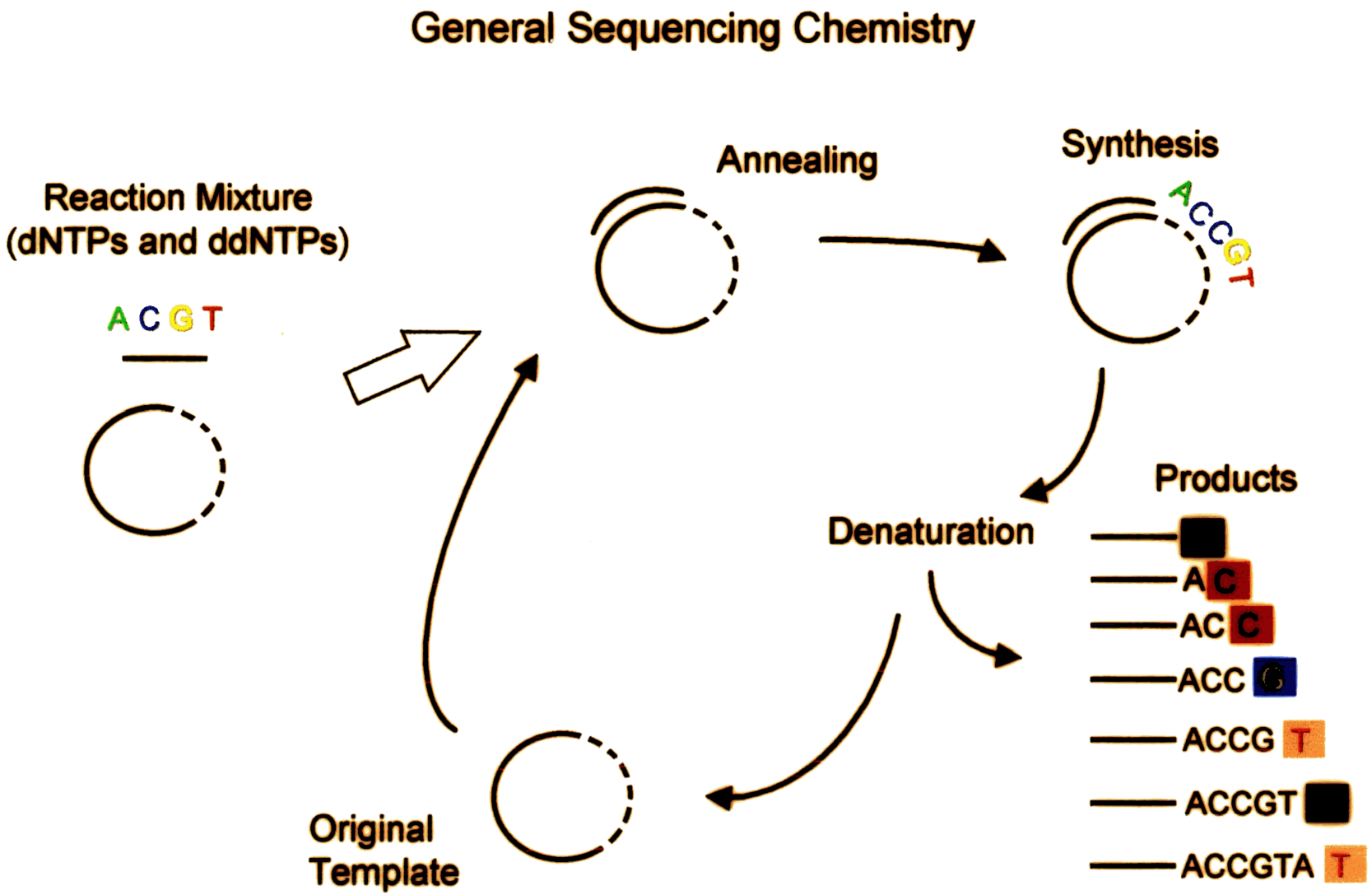


Figure 3: Diagram showing the steps involved in cycle sequencing and labeling the product with the fluorescent label di-deoxy nucleotides.

CHAPTER 2

AIMS AND OBJECTIVES

Aim and Objectives

Molecular epidemiology of wild poliovirus in India was an unexplored bur important area of research that had direct application to global polio eradication program. The main aim of the present study was ti perform nucleotide sequence analysis of genomic RNA of poliovirus isolates to generate knowledge regarding distribution and transmission of type 2 and type 3 poliovirus in the country. The information so generated will be utilized to evaluate and guide polio eradication program to reach its goal. The following objectives were set to be achieving:

1. To determine the genomic diversity of wild poliovirus type 2 and 3 in India.
2. To study transmission of wild poliovirus type 2 and 3 in different parts of India.
3. To characterize wild type 2 and 3 isolates from Mumbai to know when and where certain lineages were eliminated as polio eradication progressed.
4. To provide the information to national polio eradication program to assist in early realization of the goal.

CHAPTER 3

MATERIALS AND METHODS

Materials and Methods

Poliovirus isolation, typing and intratypic differentiation

Poliovirus was isolated from stool specimens collected from cases of acute flaccid paralysis. Briefly, a 10% (w/v) suspension of the stool specimen was prepared either in phosphate buffered saline (PBS) or Hanks balance salt solution (HBSS). Chloroform was added to it to 10% (v/v) final concentration. The suspension is vigorously shaken for 20 min and centrifuged at 3000g for 30 min. The clear aqueous layer was collected in sterile tubes. 200µl of the suspension was inoculated in duplicate in cell cultures of RD (human rhabdomyosarcoma) and L20B (a recombinant murine cell line) grown in test tubes. The cells were incubated for 7 days at 36^{0}C. During this period cells were microscopically observed for virus caused cytopathic effect (CPE). Cell cultures showing CPE were taken for virus identification. All CPE negative cell culture tubes were inoculated once more to the fresh batch of cells. The virus isolation procedure is illustrated in Figure 4.

All specimens producing CPE in RD cells were inoculated in L20B cells for selective growth of poliovirus. The poliovirus serotypes were identified by virus neutralization test. Poliovirus type 1, 2 and 3 specific rabbit anti-sera were used for virus neutralization test. Briefly, 50µl of test virus (~100$TCID_{50}$) and 50µl of each anti-serum was added to the wells of microtitre plates. The mixture was incubated at 36^{0}C for 90 min and 100µl of L20B cell suspension containing 10^5 cells/ml were seeded in each well. The plates were incubated at 36^{0}C in a CO_2 incubator. The plates were observed microscopically to record CPE. Neutralization of virus infection of the cells by specific antisera identifies the virus type. Combination of anti-serum was used to identify virus mixtures (Melnick, 1989).

Poliovirus isolates were then differentiated into virus of Sabin attenuated live oral poliovirus vaccine origin (Sabin vaccine-like) or wild type by enzyme linked Immunosorbent assay (ELISA) (van Wezel and

Hazendonk 1979) and RNA-RNA probe hybridization tests (De et al, 1995). Virus isolates used in this study were obtained from virus bank maintained at the Centre for the molecular epidemiology studies presented in this thesis.

Poliovirus type 2

A total of 44 wild poliovirus type 2 isolates were studied for determining genetic relationship by partial genomic sequencing. These included, 14 isolates of the period 1971-89 and 30 isolates of the period 1991-95 obtained from cases of paralytic poliomyelitis reported from Mumbai (Tables 3 and 4). All isolates were from cases of acute flaccid paralysis. In addition, 37 isolates from paralytic cases from Uttar Pradesh and Bihar (1998-99) representing the last circulating wild poliovirus 2 in India were also studied. Details of these isolates are presented in Table 5.

Poliovirus type 3

A total of 96 wild poliovirus type 3 isolates isolated from cases of acute flaccid paralysis reported from Mumbai in the years 1972 to 2000 were studied for determining genetic relationship of viruses detected in Mumbai. Of these 30 isolates represented the period from 1972 to 1989 and 66 were from years 1992 to 2000. Details of the poliovirus type 3 isolates are given in Table 6 and 7. In addition, 135 wild poliovirus type 3 isolated from different states in the years 1997 to 2001 were also sequenced to determine genetic biodiversity of wild polio 3 in India. Table 8 lists the isolates from other states used in this study.

Complete VP1 sequences of a total of 79 wild poliovirus type 3 isolated from 1998 to 2002 were determined for the high-resolution molecular epidemiology. The details of these isolates are presented in Table 9.

Each poliovirus isolate was inoculated in monolayer test tube cultures of RD cell line. When all the cells showed CPE, the cultures were

freeze-thawed to release the virus. The culture supernatant was collected in sterile cryo-vials and stored at –20^{0}C until used.

Sequencing poliovirus RNA

The following steps are involved in the viral RNA sequencing,

1. Extraction of RNA from the virus particles (infected culture fluid).
2. Reverse Transcription to convert RNA to cDNA.
3. Polymerase Chain Reaction (PCR) for cDNA amplification.
4. Cycle sequencing using di-deoxy chain termination reaction.
5. Determining the sequences by electrophoresis.
6. Sequence editing and phylogenetic analysis.

Extraction of poliovirus RNA

1. To 250µl of virus suspension in a 1.5ml eppendorf tube add 750µl of Tri-pure reagent (1667157, Roche), mix thoroughly and incubate at room temperature for 10 min.
2. Add 200µl of chloroform, mix on vortex mixture and then incubate at room temperature for 10 min.
3. Centrifuge the tubes at 12000 rpm in a refrigerated micro-centrifuge at 4^{0}C for 10 min.
4. Carefully collect 500µl of the top aqueous layer in a fresh eppendorf tube.
5. To the aqueous layer add 500µl isopropanol and 5µl 3M sodium acetate. Vortex to mix, incubate the tubes for 30min on ice to facilitate the RNA precipitation.
6. Centrifuge the tubes at 12000 rpm at 4^{0}C for 30 min.
7. Carefully drain off the supernatant fluid. Wash the RNA pellet with 1ml of 75% ethanol.
8. Centrifuge as above for 10min and drain off the supernatant and carefully blot the last drop of ethanol.
9. Dry the RNA pellet in a DAN-RNA concentrator (Speed Vac, Savant).

10. Dissolve the RNA pellet in 20µl of sterile DEPC treated water and store at minus 20^0C until used.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR)

PCR primers:

The following primers were used for RT-PCR and sequencing of poliovirus RNA,

1. **Q8**

   Location: Nucleotide numbers 3485 to 3504

   Orientation: Reverse primer

   Sequence: 5' AAG AGG TCT CTA TTC CAC AT 3'

   Function: The Q8 primer is used as a universal primer to convert viral RNA of poliovirus types 1, 2 and 3 to cDNA. The primer interacts with sequences in the 2A region, thus is useful for sequencing VP1/2A region as well as complete VP1 gene.

2. **S14F**

   Location: Nucleotide numbers 3209 to 3228

   Orientation: Forward primer

   Sequence: 5' GTG GTT AAT GAT CAC AAC CC 3'

   Function: The S14F primer is used as a universal primer to all the three poliovirus serotypes to convert viral RNA to cDNA. The primer interact in the VP1 region of viral RNA and useful for sequencing of VP1/2A region.

3. **P3/PCR4**

   Location: Nucleotide numbers 3235 to 3252

   Orientation: Forward primer

   Sequence: 5' GTT ACA TCA AAG GTG CGA 3'

Function: This primer was designed for the selective amplification of wild poliovirus type 3 isolates (Nadkarni, 2000). The primer interacts in the VP1 gene of the wild type 3 poliovirus only and useful in sequencing the VP1/2A region of wild type 3 poliovirus RNA.

4. **S2B**

Location: Nucleotide numbers 2852 to 2871.

Orientation: Forward primer

Sequence: 5' TTC ACA TAC TCC CGT TTT GA 3'

Function: The S2B primer interacts in the VP1 gene of the poliovirus serotype 2 and 3 and is useful in the sequencing of the full length sequencing of poliovirus serotype 2 and 3.

5. **Y7**

Location: Nucleotide numbers 2399 to 2421

Orientation: Forward primer

Sequence: 5' GGG TTT GTG TCA GCC TGT AAT GA 3'

Function: The Y7 primer is a universal primer and interacts in the 3' end of the VP3 gene of all the poliovirus serotypes. This primer is useful in the full length sequencing of VP1 gene.

6. **S1**

Location: Nucleotide numbers 2987 to 3006

Orientation: Reverse primer

Sequence: 5' TGG GAC GAC TAC ACA TGG CA 3'

Function: The s1 primer is a universal primer and interacts in the VP1 gene of all the poliovirus serotypes. This primer is useful in the full length sequencing of VP1 gene.

All the primers except P3/PCR4 were obtained from CDC, Atlanta. Primer P3/PCR4 was synthesized by National Chemical Laboratory, Pune

as per sequence information provided to them for the amplification of wild poliovirus type 3 (Nadkarni ; 2000)

Calculating the primer concentration:

Each primer was dissolved in 1ml DEPC treated sterile distilled water. A 10µl of the stock primer is diluted 100 fold in DEPC treated water and its optical density was measured in a UV spectrophotometer (UVSpec, LKB). Primer concentration was determined using 1unit OD_{260nm} as equivalent to 33µg/ml of the ssRNA. The Pico mole primer concentration was determined by using the following formula:

$$\text{Pico mol primer} = \frac{\mu g \text{ primer} \times 10^6}{\text{Length of primer} \times 325}$$

Reverse Transcription and PCR (RT-PCR) for VP1/2A region sequencing

The primer pair Q8/ S14F was used to amplify a 296bp segment encompassing the 150 nucleotides of VP1/2A region for genetic analysis of wild poliovirus type 2 and 3 isolates. Alternatively, the Q8/P3-PCR4 primer pair was used in place of S14F for amplifying only wild poliovirus type 3 isolates. The Q8/P3-PCR4 pair produced an amplificate of 270bp. One tube RT-PCR was used throughout this work.

1. 2µl of viral RNA was mixed with 2µl of 20ng reverse primer (Q8) in a 0.2ml PCR tube, denatured at 95^0C for 5min and then quickly chilled on ice for the re-annealing.
2. 1µl of enzyme mix was then added to the annealed template primer reaction tube (final volume 25µl). The enzyme mix had the following compositin: 20ng of forward primer (S14F or P3/PCR4), 5X PCR I buffer (50mM Tris pH 8.3, 70mM KCl, 5mM $MgCl_2$, 0.5% NP40, and 10mM DTT), 125U/µl of AMV-RT, 1.25U/µl of Taq-DNA polymerase,

5U/µl of Rnase inhibitor, 1mM of each of the four nucleotides and the final volume was made by adding DEPC treated distilled water.

3. Tubes were vortexed to mix the reagent properly and centrifuged for 10 sec to recover the contents at the bottom of the tubes.
4. RT-PCR reactions were incubated in PCR thermal cycler (Model 2400, Perkin-Elmer) using the following program for reverse transcription followed by 30 cycles of PCR.

Reverse transcription	42°C for 60 min
Denaturation to stop RT	95°C, 3 min
30 cycles of PCR:	
Denaturation	95°C, 1 min
Annealing	55°C, 1 min
Extension	72°C, 2 min
Completion of extension	72°C, 10 min
Hold	4°C, infinite (until removed from cycler)

5. RT-PCR product was detected by performing agarose gel electrophoresis.

RT- PCR for complete VP1 gene sequencing

The VP1 gene of wild Poliovirus type 2 (903bp) and 3 (900bp) was amplified in two fragments. The first fragment was amplified using Y7 and S1 pairs with the product size of 608bp and the second fragment were amplified using Q8 and S2B pairs with the product size of 653bp.

1. 2µl viral RNA was transferred to two PCR tubes containing 2µl (50ng) of the prime pair (Y7/S1) or (Q8/ S2B), respectively.
2. The tubes were heated at 95^0C for 5min and then quickly chilled on ice for denaturation of RNA and annealing with primers.

3. 46µl of the enzyme mixture was added to the reaction tubes (final volume 50µl). The enzyme mix had the following composition: 10X PCR III buffer (100MTris pH 9.0, 50mM KCl, 2.5mM $MgCl_2$, 2.5mM DTT, 0.1% TritionX100), 6.25U/µl AMV-RT, 2.5U/µl Taq-DNA polymerase, 10 U/µl RNase inhibitor, 10mM each dNTP and water.
4. Contents of the tubes were mixed and centrifuged briefly.
5. RT-PCR reactions were incubated in PCR thermal cycler (Model 2400, Perkin-Elmer) using the following program for reverse transcription followed by 30 cycles of PCR.

Reverse transcription	42°C for 60 min
Denaturation to stop RT	95°C, 3 min
30 cycles of PCR:	
Denaturation	95°C, 1 min
Annealing	55°C, 1 min
Extension	72°C, 2 min
Completion of extension	72°C, 10 min
Hold	4°C, infinite (until removed from cycler)

6. After the RT-PCR the product was detected by performing agarose gel electrophoresis and the product was purified using QIA quick gel extraction kit.

Agarose gel electrophoresis

1. 1% Agarose was prepared in 0.5X TBE buffer by heating the contents in a microwave oven.
2. 1µl/ml of Ethidium Bromide was added to the agarose solution just before pouring the gel in a UV transparent submerging gel tray.
3. A 15 well (1mm x 3mm) comb was used to make sample wells.
4. Test samples were prepared by mixing 5µl of RT-PCR reaction and 1µl of gel loading buffer.

5. Samples were loaded using micro tips.
6. Each run contained a lane loaded with molecular weight marker.
7. Agarose gel electrophoresis was carried out at 100V for 30min.
8. After the electrophoresis the gels were observed using a UV trans-illuminator.
9. Specific amplification products were identified as brightly fluorescent bands of expected size.
10. Photographs were taken using *BIO RAD* Gel documentation system 2000 for documentation purpose.

Extraction of bands from agarose gels

Agarose gel electrophoresis was performed as described above but using the larger wells (comb size 2mm x 6mm) so that the entire (50µl) reaction volume could be loaded in a well.

1. Electrophoresis was carried out at 110V for 30min.
2. The specific PCR product (band) was located by using UV Trans-illuminator.
3. The band was excised using a sterile scalpel blade and transferred to a 1.5ml eppendorf tube.
4. QIAquik Gel Extraction Kit (QIAGEN, Germany Cat no. 28076) kit reagents were used for extracting DNA from the gel as per the manufacturers instructions.
5. DNA was extracted in approximately 30-40µl of the elution buffer.
6. Recovery of the PCR product from each extraction was checked by electrophoresis before proceeding to the sequencing step.

Cycle Sequencing:

PCR product (DNA) was sequenced by dideoxy chain termination cycle sequencing reaction using florescent-labeled dideoxy nucleotides (Perkin-Almer cat no. 4303049). The method is the modified form of the Sanger's dideoxy chain termination method (Sanger et al 1975). The cycle

sequencing method modified for automated DNA sequencing utilizes a one-tube reaction containing fluorescent-labeled di-deoxy nucleotides, which gives different spectral color when exited by the laser beams. The following steps are involved:

1. 1μl of 3.2 Pico mole of each forward and reverse primer was taken in a separate 0.2ml of tubes.
2. Then 8μl of the Big dye ready reaction mix (Perkin-Elmer Cat no.4303049) was added to each tube.
3. 4-5μl of the purified RT-PCR product containing 20-50ng of DNA was added as per the protocol made.
4. The final volume was made to 20μl by adding MilliQ water.
5. All the tubes were vortex mixed thoroughly and centrifuge for 10 sec.
6. All the tubes were kept in the thermal cyclar under the following program of cycle sequencing.

25 cycles of	
Denaturation	95°C, 10 sec
Annealing	50°C, 5 sec
Extension	60°C, 4 min
Hold	4°C, infinite (until removed from cycler)

Purification of the cycle sequenced product:

1. To the 20μl cycle sequencing reaction mixture 80μl of the distilled water was added to make the final volume to 100μl.
2. Reaction mixture was then transferred to 1.5ml eppendorf tubes and 10μl of 3M sodium acetate and 250μl of the 95% ethanol was added.
3. The tubes were centrifuged at 14,000 rpm for 20 min at room temperature.
4. Supernatant was discarded and the pellet was washed with 0.5ml 0f 70% ethanol.
5. The tubes were centrifuged at 14,000 rpm for 10min.

6. Supernatant was discarded and the washing and centrifugation was repeated once more.
7. Finally supernatant was discarded and the pellet was dried DNA concentrator for 5 min at room temperature.
8. The pellet was re-suspend in the Template Suppression Reagent (TSR).
9. The samples were denatured at 95^0C for 3 min and the quickly chilled on ice.
10. The denatured samples were transferred to the 0.5ml sequencing tubes (Perkin-Elmer Cat no. 401957).
11. The samples were arranged on the 48-well sample tray and transferred to the ABI 310 Genetic Analyzer (Perkin-Elmer) for automated DNA sequencing by the capillary electrophoresis.

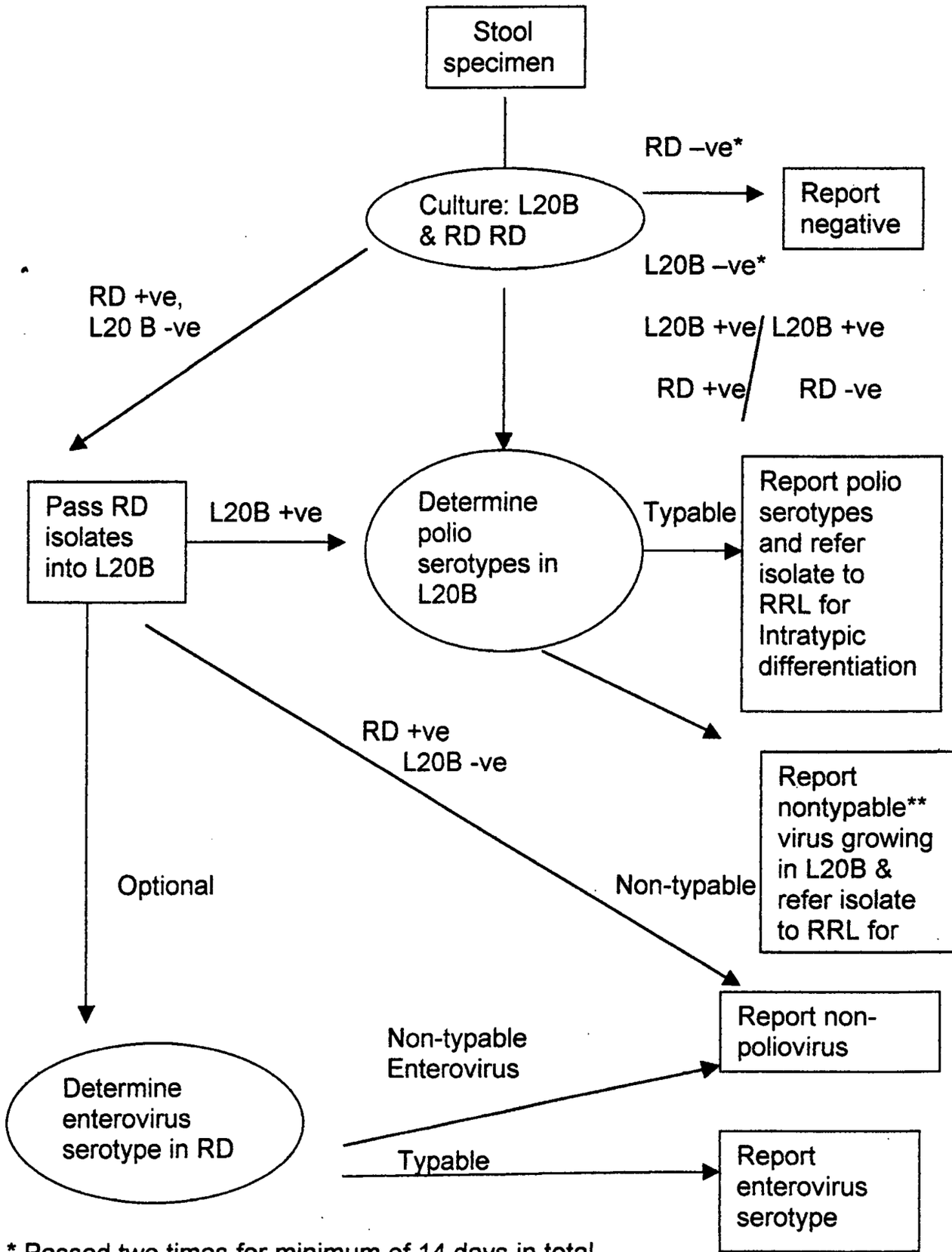
Electrophoresis on DNA sequencer:

The electrophoresis was carried out as per the manufacturers instruction. Briefly the 47cm long x 50μm diameter capillary (Perkin-Elmer cat no. 402839) was used for running the fragment size of 300-400bp and 61cm long x 50μm diameter capillary (Perkin-Elmer cat no. 402840) was used for running fragment size of 550-650bp. The POP6 (Perkin-Elmer cat no. 402844) was used as gel matrix for fragment separation. The samples were electrophorazed at 50^0C at 12 KV and 7μA current for about 1 hour for the small size fragments and 2 hours for the long fragments.

Sequence alignment and analysis

1. Sequences obtained from the ABI Prism 310 genetic analyzer were exported to a floppy disk using the Sequence Navigator 1.0.1.
2. Sequences are edited using the computer software DNASIS. Both forward and reverse sequences were used to correct any ambiguity.
3. The sequences were aligned in the clustal format.
4. Distances were calculated using Kimura-2-Parameter method and translation-transversion ratio of 10.
5. The genetic relationship was determined by Neighbor-Joining method, provided in the program package PHYLIP v3.5c (Felsenstein J, 1993).
6. The dendrograms representing the trees were drawn using the computer software Tree-View.
7. Where necessary bootstrapping was done for statistical analysis.

Figure 4: Flowchart for Poliovirus isolation in RD and L20B cells



* Passed two times for minimum of 14 days in total

** Inform regional laboratory coordinator and national program if non-typable isolate obtained in L20B cells from samples from non-endemic or recently endemic country.

CHAPTER 4

REAGENTS AND CHEMICALS

Reagents and Chemicals

Reagents for RNA extraction

1. RNase free water

   Milli Q purified water 1000ml

   Diethyl Pyrocarbonate (Sigma Cat No. D-5758) 1ml

   Take Milli Q purified water in a glass bottle (New) or sterile disposable plastic bottle and add 1ml DEPC. Stir the solution vigorously (overnight) at room temperature using a magnetic stirrer. Sterilize by autoclaving. Aliquots may be prepared in RNase free containers, such as sterile disposable vials, tubes and flasks. This preparation is referred to as DEPC treated water.
2. Isopropyl Alcohol (SRL Cat no. -092956)
3. Chloroform (Sigma Cat no. 27063-6)
4. 3M Sodium acetate, pH 4.6-4.9

   Sodium acetate (Sigma, Cat no. S-2889) 12.3gm

   DEPC treated distilled water 25ml

   Acetic acid (Qualigens, Cat no. 21057) 15ml

   Adjust the pH to 4.6-4.9 with 1N HCl and make the volume to 50ml by adding DEPC Milli Q distilled water.
5. 75% alcohol

   Take 75ml of distilled Ethanol and make the volume to 100ml by adding DEPC treated Milli Q distilled water.
6. Tri-Pure isolating reagent (Roche Cat no., 1667157)

Reagents for PCR

1. Tris HCl, 1M pH 8.3

   Trizma base (Sigma, Cat no.T-6066) 12.1 gm

   DEPC water to 100ml

   Dissolve Tris powder in approximately 80ml of DEPC water. Adjust the pH to 8.3 by adding 1N HCl. Adjust the volume to 100ml by adding DEPC treated MilliQ water.

2. 1.4 M KCl

   KCl 10.438gm

   Dissolve the powder in 80 ml of DEPC treated MilliQ water and make the volume to 100ml.

3. 240mM $MgCl_2$

   $MgCl_2$ (Sigma Cat no.M-0250) 40.48gms

   Dissolve the powder in 80ml of DEPC treated Milli Q water and then make the volume to 100ml by DEPC treated MilliQ water. Check the molarity of the solution by titration. Sterilize by filtration and store the aliquots at 4^0C

4. 1M Dithiothreitol (DTT)

   DTT (Sigma Cat no. D-5545) 0.925gm

   Dissolve the salt in 6ml of 0.01M Sodium Acetate. Sterilize by filtration and store the aliquots at -20^0C.

5. 25% Nonidet P 40 (BDH Cat no. 56009)

   Add 2.5ml of NP40 in 7.5 ml of DEPC treated Milli Q water. Store at 4^0C.

6. 1M Tris-HCl pH 9.0

   Trizma base (Sigma, Cat no. T-6066) 12.1 gm

   DEPC water to 100ml

   Dissolve Tris powder in approximately 80ml of DEPC water. Adjust the pH to by adding 1N HCl. Adjust the volume to 100ml by adding DEPC treated MilliQ water.

7. 10% TritionX100 (Sigma, Cat no. T-6878,)

   Take 1ml of Txition X100 and make the volume to 10ml by adding DEPC treated MilliQ water.

8. 5X PCR I Buffer to make 5ml

1M Tris-HCl pH 8.3	1.0ml
1.4M KCl	1.0ml
240mM $MgCl_2$	0.464µl
25% NP40	0.400µl
1M DTT	0.200µl
DEPC MilliQ water	0.936µl

9. 10X PCR III Buffer to make 10ml

1M Tris-HCl pH 9.0	1.50ml
1.4M KCl	3.57ml
240mM $MgCl_2$	1.04ml
1M DTT	0.250µl
DEPC MilliQ water	2064ml

10. Reverse Trasnscriptase AMV (Roche, Cat no.1495062)

RT-AMV 500units	20U/µl

11. Taq DNA Polymerase (Roche, Cat no. 14184432)

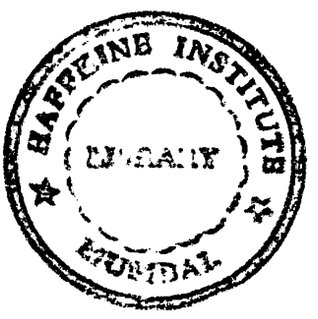
Taq DNA Pol	5U/µl

12. 10mM dNTPs (Roche, Cat no. 1814362)

    Containing 10mM of each dATP, dGTP, dCTP, dTTP

13. Rnase Inhibitor (Roche, Cat no. 799017)

RNase Inhibitor 2000U	25U/µl



Reagents for electrophoresis

1. 0.5M EDTA pH 8.0

EDTA (Sigma Cat no. E-5134)	18.12gm
NaOH pellets (Qualigens; Cat no.27815)	0.2gm

   Adjust the pH to 8.0 by adding 1N NaOH and make the volume to 100ml by adding MilliQ water.

2. Tris borate buffer (TBE) 5X concentration

Tris base (Sigma, Cat no. T-6066)	54.0gm
Boric acid (Sigma, Cat no. B-6788)	2705gm
0.5M EDTA	20ml

3. 1% Agarose

Agarose (Roche, Cat no. 1388983)	1gm
0.5X TBE	100ml

   Dissolve agarose by heating in microwave oven or boiling water bath. Cool and add 1μg/ml Ethidium bromide.

4. Ethidium Bromide (Sigma; Cat no. E-8715)

Ethidium Bromide	10mg
MilliQ water	1ml

5. 1% Bromophenol blue (Sigma Cat no. B-0630)

Bromophenol blue	1gm
MilliQ water	100ml

6. 1% Xylene cyanol FF (Sigma Cat no. X-2751)

Xylene cyanol FF	1gm
MilliQ water	100ml

7. 6X Gel Loading dye

1% Bromophenol blue	12.5ml
1% Xylene cyanol FF	12.5ml

8. DNA molecular weight marker VIII (Roche Cat no. 1336045)

   DNA fragment of bp 19, 26, 34, 37, 67, 110, 124, 147, 190, 242, 320, 404, 489, 501, 692, 900, 1114.

Reagents and chemicals for cycle sequencing

1. 70% Ethanol

   To 70ml of distilled ethanol add 30ml of MilliQ water and mix thoroughly.

2. Big dye terminator ready reaction kit

   Perkin-Almer Cat no. 4303149.

3. Performance Optimized Polymer (POP6)

   Perkin-Elmer Cat no. 402844

4. Genetic Analyzer EDTA buffer

   Perkin-Elmer Cat no. 402824.

CHAPTER 5

RESULTS

Results

Stool specimens from cases of AFP are referred to Enterovirus Research Center (ERC) for isolation and identification of polioviruses. Polioviruses differentiated, as wild type 2 and 3 are the materials for the present study. Historical isolates of the yester years were obtained from the virus bank maintained in the center.

Viral RNA was extracted using Tri-pure isolating reagent and precipitated by isopropyl alcohol and sodium acetate. The VP1/2A junction region of the viral genome was amplified using Q8/S14F pair of primers for all the wild poliovirus type 2 poliovirus. The wild type 3 polioviruses were amplified using Q8/P3-PCR4 pair of primers.

RT-PCR amplification reaction of poliovirus type 2 and type 3 in VP1\2A junction region of viral RNA is shown in Figure 5. For poliovirus type 2 the Q8\S14F primer pair amplified a 296bp segment of VP1\2A region of the genome. Lanes 2 to 8 show the 296bp segment of poliovirus type 2 amplified by Q8\S14F primer pair and lane 9 to 15 show 270bp segment of poliovirus type 3 amplified by Q8\P3-PCR4 primer pair.

VP1 capsid protein of poliovirus type 2 and type 3 are coded by 903 and 900 nucleotides respectively. For the purpose of sequencing of VP1 region a 1100bp bases long segment of viral RNA was amplified in two overlapping fragments using Y7/S1 (608bp; nt no. 2399 to nt no. 3006) and S2B/Q8 (653bp; nt no. 2852 to nt no. 3504). Figure 6 shows the amplification product obtained from poliovirus type 3 RNA.

A closer look at the Figure 6 indicates the contamination due to the non-specific products. The amplification product was purified by cutting the specific band from the gel followed by extracting the DNA using QIA quick gel extraction kit. Figure 7 shows the same amplicons after purification.

Figure 8 shows the electrophorogram of sequencing reactions obtained from ABI Prism 310 genetic analyzer. Panel A shows the sequence of fragment Y7/S1 cycle sequenced using Y7 primer. The first

nucleotide of VP1 protein coding region is marked by arrow and the end of the fragment needed for joining the 5' end of the S2 reaction is indicated by a horizontal line. Panel B (Figure 8) shows the S2B/Q8 fragment sequenced using S2B primer. As in Panel A, the connecting (overlapping) sequences are indicated by horizontal line and the last nucleotide of VP1 by an arrow. Joining of the Y7 fragment with S2B yielded complete VP1 (900bp) sequence of poliovirus type 3.

Sequences were edited using the computer software DNASIS. The phylogenetic relationship was determined employing program available in software PHYLIP version 3.5c. For analysis Kimura 2 parameter algorithms were utilized employing the Neighboring Joining (NJ) method for tree construction.

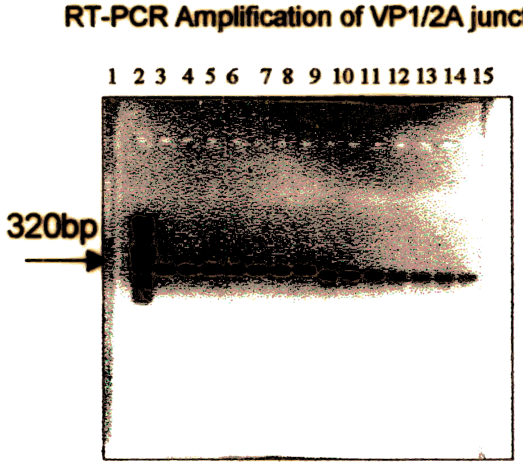


Figure 5: RT-PCR Amplification of VP1/2A junction region of viral RNA using Q8/S14F and Q8/P3-PCR4 pairs of primers. Lane 1: molecular weight marker VIII. Lane 2 to 8: a 296bp product of wild type 2 strains amplified with Q8/S14F primer pair. Lane 9 to 15:a 270bp product of wild type 3 poliovirus amplified with Q8/P3-PCR4 primer pair

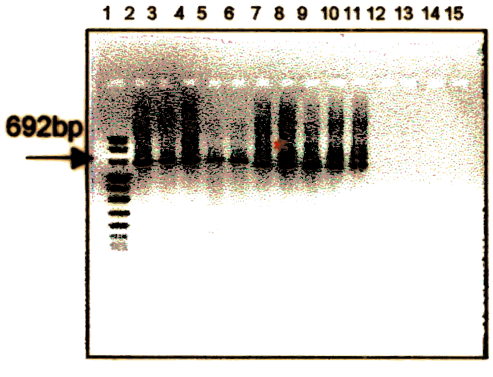


Figure 6: RT-PCR for the amplification of VP1 gene of poliovirus type 3 RNA using Q8/S2B and Y7/S1 pair of primers. Lane 1: DNA molecular weight marker VIII. Lane 2 to 6: amplified using Q8/S2B pairs of primer which yielded a 653bp product. Lane 7 to 11 was amplified using Y7/S1 pairs of primers which yielded 608 bp product. Star in red indicates the non-specific band.

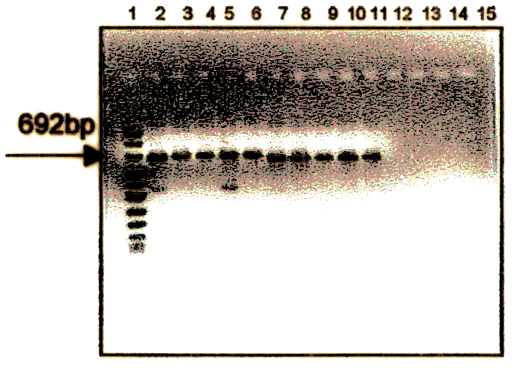
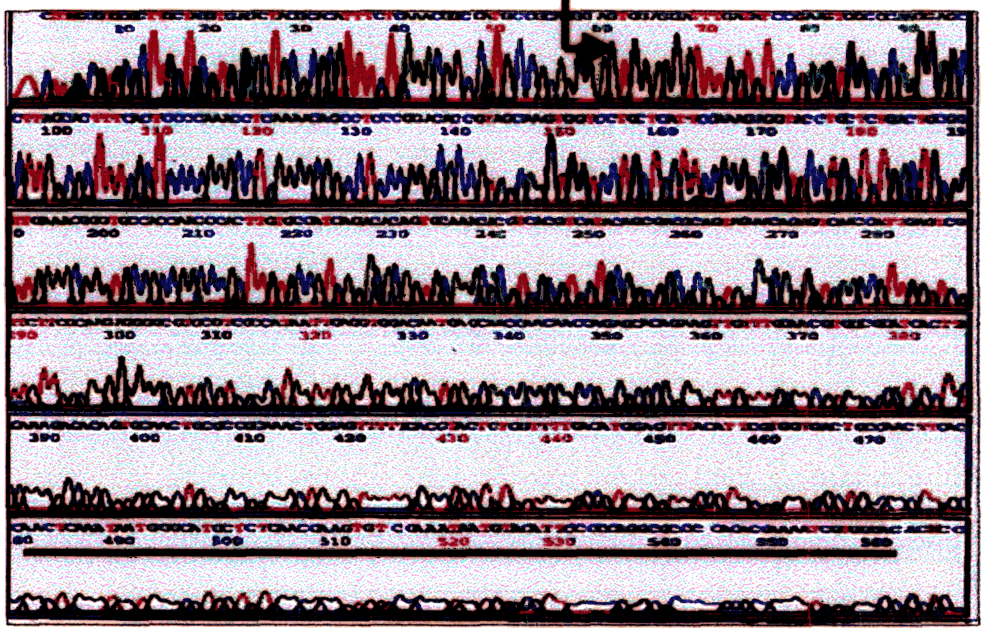


Figure 7: the amplified VP1 gene after purification with the Qiagen Gel extraction kit. Lane 1: Molecular weight marker number VIII. Lane 2 to 6: A 653bp purified product of Q8/S2B pairs of primers. Lane 7 to 11: A 608bp product of Y7/S1 pairs of primers.

Electropherogram showing nucleotide sequence of VP1 gene of wild type 3 poliovirus

A



B

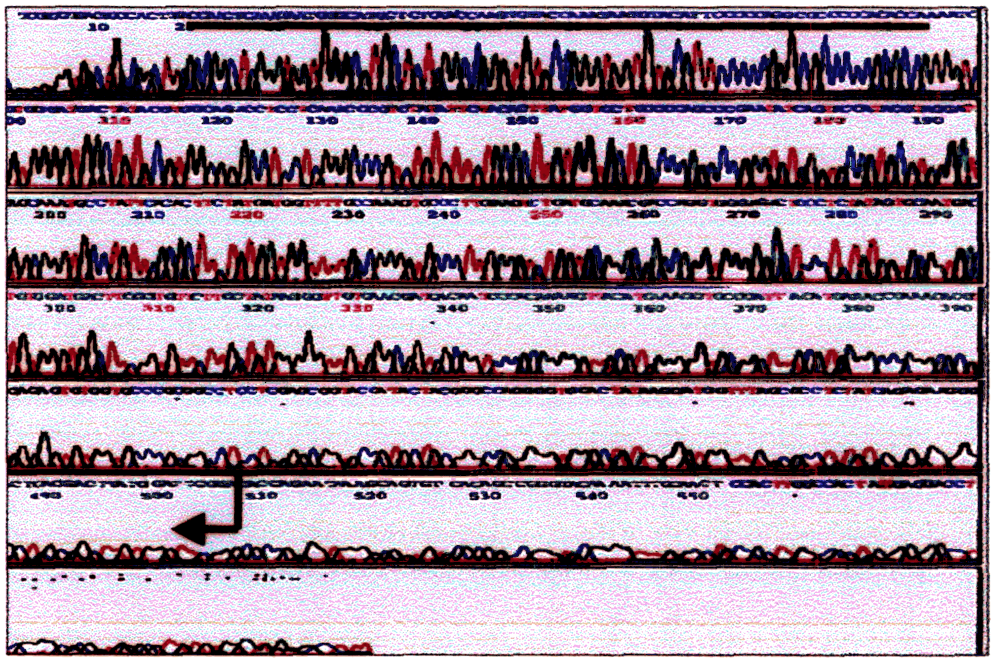


Figure 8: electropherogram of wild type 3 poliovirus in VP1 region. Panel A shows sequences of Y7/S1 fragment using Y7 primer. The curved arrow shows the start of VP1 gene and horizontal line at the 3' end shows the connecting sequences with S2B primer. Panel B shows the S2B/Q8 segment sequenced with S2B primer. The horizontal line at the 5' shows the connecting sequences with Y7 primer and curved line at the 3' end indicates the end of the VP1sequences

Phylogenetic analysis of wild type 2 poliovirus from Mumbai based on VP1\2A sequence data:

A total of 44 wild type 2 strains from Mumbai were sequenced in the VP1/2A junction region of the viral RNA. Out of the 44 strains sequenced, 14 were from the year 1971 to 1986 and 30 belonged to the years 1991 to 1995. The details of the isolates are given in Tables 3 and 4. All the strains were compared with the poliovirus type 2 Sabin oral polio vaccine strain to confirm the wild type origin. Figure 9 shows the sequences alignment of the isolates from the years 1971 to 1986. Genomic distances calculated using the Kimura 2 parameter algorithm given in Figure 10.

Genetic relationship of the 14 isolates is presented in the form of a dendrogram in Figure 11. The 14 isolates show more than 85% sequence similarity and hence belong to one genotype. It can be seen that the 14 isolates evolved from V9 (1971) due to nucleotide substitution over time. Nucleotide substitution rate of poliovirus RNA is about 2% per year in the entire genome (Nottay et al, 1981). In the absence of polio vaccination program in the 70s, virus had a chance to continue infecting the susceptible children and thus diverged sufficiently to form separate lineages. Isolates V340 (1973), V813 (1976) and V855 (1977) are related to the ancestral V9 showing more than 98% sequence similarity. The isolate V769 (1975) diverged early with approximately 4% sequence divergence with non-synonymous substitution. In 1978-79 two lineages were detected viz., a) isolate V964 from L ward and b) V1050 (E ward, 1979), V1088 (1979) and V1128 (G ward, 1979). The isolate from a case from Nasik was found to be very closely related to viruses from wards E and G in Mumbai.

By the year 1982-83 three more lineages were detected from P ward (V2838), F ward (V2909) and R ward (V3056) as they are more than 5% diverged from each other. Isolates V2909 (1983) and V3056 (1986) diverged to the extant of becoming separate genotypes as the genomic distance was found to be more than 15%. The isolate V5435 (1986) from a

case from Gonda district of Utter Pradesh and isolates V813 and V2909 showed less than 15% sequence divergence. It is therefore possible that this particular genotype also circulated in other parts of the country.

The dendrogram showing the phylogenetic relationship of the 30 wild type 2 poliovirus is shown in Figure 12. The V9 virus was used to draw an out-group rooted tree. All isolates of the years 1992-95 except one isolate of the year 1992 (a mixture of polioviruses) were sequenced. The 30 isolates grouped into two clusters (A and B). The eight isolates of the year 1991 formed three lineages, one in cluster A and two in cluster B. Within the cluster B, the viruses showed minimal genetic distances in the respective lineages. The isolate from a child from Varanasi UP (V8895) was genetically close to 1991 Mumbai isolates indicating that the child acquired infection in Mumbai. In contrast the 1991 case from Patna Bihar (V8928) appears to be an infection outside Mumbai.

The three isolates of the year 1992 represent three independent lineages. A single lineage of wild poliovirus type 2 was observed in Mumbai in the year 1993. Isolates of the same lineage were detected in 1994. There was only one case of paralytic poliomyelitis due to wild poliovirus type 2 in the year 1995. In addition there were 5 five cases of poliovirus type 2 infection in the Buldhana district of Maharashtra state. The Buldhana isolates shows genetic relationship with 1994 cases in Mumbai, however in the absence of additional earlier strains from Buldhana further comments can not be made. Notably the last case of poliovirus type 2 infection in Mumbai residents was not related to the Buldhana isolates. However it appears that the M ward 1991, R ward 1994 and the T ward 1995 isolates belong to the same lineage but the intermediate linkages were not detected.

Considering the genetic distances between temporal proximity of the isolates it could be said that between 1993 and 1994 wild poliovirus type 2 strains had diverged to form two separate genotypes A and B.

Amino acid sequences of the VP1/2A region were deduced from the nucleotide sequences and are shown in Figure 13. All wild virus isolates showed a common amino acid difference of phenylalanine verses tyrosine at 280th amino acid in VP1 compared to Sabin poliovirus type 2 vaccine strain. The isolate V769 shows two amino acids substitutions, phenylalanine to leucine (3rd amino acid) and tyrosine to phenylalanine (15th amino acid), in 2A region when compared to V9. Single additional amino acid substitutions were observed in isolates V9969 (glycine to glutamic acid at 297th amino acid in the VP1 region), V9495 (threonine to serine at 291st amino acid in the VP1 region), V9802 (phenylalanine to tyrosine at the 3rd amino acid in 2A region) and V9057 (histidine to asparagine at the 5th amino acid in 2A region).

Phylogenetic analysis of wild poliovirus type 2 from Utter Pradesh and Bihar using complete VP1 sequences:

A total of 79 cases in the year 1998 and 11 cases in the year 1999 were due to wild poliovirus type 2 infection. Except two cases, one each from Gujarat and West Bengal, all other cases were from Utter Pradesh and Bihar. Complete VP1 sequence of a total of 28 isolates from the year 1998 and all of the year 1999 were determined for genetic analysis. The details of these isolates are presented in Table 5. Sequences of these isolates were compared with the poliovirus type 2 Sabin vaccine strain to confirm their wild origin. The aligned sequences are presented in Figure 14. The genetic distances, calculated by Kimura-2-parameters are shown in Figure 15. Phylogenetic relationship is presented in the form of dendrogram in Figure 16.

The laboratory number as well as the EPID number in the dendrogram shows each virus isolate. Each AFP case is assigned a unique case identification number. The EPID number is constructed to identify the case by country, state, district, year and the serial number of the case. For example IND UP ALG 99 044 indicates 44th case of the year 1999 from Aligarh district, Utter Pradesh, India. For simplistic reasons the first three letter code for the country has been omitted in the Figure 16.

All the isolates represent a single genotype since the genomic distances are within 15% divergence. Isolate V9635 (Mumbai, 1994) was used as an out-group, this isolate shows approximately 9-10% distance from the 1998-99 isolates. All the 1998-99 isolates shows more than 95% sequence similarity indicating a single lineage. However, at least 4 clusters indicating transmission chains of the wild virus were identified in the year 1998. Multiple transmission chains were observed in western Utter Pradesh (Meerut, Muzzaffernager, Bareilly) and also in Bihar. The single isolate from Gujarat had close relationship with isolates ((UP MZN 98 081 genetic distance 1%, UP BLS 98 022 genetic distance >1%). In the year 1999, at least 5 transmission chains were identified. The results also show

elimination of circulation of at least one transmission chain of the year 1998. The relationship of the isolates of 1998 and 1999 is clearly documented. The dendrogram also shows that the West Bengal case had a very close genetic similarity with virus from Sultanpur, Utter Pradesh. The last case of wild poliovirus type 2 infection in India (and also from the world) was from district Aligarh Utter Pradesh [illegible]

Considering the information gathered from isolates from Mumbai (multiple lineages) it was found that a significant reduction in genetic diversity occurred during the final stages of eradication of wild poliovirus type 2.

The amino acid sequence of the complete VP1 protein was deduced from the nucleotide sequences (Figure 17). The wild poliovirus type 2 (India) genotype shows at least 19 amino acid differences with Sabin vaccine strain. 13 out of the 19 differences were at the end terminus of VP1 protein. The amino acids 89-100 comprise the major neutralizing antigenic site of poliovirus type 2. No change of amino acid was detected in the wild type 2 poliovirus when compared with the Sabin vaccine strain.

Table 3: List of wild poliovirus type 2 isolates from Mumbai (1971-86) used for generating sequence.

Sr. No.	V No.	Month and Year of isolation	Ward
1	V9	March 1971	L
2	V340	April 1973	Thane
3	V769	February 1975	L
4	V813	April 1976	G
5	V855	May 1977	A
6	V1010	November 1978	Nasik
7	V964	October 1978	L
8	V1050	July 1979	E
9	V1088	1979	Not Known
10	V1128	September 1979	G
11	V2838	1982	P
12	V2909	April 1983	F
13	V3056	August 1983	R
14	V5435	January 1986	Gonda

Table 4: The wild type 2 isolates from Mumbai 1991 to 1995 studied in the VP1/2A junction region of viral RNA to establish phylogenetic relationship.

Sr. No.	V No.	Month and Year of isolation	Ward
1	V8817	May 1991	G
2	V8840	July 1991	R
3	V8855	July 1991	Thane
4	V8884	August 1991	M
5	V8895	August 1991	Varansi
6	V8925	October 1991	M
7	V8928	October 1991	Patna, Bihar
8	V8930	October 1991	B
9	V9027	January 1992	H
10	V9057	February 1992	N
11	V9064	February 1992	D
12	V9504	June 1993	M
13	V9510	June 1993	M
14	V9538	June 1993	K
15	V9495	July 1993	M
16	V9521	August 1993	S
17	V9606	1994	F
18	V9635	January 1994	K
19	V9646	January 1994	F
20	V9668	April 1994	P
21	V9672	April 1994	K
22	V9695	June 1994	M
23	V9705	June 1994	L
24	V9802	September 1994	R
25	V9885	1994	Jamnagar
26	V9969	July 1995	T
27	V10048	November 1995	Buldhana,
28	V10089	November 1995	Buldhana,
29	V10090	November 1995	Buldhana,
30	V10092	November 1995	Buldhana,

Table 5: List of wild poliovirus type 2 used for complete VP1 sequence analysis.

Sr.No.	Eipid Number	Lab number	Month and year of isolation
1	BI GYA 98 026	R-5691	July 1998
2	BI GYA 98 071	R-4908	December 1998
3	BI PTN 98 022	R-5584	June 1998
4	BI PTN 98 037	R-3674	September 1998
5	DL DLH 98 058	R98-2537	October 1998
6	GU AMC 98 004	R98-239	January 1998
7	UP AGR 98 009	R98-2494	August 1998
8	UP ALG 98 027	R98-2643	August 1998
9	UP BLS 98 022	R-4111	July 1998
10	UP BLS 98 058	R-3628	September 1998
11	UP BRL 98 014	R98-2489	July 1998
12	UP BRL 98 023	R-4703	October 1998
13	UP HMP 98 013	R98-2464	July 1998
14	UP MAU 98 005	R98-2466	July 1998
15	UP MRD 98 012	R98-2813	July 1998
16	UP MRD 98 026	R98-2885	August 1998
17	UP MRD 98 031	R98-2889	August 1998
18	UP MRT 98 014	R-5621	July 1998
19	UP MRT 98 052	R-3776	October 1998
20	UP MZN 98 037	R98-2474	July 1998
21	UP MZN 98 040	R98-2510	July 1998
22	UP MZN 98 055	R98-2865	July 1998
23	UP MZN 98 081	R-3588	August 1998
24	UP PTG 98 266	R98-2839	August 1998
25	UP RBL 98 018	R98-2396	July 1998
26	UP RMP 98 043	R-4487	November 1998
27	UP ALG 99 044	R-7773	October 1999
28	UP BJN 99 004	R-5027	January 1999
29	UP BJN 99 087	R-6555	September 1999
30	UP BRC 99 003	R-5028	January 1999
31	UP BRL 99 004	R-5029	February 1999
32	UP JPN 99 011	R-6546	February 1999
33	UP MAU 99 001	R-5205	February 1999
34	UP MRT 99 001	R-5009	January 1999
35	UP MRT 99 002	R-5033	February 1999
36	UP SUL 99 021	R-7283	September 1999
37	WB CAL 99 119	R-6099	July 1999

Figure 9: Aligned nucleotide sequences (VP1/2A) of wild poliovirus type 2 from Mumbai (1971-1995).

```
V9          CGTCCCCCTC GTGCGGTCCC ATACTACGGA CCGGGAGTTG ACTACAAAGA TGGGCTAACC
V340        .......... .......... G......... .......... .......G.. ..........
V769        ..C..T.... .......... .........T ..A....... .......... ..........
V813        .......... .......... .......... .......... .......... ....T.....
V855        .......... .......... .......... .......... .......... ..........
V964        ..A....... ....A..... .......... .......... ....T..... ..........
V1010       .......... .......... .......... .......... .......... ...A......
V1050       .......... .......... .......... .......... .......... ...A......
V1088       .......... .......... .......... .......... .......... ...A......
V1128       .......... .......... .......... .......... .......... ...A......
V2838       .......... ....A..... .........G ..A..G.... .......... ..........
V2909       .......... ....A..... .....T.... .....G.... .......... ....T.....
V3056       .......... .C........ .......... ..A.....C. .T.....G.. ...A......
V5435       .....T.... ....A..... G..T...... .....G.... .T..T..... C.........
V8925       ..G....... .C..A..... G......... .......... .T........ C..A......
V8928       ..G....... .C..A..... .......... .......... .T........ ...A......
V8930       ..G....... .C..A..... G......... .......... .T........ C..A......
V9064       ..G....... ....A..... .......... .......... .T........ ......G..T
V8884       .......... ....A..... G.....T... .....G.... ....T..... ..........
V9969       .......... ....A..... G.....T..T .....G.... .......... ..........
V9802       .......... ....A..... G.....T... .....G.... .......... ..........
V9027       .......... .......... G.....T..G .....G.... .T........ ...A......
V8817       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V8840       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V8855       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V8895       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9057       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9495       ..G....... ....A..T.. ......T..G .......... .......... ...A...T.T
V9510       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9646       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9668       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9504       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9521       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9538       ..G....... ....A..T.. ......T..G .......... .......... ...A......
V9885       ..G..T.... ....A..T.. ......T..G .......... .......... ..........
V9635       ..C....... ....A..... G.....T... .....G.... .......... ..........
V9672       ..C....... ....A..... G.....T... .....G.... .......... ..........
V9606       .......... ....A..... G.....T... .....G.... .......... ..........
V9695       .......... ....A..... G.....T... .....G.... .......... ..........
V9705       .......... ....A..... G.....T... .....G.... .......... ..........
V10048      ..C....... ....A..... G.....T... .....G.... .......... ..........
V10089      ..C..T.... ....A..... G.....T... .....G.... .......... ..........
V10090      ..C..T.... ....A..... G.....T... .....G.... .......... ..........
V10092      ..C..T.... ....A..... G.....T... .....G.... .......... ..........
SAB2        ..A..T..A. .A..A..... .....T.... ..A..T.... .T..T..... ......C...

V9          CCCTTACCAG AAAAAGGGTT GACCACATAC GGATTTGGGC ACCAGAATAA AGCAGTCTAC
V340        .......... .......... .......... .......... .......... ..........
V769        .......... .......... .......... ..GC...... .......... ..........
V813        .......... .......... .......... .......... .......... ..........
V855        .......... .G........ .......... ..G....... .......... ..........
V964        .......... .......... ...T...... ..G..C.... .T..A..... ..........
V1010       ..T....... .......... .......... ..T....... ....A..... G.........
V1050       ..T....... .......... .......... ..T....... ....A..... G.........T
V1088       .......... .G........ .......... ..T....... ....A..... G.........T
V1128       .......... .......... .......... ..T....... ....A..... G.........T
V2838       .......... .......... ...T...... ..G....... .......... ..........
V2909       .......... .......... ......G... .....C.... .......... ......A...
V3056       .......... .......A.. ......T... .......... .T..A..... .........T
V5435       .......... .......... .......... .......... .......C.. ..........
V8925       ...C....T. .G......C. ......G..T ...A...... .T....GC.. ......T...
V8928       ...C....T. ........C. ......G..T .......... .T........ G.....T...
V8930       ...C....T. .G......C. ......G..T ...A...... .T.....C.. ......T...
V9064       ...C....T. ........C. ......G..T ..G....... .T.....C.. G.....T...
V8884       .......... .G........ ......G... .......... .T.....C.. ..........
V9969       ...C...... .G....A... ......G... .......... .T.....C.. ..........
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V9802       .......... .G........ ......G... ....A..... .T........ ..........
V9027       .......... .G......C. ...T..G... .......... .T.....C.. ..........
V8817       ...C....G. .G......C. ......G..T .......... .T.....C.. ......T...
V8840       ...C....G. .G......C. ......G..T .......... .T.....C.. ......T...
V8855       ...C....G. .G......C. ......G..T .......... .......C.. ......T...
V8895       ...C....G. .G......C. ......G..T ........A. .T.....C.. ......T...
V9057       ...C....G. .G......C. ......G..T .........A .T.....C.. ......T...
V9495       ...C....G. ........C. ......G..T ........A. .T.....C.. ......G...
V9510       ...C....G. ........C. ......G..T .......... .T.....C.. ......G...
V9646       ...C....G. .G......C. ......G..T .......... .T.....C.. ......G...
V9668       ...C....G. .G......C. ......G..T .......... .T.....C.. ......G...
V9504       ...C....G. .G......C. ...T..G..T .......... .T..A..C.. ......G...
V9521       ...C....G. .G......C. ...T..G..T .......... .T.....C.. ......G..T
V9538       ...C....G. .G......C. ...T..G..T .......... .T.....C.. ......G...
V9885       ...C....G. .G......C. ......G..T ..G....... .T.....C.. ......G...
V9635       ........T. .G........ ...T..G... ..T....... .......C.. ...G......
V9672       ........T. .G........ ...T..G... ..T....... .......C.. ...G......
V9606       .......... .G........ ......G... ..T....... .......C.. ...G......
V9695       .......... .G........ ......G... ..T..C.... .......C.. ...G......
V9705       .......... .G........ ......G... ..T..C.... .......C.. ...G......
V10048      ........T. .G........ ...T..G... ..T....... .......C.. ...G......
V10089      .....G.... .G........ ...T..G... ..T....... .T.....C.. ...G......
V10090      .....G.... .G........ ...T..G... ..T....... .T.....C.. ...G......
V10092      .....G.... .G........ ...T..G... ..T....... .T.....C.. ...G......
SAB2        ..AC...... ....G..A.. A..G..T..T ........A. ....A..C.. ...T..G...

V9          ACCGCGGGGT ATAAAATATG TAACTATCAT
V340        .......... .......... ..........
V769        .......... T......... ..........
V813        .......... .......... C........C
V855        .......... .C........ ..........
V964        .......... .......... ..........
V1010       .......... .......... C.........
V1050       .......... .......... C.........
V1088       .......... .......... C.........
V1128       .......... .......... C.....C...
V2838       .....A.... .......... ..........
V2909       .......... .......... ......C..C
V3056       .......... .C........ ...T......
V5435       .....A.... .C........ .........C
V8925       ..T....... .C........ ......C..C
V8928       ..T....... .C........ ......C..C
V8930       ..T....... .C........ ......C..C
V9064       ..T....... .C........ ......C..C
V8884       .....T.... .C........ C.....C...
V9969       .....T.... .C........ C.....C...
V9802       .....T.... .C........ C.....C..C
V9027       .....T.... .C........ C..T..C...
V8817       ..T....... .C........ .........C
V8840       ..T....... .C........ .........C
V8855       ..T....... .C........ .........C
V8895       ..T....... .C........ .........C
V9057       ..T....... .C........ .........C
V9495       ..A..A..C. .C..G..T.. C..T..C..C
V9510       ..A..A..C. .C..G..T.. C..T.....C
V9646       ..A..A..C. .C..G..T.. C..T.....C
V9668       ..A..A..C. .C..G..T.. C..T.....C
V9504       ..A..A..C. .C..G..T.. C..T.....C
V9521       ..A..A..T. .C..G..T.. C..T.....C
V9538       ..A..A..T. .C..G..T.. C..T.....C
V9885       ..G..A..T. .C..G..T.. C..T.....C
V9635       .....T.... .......... C..T..C...
V9672       .....T.... .......... C..T..C...
V9606       .....T.... .......... C.....C...
V9695       .....T.... .......... C.....C...
V9705       .....T.... .......... C.....C...
V10048      .....T.... .......... C..T..C...
V10089      .....T.... .......... C.....C...
V10090      .....T.... .......... C.....C...
V10092      .....T.... .......... C.....C...
SAB2        ..A..T..C. .C.....T.. C..T..C..C
```

Figure 10: Genetic distance of 44 wild poliovirus type 2 isolates from Mumbai (1971 to 1995) for 150 nucleotides of VP1/2A region calculated by Kimura 2 Parameter method.

V9	0.0000	0.0135	0.0492	0.0203	0.0203	0.0562	0.0417	0.0489
0.0489	0.0489	0.0486	0.0636	0.0942	0.0858	0.1716	0.1272	0.1627
0.1441	0.0942	0.1108	0.0948	0.1263	0.1346	0.1346	0.1263	0.1430
0.1441	0.2239	0.1836	0.1930	0.1930	0.2122	0.2122	0.2025	0.2025
0.1198	0.1198	0.0870	0.0948	0.0948	0.1198	0.1272	0.1272	0.1272
0.3146								
V340	0.0135	0.0000	0.0640	0.0343	0.0343	0.0711	0.0562	0.0636
0.0636	0.0636	0.0632	0.0787	0.0942	0.0858	0.1716	0.1441	0.1627
0.1614	0.0942	0.1108	0.0948	0.1263	0.1516	0.1516	0.1430	0.1602
0.1614	0.2443	0.2025	0.2122	0.2122	0.2320	0.2320	0.2220	0.2220
0.1198	0.1198	0.0870	0.0948	0.0948	0.1198	0.1272	0.1272	0.1272
0.3404								
V769	0.0492	0.0640	0.0000	0.0716	0.0566	0.0876	0.0876	0.0955
0.0955	0.0955	0.0640	0.1198	0.1366	0.1272	0.2220	0.1821	0.2122
0.1821	0.1539	0.1527	0.1551	0.1807	0.1807	0.1807	0.1716	0.1899
0.1914	0.2813	0.2361	0.2465	0.2465	0.2677	0.2677	0.2570	0.2158
0.1563	0.1563	0.1376	0.1463	0.1463	0.1563	0.1463	0.1463	0.1463
0.3484								
V813	0.0203	0.0343	0.0716	0.0000	0.0414	0.0787	0.0489	0.0562
0.0562	0.0562	0.0706	0.0562	0.1181	0.0935	0.1807	0.1356	0.1716
0.1527	0.1020	0.1190	0.0870	0.1346	0.1430	0.1430	0.1346	0.1516
0.1527	0.2140	0.1743	0.1836	0.1836	0.2025	0.2025	0.1930	0.1930
0.1282	0.1282	0.0948	0.1028	0.1028	0.1282	0.1356	0.1356	0.1356
0.3021								
V855	0.0203	0.0343	0.0566	0.0414	0.0000	0.0636	0.0562	0.0636
0.0489	0.0636	0.0558	0.0864	0.1020	0.0935	0.1627	0.1356	0.1539
0.1356	0.0864	0.1028	0.0870	0.1181	0.1263	0.1263	0.1181	0.1346
0.1356	0.2340	0.1930	0.1836	0.1836	0.2025	0.2025	0.1930	0.1743
0.1198	0.1198	0.0870	0.0948	0.0948	0.1198	0.1272	0.1272	0.1272
0.3274								
V964	0.0562	0.0711	0.0876	0.0787	0.0636	0.0000	0.0792	0.0870
0.0870	0.0870	0.0636	0.0948	0.1272	0.1181	0.1976	0.1516	0.1883
0.1516	0.1108	0.1452	0.1282	0.1614	0.1590	0.1590	0.1677	0.1677
0.1690	0.2524	0.2104	0.2201	0.2201	0.2008	0.2201	0.2104	0.2104
0.1376	0.1376	0.1282	0.1198	0.1198	0.1376	0.1282	0.1282	0.1282
0.2989								
V1010	0.0417	0.0562	0.0876	0.0489	0.0562	0.0792	0.0000	0.0067
0.0203	0.0203	0.0864	0.1108	0.1108	0.1346	0.2104	0.1452	0.2008
0.1716	0.1272	0.1452	0.1282	0.1441	0.1703	0.1703	0.1614	0.1792
0.1807	0.2465	0.2042	0.2140	0.2140	0.2140	0.2340	0.2239	0.2340
0.1356	0.1356	0.1020	0.1100	0.1100	0.1356	0.1430	0.1430	0.1430
0.3311								
V1050	0.0489	0.0636	0.0955	0.0562	0.0636	0.0870	0.0067	0.0000
0.0135	0.0135	0.0942	0.1190	0.1028	0.1430	0.2201	0.1539	0.2104
0.1807	0.1356	0.1539	0.1366	0.1527	0.1792	0.1792	0.1703	0.1883
0.1899	0.2570	0.2140	0.2239	0.2239	0.2239	0.2239	0.2340	0.2443
0.1441	0.1441	0.1100	0.1181	0.1181	0.1441	0.1516	0.1516	0.1516
0.3444								
V1088	0.0489	0.0636	0.0955	0.0562	0.0489	0.0870	0.0203	0.0135
0.0000	0.0135	0.0942	0.1190	0.1028	0.1430	0.2008	0.1539	0.1914
0.1807	0.1190	0.1366	0.1198	0.1356	0.1614	0.1614	0.1527	0.1703
0.1716	0.2570	0.2140	0.2042	0.2042	0.2042	0.2042	0.2140	0.2239
0.1272	0.1272	0.0942	0.1020	0.1020	0.1272	0.1346	0.1346	0.1346
0.3580								
V1128	0.0489	0.0636	0.0955	0.0562	0.0636	0.0870	0.0203	0.0135
0.0135	0.0000	0.0942	0.1028	0.1028	0.1430	0.2008	0.1366	0.1914
0.1627	0.1190	0.1366	0.1198	0.1356	0.1792	0.1792	0.1703	0.1883
0.1899	0.2361	0.2140	0.2239	0.2239	0.2239	0.2239	0.2340	0.2443
0.1272	0.1272	0.0942	0.1020	0.1020	0.1272	0.1346	0.1346	0.1346
0.3311								
V2838	0.0486	0.0632	0.0640	0.0706	0.0558	0.0636	0.0864	0.0942
0.0942	0.0942	0.0000	0.0864	0.1346	0.0935	0.2183	0.1703	0.2086
0.1703	0.1100	0.1190	0.1108	0.1263	0.1602	0.1602	0.1516	0.1690
0.1703	0.2340	0.1930	0.2025	0.2025	0.2025	0.2025	0.1930	0.1930

```
  0.1116  0.1116  0.0948  0.1028  0.1028  0.1116  0.1190  0.1190  0.1190
  0.3146
V2909       0.0636  0.0787  0.1198  0.0562  0.0864  0.0948  0.1108  0.1190
  0.1190  0.1028  0.0864  0.0000  0.1614  0.1100  0.1730  0.1282  0.1640
  0.1452  0.0870  0.1035  0.0721  0.1356  0.1356  0.1356  0.1272  0.1441
  0.1452  0.2025  0.1821  0.1914  0.1914  0.2104  0.2104  0.2008  0.2008
  0.1124  0.1124  0.0798  0.0721  0.0721  0.1124  0.1198  0.1198  0.1198
  0.3112
V3056       0.0942  0.0942  0.1366  0.1181  0.1020  0.1272  0.1108  0.1028
  0.1028  0.1028  0.1346  0.1614  0.0000  0.1602  0.1914  0.1452  0.1821
  0.1992  0.1614  0.1807  0.1627  0.1441  0.1883  0.1883  0.1976  0.1976
  0.1992  0.2677  0.2239  0.2340  0.2340  0.2340  0.2340  0.2443  0.2653
  0.2104  0.2104  0.1899  0.1992  0.1992  0.2104  0.2183  0.2183  0.2183
  0.2868
V5435       0.0858  0.0858  0.1272  0.0935  0.0935  0.1181  0.1346  0.1430
  0.1430  0.1430  0.0935  0.1100  0.1602  0.0000  0.1539  0.1614  0.1452
  0.1614  0.0864  0.1190  0.1028  0.1346  0.1690  0.1690  0.1602  0.1778
  0.1792  0.2443  0.2025  0.2122  0.2122  0.2320  0.2320  0.2220  0.2025
  0.1452  0.1452  0.1108  0.1190  0.1190  0.1452  0.1356  0.1356  0.1356
  0.2660
V8925       0.1716  0.1716  0.2220  0.1807  0.1627  0.1976  0.2104  0.2201
  0.2008  0.2008  0.2183  0.1730  0.1914  0.1539  0.0000  0.0489  0.0067
  0.0787  0.1463  0.1474  0.1474  0.1463  0.0792  0.0792  0.0870  0.0870
  0.0876  0.1772  0.1575  0.1486  0.1486  0.1666  0.1666  0.1575  0.1758
  0.1821  0.1821  0.1743  0.1836  0.1836  0.1821  0.1930  0.1930  0.1930
  0.3311
V8928       0.1272  0.1441  0.1821  0.1356  0.1356  0.1516  0.1452  0.1539
  0.1539  0.1366  0.1703  0.1282  0.1452  0.1614  0.0489  0.0000  0.0417
  0.0414  0.1539  0.1551  0.1376  0.1539  0.0711  0.0711  0.0787  0.0787
  0.0792  0.1486  0.1301  0.1387  0.1387  0.1563  0.1563  0.1474  0.1652
  0.1899  0.1899  0.1821  0.1914  0.1914  0.1899  0.2008  0.2008  0.2008
  0.2898
V8930       0.1627  0.1627  0.2122  0.1716  0.1539  0.1883  0.2008  0.2104
  0.1914  0.1914  0.2086  0.1640  0.1821  0.1452  0.0067  0.0417  0.0000
  0.0711  0.1376  0.1387  0.1387  0.1376  0.0716  0.0716  0.0792  0.0792
  0.0798  0.1679  0.1486  0.1398  0.1398  0.1575  0.1575  0.1486  0.1666
  0.1730  0.1730  0.1652  0.1743  0.1743  0.1730  0.1836  0.1836  0.1836
  0.3181
V9064       0.1441  0.1614  0.1821  0.1527  0.1356  0.1516  0.1716  0.1807
  0.1807  0.1627  0.1703  0.1452  0.1992  0.1614  0.0787  0.0414  0.0711
  0.0000  0.1539  0.1551  0.1551  0.1716  0.0864  0.0864  0.0942  0.0942
  0.0948  0.1486  0.1474  0.1563  0.1563  0.1743  0.1743  0.1652  0.1474
  0.1807  0.1807  0.1730  0.1821  0.1821  0.1807  0.1914  0.1914  0.1914
  0.2898
V8884       0.0942  0.0942  0.1539  0.1020  0.0864  0.1108  0.1272  0.1356
  0.1190  0.1190  0.1100  0.0870  0.1614  0.0864  0.1463  0.1539  0.1376
  0.1539  0.0000  0.0275  0.0275  0.0558  0.1272  0.1272  0.1356  0.1356
  0.1366  0.1866  0.1666  0.1575  0.1575  0.1758  0.1758  0.1666  0.1666
  0.0640  0.0640  0.0345  0.0417  0.0417  0.0640  0.0562  0.0562  0.0562
  0.2750
V9969       0.1108  0.1108  0.1527  0.1190  0.1028  0.1452  0.1452  0.1539
  0.1366  0.1366  0.1190  0.1035  0.1807  0.1190  0.1474  0.1551  0.1387
  0.1551  0.0275  0.0000  0.0419  0.0636  0.1198  0.1198  0.1282  0.1282
  0.1291  0.1787  0.1587  0.1497  0.1497  0.1679  0.1679  0.1587  0.1587
  0.0798  0.0798  0.0492  0.0566  0.0566  0.0798  0.0716  0.0716  0.0716
  0.3021
V9802       0.0948  0.0948  0.1551  0.0870  0.0870  0.1282  0.1282  0.1366
  0.1198  0.1198  0.1108  0.0721  0.1627  0.1028  0.1474  0.1376  0.1387
  0.1551  0.0275  0.0419  0.0000  0.0711  0.1282  0.1282  0.1366  0.1366
  0.1376  0.1882  0.1679  0.1587  0.1587  0.1772  0.1772  0.1679  0.1679
  0.0798  0.0798  0.0492  0.0566  0.0566  0.0798  0.0716  0.0716  0.0716
  0.3021
V9027       0.1263  0.1263  0.1807  0.1346  0.1181  0.1614  0.1441  0.1527
  0.1356  0.1356  0.1263  0.1356  0.1441  0.1346  0.1463  0.1539  0.1376
  0.1716  0.0558  0.0636  0.0711  0.0000  0.1272  0.1272  0.1356  0.1356
  0.1366  0.1679  0.1486  0.1398  0.1398  0.1398  0.1398  0.1311  0.1666
  0.0792  0.0792  0.0787  0.0864  0.0864  0.0792  0.0864  0.0864  0.0864
  0.3112
V8817       0.1346  0.1516  0.1807  0.1430  0.1263  0.1590  0.1703  0.1792
  0.1614  0.1792  0.1602  0.1356  0.1883  0.1690  0.0792  0.0711  0.0716
  0.0864  0.1272  0.1198  0.1282  0.1272  0.0000  0.0000  0.0067  0.0067
  0.0067  0.0969  0.0649  0.0573  0.0573  0.0726  0.0726  0.0649  0.0803
```

0.1730 0.1730 0.1539 0.1627 0.1627 0.1730 0.1716 0.1716 0.1716
0.3366
V8840 0.1346 0.1516 0.1807 0.1430 0.1263 0.1590 0.1703 0.1792
0.1614 0.1792 0.1602 0.1356 0.1883 0.1690 0.0792 0.0711 0.0716
0.0864 0.1272 0.1198 0.1282 0.1272 0.0000 0.0000 0.0067 0.0067
0.0067 0.0969 0.0649 0.0573 0.0573 0.0726 0.0726 0.0649 0.0803
0.1730 0.1730 0.1539 0.1627 0.1627 0.1730 0.1716 0.1716 0.1716
0.3366
V8855 0.1263 0.1430 0.1716 0.1346 0.1181 0.1677 0.1614 0.1703
0.1527 0.1703 0.1516 0.1272 0.1976 0.1602 0.0870 0.0787 0.0792
0.0942 0.1356 0.1282 0.1366 0.1356 0.0067 0.0067 0.0000 0.0135
0.0136 0.1050 0.0726 0.0649 0.0649 0.0803 0.0803 0.0726 0.0882
0.1640 0.1640 0.1452 0.1539 0.1539 0.1640 0.1807 0.1807 0.1807
0.3238
V8895 0.1430 0.1602 0.1899 0.1516 0.1346 0.1677 0.1792 0.1883
0.1703 0.1883 0.1690 0.1441 0.1976 0.1778 0.0870 0.0787 0.0792
0.0942 0.1356 0.1282 0.1366 0.1356 0.0067 0.0067 0.0135 0.0000
0.0136 0.0888 0.0726 0.0649 0.0649 0.0803 0.0803 0.0726 0.0882
0.1821 0.1821 0.1627 0.1716 0.1716 0.1821 0.1807 0.1807 0.1807
0.3238
V9057 0.1441 0.1614 0.1914 0.1527 0.1356 0.1690 0.1807 0.1899
0.1716 0.1899 0.1703 0.1452 0.1992 0.1792 0.0876 0.0792 0.0798
0.0948 0.1366 0.1291 0.1376 0.1366 0.0067 0.0067 0.0136 0.0136
0.0000 0.1058 0.0731 0.0653 0.0653 0.0809 0.0809 0.0731 0.0888
0.1836 0.1836 0.1640 0.1730 0.1730 0.1836 0.1821 0.1821 0.1821
0.3538
V9495 0.2239 0.2443 0.2813 0.2140 0.2340 0.2524 0.2465 0.2570
0.2570 0.2361 0.2340 0.2025 0.2677 0.2443 0.1772 0.1486 0.1679
0.1486 0.1866 0.1787 0.1882 0.1679 0.0969 0.0969 0.1050 0.0888
0.1058 0.0000 0.0275 0.0345 0.0345 0.0489 0.0562 0.0489 0.0711
0.2196 0.2196 0.2177 0.2279 0.2279 0.2196 0.2382 0.2382 0.2382
0.2558
V9510 0.1836 0.2025 0.2361 0.1743 0.1930 0.2104 0.2042 0.2140
0.2140 0.2140 0.1930 0.1821 0.2239 0.2025 0.1575 0.1301 0.1486
0.1474 0.1666 0.1587 0.1679 0.1486 0.0649 0.0649 0.0726 0.0726
0.0731 0.0275 0.0000 0.0067 0.0067 0.0203 0.0273 0.0203 0.0414
0.1979 0.1979 0.1962 0.2059 0.2059 0.1979 0.2158 0.2158 0.2158
0.2534
V9646 0.1930 0.2122 0.2465 0.1836 0.1836 0.2201 0.2140 0.2239
0.2042 0.2239 0.2025 0.1914 0.2340 0.2122 0.1486 0.1387 0.1398
0.1563 0.1575 0.1497 0.1587 0.1398 0.0573 0.0573 0.0649 0.0649
0.0653 0.0345 0.0067 0.0000 0.0000 0.0135 0.0203 0.0135 0.0343
0.1882 0.1882 0.1866 0.1962 0.1962 0.1882 0.2059 0.2059 0.2059
0.2644
V9668 0.1930 0.2122 0.2465 0.1836 0.1836 0.2201 0.2140 0.2239
0.2042 0.2239 0.2025 0.1914 0.2340 0.2122 0.1486 0.1387 0.1398
0.1563 0.1575 0.1497 0.1587 0.1398 0.0573 0.0573 0.0649 0.0649
0.0653 0.0345 0.0067 0.0000 0.0000 0.0135 0.0203 0.0135 0.0343
0.1882 0.1882 0.1866 0.1962 0.1962 0.1882 0.2059 0.2059 0.2059
0.2644
V9504 0.2122 0.2320 0.2677 0.2025 0.2025 0.2008 0.2140 0.2239
0.2042 0.2239 0.2025 0.2104 0.2340 0.2320 0.1666 0.1563 0.1575
0.1743 0.1758 0.1679 0.1772 0.1398 0.0726 0.0726 0.0803 0.0803
0.0809 0.0489 0.0203 0.0135 0.0135 0.0000 0.0203 0.0135 0.0486
0.1882 0.1882 0.2059 0.2158 0.2158 0.1882 0.2059 0.2059 0.2059
0.2534
V9521 0.2122 0.2320 0.2677 0.2025 0.2025 0.2201 0.2340 0.2239
0.2042 0.2239 0.2025 0.2104 0.2340 0.2320 0.1666 0.1563 0.1575
0.1743 0.1758 0.1679 0.1772 0.1398 0.0726 0.0726 0.0803 0.0803
0.0809 0.0562 0.0273 0.0203 0.0203 0.0203 0.0000 0.0067 0.0414
0.1882 0.1882 0.2059 0.2158 0.2158 0.1882 0.2059 0.2059 0.2059
0.2869
V9538 0.2025 0.2220 0.2570 0.1930 0.1930 0.2104 0.2239 0.2340
0.2140 0.2340 0.1930 0.2008 0.2443 0.2220 0.1575 0.1474 0.1486
0.1652 0.1666 0.1587 0.1679 0.1311 0.0649 0.0649 0.0726 0.0726
0.0731 0.0489 0.0203 0.0135 0.0135 0.0135 0.0067 0.0000 0.0343
0.1787 0.1787 0.1962 0.2059 0.2059 0.1787 0.1962 0.1962 0.1962
0.2755
V9885 0.2025 0.2220 0.2158 0.1930 0.1743 0.2104 0.2340 0.2443
0.2239 0.2443 0.1930 0.2008 0.2653 0.2025 0.1758 0.1652 0.1666
0.1474 0.1666 0.1587 0.1679 0.1666 0.0803 0.0803 0.0882 0.0882
0.0888 0.0711 0.0414 0.0343 0.0343 0.0486 0.0414 0.0343 0.0000

0.1882 0.1882 0.1866 0.1962 0.1962 0.1882 0.1866 0.1866 0.1866
0.2755
V9635 0.1198 0.1198 0.1563 0.1282 0.1198 0.1376 0.1356 0.1441
0.1272 0.1272 0.1116 0.1124 0.2104 0.1452 0.1821 0.1899 0.1730
0.1807 0.0640 0.0798 0.0798 0.0792 0.1730 0.1730 0.1640 0.1821
0.1836 0.2196 0.1979 0.1882 0.1882 0.1882 0.1882 0.1787 0.1882
0.0000 0.0000 0.0275 0.0345 0.0345 0.0000 0.0345 0.0345 0.0345
0.3054
V9672 0.1198 0.1198 0.1563 0.1282 0.1198 0.1376 0.1356 0.1441
0.1272 0.1272 0.1116 0.1124 0.2104 0.1452 0.1821 0.1899 0.1730
0.1807 0.0640 0.0798 0.0798 0.0792 0.1730 0.1730 0.1640 0.1821
0.1836 0.2196 0.1979 0.1882 0.1882 0.1882 0.1882 0.1787 0.1882
0.0000 0.0000 0.0275 0.0345 0.0345 0.0000 0.0345 0.0345 0.0345
0.3054
V9606 0.0870 0.0870 0.1376 0.0948 0.0870 0.1282 0.1020 0.1100
0.0942 0.0942 0.0948 0.0798 0.1899 0.1108 0.1743 0.1821 0.1652
0.1730 0.0345 0.0492 0.0492 0.0787 0.1539 0.1539 0.1452 0.1627
0.1640 0.2177 0.1962 0.1866 0.1866 0.2059 0.2059 0.1962 0.1866
0.0275 0.0275 0.0000 0.0067 0.0067 0.0275 0.0343 0.0343 0.0343
0.3021
V9695 0.0948 0.0948 0.1463 0.1028 0.0948 0.1198 0.1100 0.1181
0.1020 0.1020 0.1028 0.0721 0.1992 0.1190 0.1836 0.1914 0.1743
0.1821 0.0417 0.0566 0.0566 0.0864 0.1627 0.1627 0.1539 0.1716
0.1730 0.2279 0.2059 0.1962 0.1962 0.2158 0.2158 0.2059 0.1962
0.0345 0.0345 0.0067 0.0000 0.0000 0.0345 0.0414 0.0414 0.0414
0.3146
V9705 0.0948 0.0948 0.1463 0.1028 0.0948 0.1198 0.1100 0.1181
0.1020 0.1020 0.1028 0.0721 0.1992 0.1190 0.1836 0.1914 0.1743
0.1821 0.0417 0.0566 0.0566 0.0864 0.1627 0.1627 0.1539 0.1716
0.1730 0.2279 0.2059 0.1962 0.1962 0.2158 0.2158 0.2059 0.1962
0.0345 0.0345 0.0067 0.0000 0.0000 0.0345 0.0414 0.0414 0.0414
0.3146
V10048 0.1198 0.1198 0.1563 0.1282 0.1198 0.1376 0.1356 0.1441
0.1272 0.1272 0.1116 0.1124 0.2104 0.1452 0.1821 0.1899 0.1730
0.1807 0.0640 0.0798 0.0798 0.0792 0.1730 0.1730 0.1640 0.1821
0.1836 0.2196 0.1979 0.1882 0.1882 0.1882 0.1882 0.1787 0.1882
0.0000 0.0000 0.0275 0.0345 0.0345 0.0000 0.0345 0.0345 0.0345
0.3054
V10089 0.1272 0.1272 0.1463 0.1356 0.1272 0.1282 0.1430 0.1516
0.1346 0.1346 0.1190 0.1198 0.2183 0.1356 0.1930 0.2008 0.1836
0.1914 0.0562 0.0716 0.0716 0.0864 0.1716 0.1716 0.1807 0.1807
0.1821 0.2382 0.2158 0.2059 0.2059 0.2059 0.2059 0.1962 0.1866
0.0345 0.0345 0.0343 0.0414 0.0414 0.0345 0.0000 0.0000 0.0000
0.3146
V10090 0.1272 0.1272 0.1463 0.1356 0.1272 0.1282 0.1430 0.1516
0.1346 0.1346 0.1190 0.1198 0.2183 0.1356 0.1930 0.2008 0.1836
0.1914 0.0562 0.0716 0.0716 0.0864 0.1716 0.1716 0.1807 0.1807
0.1821 0.2382 0.2158 0.2059 0.2059 0.2059 0.2059 0.1962 0.1866
0.0345 0.0345 0.0343 0.0414 0.0414 0.0345 0.0000 0.0000 0.0000
0.3146
V10092 0.1272 0.1272 0.1463 0.1356 0.1272 0.1282 0.1430 0.1516
0.1346 0.1346 0.1190 0.1198 0.2183 0.1356 0.1930 0.2008 0.1836
0.1914 0.0562 0.0716 0.0716 0.0864 0.1716 0.1716 0.1807 0.1807
0.1821 0.2382 0.2158 0.2059 0.2059 0.2059 0.2059 0.1962 0.1866
0.0345 0.0345 0.0343 0.0414 0.0414 0.0345 0.0000 0.0000 0.0000
0.3146
SAB2 0.3146 0.3404 0.3484 0.3021 0.3274 0.2989 0.3311 0.3444
0.3580 0.3311 0.3146 0.3112 0.2868 0.2660 0.3311 0.2898 0.3181
0.2898 0.2750 0.3021 0.3021 0.3112 0.3366 0.3366 0.3238 0.3238
0.3538 0.2558 0.2534 0.2644 0.2644 0.2534 0.2869 0.2755 0.2755
0.3054 0.3054 0.3021 0.3146 0.3146 0.3054 0.3146 0.3146 0.3146
0.0000

Genomic relationship of wild poliovirus type 2 from Mumbai 1971-86

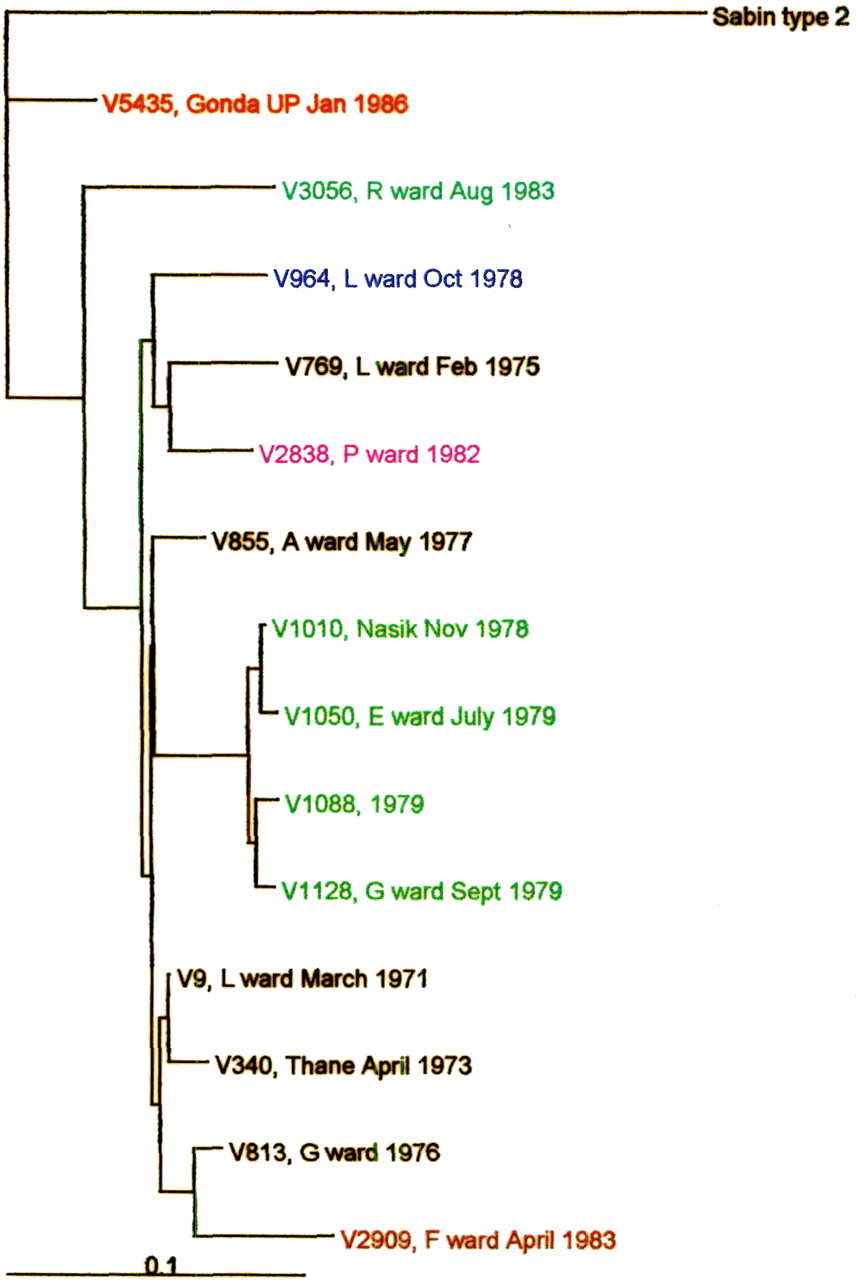

Figure 11: phylogenetic relationship of wild poliovirus type 2 (1971-86) based on VP1/2A sequence data. The relationship was determined using NJ method of package PHYLIP v3.5c.

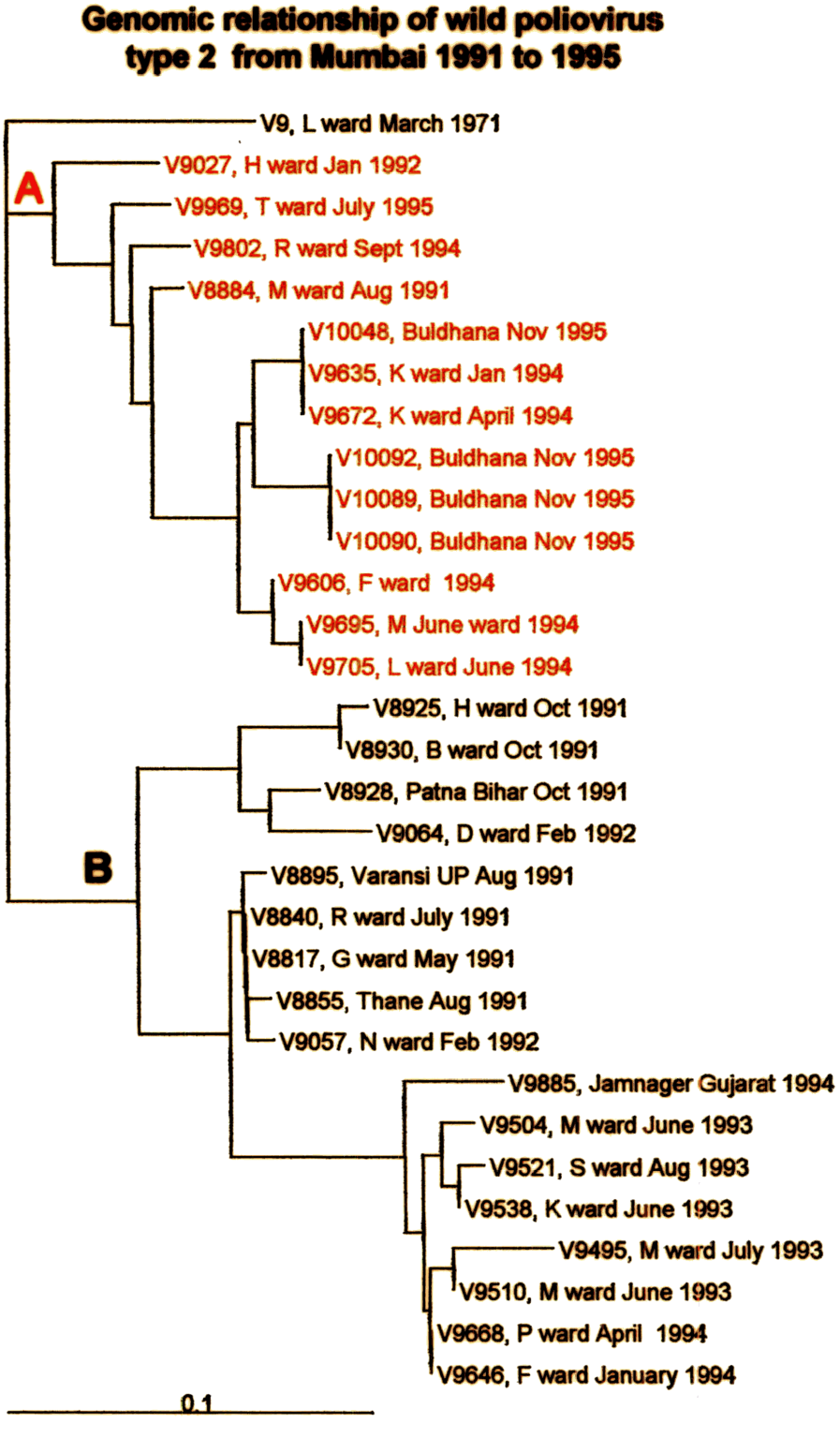

Figure 12: Phylogenetic relationship of wild type 2 poliovirus isolated from Mumbai between year 1991 and 1995 based on 150bp fragment of VP1\2A junction. The dendrogram was drawn using NJ method of package PHYLIP version 3.5c.

Figure 13: Alignment of amino acid sequences of VP1/2A junction region of wild type 2 poliovirus from Mumbai (1971 to 1995).

```
#SAB2    RPPRAVPYFG PGVDYKDGLT PLPEKGLTTY GFGHQNKAVY TAGYKICNYH
#V9      ........Y. .......... .......... .......... ..........
#V340    ........Y. .......... .......... .......... ..........
#V769    ........Y. .......... .......... .L........ ...F......
#V813    ........Y. .......... .......... .......... ..........
#V855    ........Y. .......... .......... .......... ..........
#V964    ........Y. .......... .......... .......... ..........
#V1010   ........Y. .......... .......... .......... ..........
#V1050   ........Y. .......... .......... .......... ..........
#V1088   ........Y. .......... .......... .......... ..........
#V1128   ........Y. .......... .......... .......... ..........
#V2838   ........Y. .......... .......... .......... ..........
#V2909   ........Y. .......... .......... .......... ..........
#V3056   ........Y. .......... .......... .......... ..........
#V5435   ........Y. .......... .......... .......... ..........
#V9495   ........Y. .........S .......... .......... ..........
#V9510   ........Y. .......... .......... .......... ..........
#V9646   ........Y. .......... .......... .......... ..........
#V9668   ........Y. .......... .......... .......... ..........
#V9504   ........Y. .......... .......... .......... ..........
#V9521   ........Y. .......... .......... .......... ..........
#V9538   ........Y. .......... .......... .......... ..........
#V9885   ........Y. .......... .......... .......... ..........
#V9635   ........Y. .......... .......... .......... ..........
#V9672   ........Y. .......... .......... .......... ..........
#V9969   ........Y. .......... .....E.... .......... ..........
#V9802   ........Y. .......... .......... .Y........ ..........
#V9606   ........Y. .......... .......... .......... ..........
#V9695   ........Y. .......... .......... .......... ..........
#V9705   ........Y. .......... .......... .......... ..........
#V10048  ........Y. .......... .......... .......... ..........
#V10089  ........Y. .......... .......... .......... ..........
#V10090  ........Y. .......... .......... .......... ..........
#V10092  ........Y. .......... .......... .......... ..........
#V9027   ........Y. .......... .......... .......... ..........
#V9057   ........Y. .......... .......... ...N...... ..........
#V9064   ........Y. .......... .......... .......... ..........
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Figure 14: Complete VP1 sequence alignment of wild poliovirus type 2 isolates from India (1998-1999).

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V9635VP1    GGTCTGGGAG ATCTGCTAGA GGGCGTGATT GAGAGCACCA CAAAAAACGC GTTAGTACCA
R5205VP1    .......... ..T....... ...T...... ........T. .G........ .C........
R982466VP   .......... ..T....... .......... ........T. .G........ .C........
R7773VP1    ........G. .CT....... .......... .......... .......... AC........
R3628VP1    .......... ..T....... .......... .......... .......... .C........
R4908VP1    .......... ..T....... ....A..... .......... .......... .C........
R6546VP1    .......... ..T....G.. .......... .......... .......... .C........
R3776VP1    ........G. ..T....... .......... .......... .G........ .C....G...
R4111VP1    .......... ..T....... .......... .......... .G........ .C........
R5009VP1    .......... ..T..T.... .......... ....A..... .G........ .C........
R982396VP   .......... ..T....G.. .......... .......... .G........ .C........
R98239VP    .......... ..T....... .......... ........T. .G........ .C........
R982865VP   .......... ..T....... .......... ........T. .G........ .C........
R4703VP1    .......... ..T....... ...T...... ........T. .G........ .C........
R6099VP1    .......... ..T....... .......... .......... .G........ .C........
R7283VP1    .......... ..T....... .......... .......... .G........ .C........
R5033VP1    .......... ..T....... .......... ........T. .G........ .C........
R982813VP   ..CT...... ..T....... .......... ........T. .G........ .C....G...
R3588VP1    .......... ..T....... .......... ........T. .G........ .C....G...
R982889VP   .......... ..T.A..... .......... ........T. .T........ .C.......G
R4487VP1    .......... ..T.A..... .......... ........T. .T........ .A.......G
R982537VP   .......... ..T.A..... .......... ........T. .T........ .C.......G
R5584VP1    .......... ..T.A..... .......... ........T. .T........ .C.......G
R982643VP   .......... ..T.A..... .......... .....T..T. .T........ .C.......G
SABP2VP1    ..AA.T..T. .CA..A.T.. A..G.CCG.. ..AG.G.TT. .T.....T.. A..G..T..C

V9635VP1    CTGGCATCCA CTAACAACCT GCCTGATACT CAACCAAGTG GTCCAGCACA TTCCAAAGAG
R5205VP1    ...T...... ....T..... ......C... .......... .......... .........A
R982466VP   ...A...... ....T..... ......C... .......... .C........ .........A
R7773VP1    ...A..A.T. .......... ......C... ..G....... .C........ ..........
R3628VP1    ...A...... ....T..... ......C... .......... .C........ ......G..A
R4908VP1    ...C...... ....T..... ......C... .......... .C........ ......G..A
R6546VP1    ...C...... ....T..... ......C... .......... .C........ ......G..A
R3776VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R4111VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R5009VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R982396VP   ...A...... .......... ......C... .......... .C........ .........A
R98239VP    ...A...... ....T...T. ......C... .......... .C........ .........A
R982865VP   A..A...... ....T..... ......C... ........C. .C........ .........A
R4703VP1    ...A...... ....T..... ......C... ........C. .C........ .........A
R6099VP1    ...A...... ....T..... ......C... ........C. .C........ .........A
R7283VP1    ...A...... ....T..... ...A..C... ........C. .C........ .........A
R5033VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R982813VP   ...A...... ...GT..... ......C... .......... .C........ .........A
R3588VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R982889VP   ...A.G.... ....T..... ......C... .......... .C........ .........A
R4487VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R982537VP   ...A...... ....T..... ......C... .......... .C........ .........A
R5584VP1    ...A...... ....T..... ......C... .......... .C........ .........A
R982643VP   ...A...... ....T..... ......C... .......... .C........ .........A
SABP2VP1    .C.A.T.... .C..T.G... .....GACAC A.G..G..C. .......C.. C.....G...

V9635VP1    ATTCCTGCGT TAACGGCAGT GGAGACCGGG GCCACCAACC CGTTGGTACC GTCTGACACA
R5205VP1    .......... .G........ .......... .......... .......G.. ...C......
R982466VP   .......... .G........ .......... .......... .......G.. ...C......
R7773VP1    .......... .G.....T.. .......... .......... .......G.. ...C......
R3628VP1    .......... .G........ .......... .......... .......G.. ...C......
R4908VP1    .......... .G........ .......... .......... .......G.. ...C......
R6546VP1    .......... .G........ .......... .......... .......G.. ...C......
R3776VP1    .......... .G........ .......... .......... .......G.. ...C......
R4111VP1    .......... .G........ .......... .......... .......G.. ...C......
R5009VP1    .......... .G........ .......... .......... .......G.. ...C......
R982396VP   .......... .G........ .......... .......... .......G.. ...C......
R98239VP    .......... .G........ .......... .......... .......G.. ...C......
R982865VP   .......... .G........ ......T... .......... .......G.. ...C..T...
R4703VP1    .......... .G........ ......T... .......... .A.....G.. ...C..T...
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R6099VP1     .....C.... .G........ A.......... .......... .......G.. ...C......
R7283VP1     .....C.... .G........ A.....T... .......... .......G.. ...C......
R5033VP1     .......... .G........ .......... .......... .......... ...C.....C
R982813VP    .......... .G........ .......... .......... .......G.. ...C.....G
R3588VP1     .......... .G........ .......... .......... .......G.. ...C......
R982889VP    .......... .G........ .......... .......... .......G.. ...C......
R4487VP1     .......... .G........ .......... .......... .......G.. ...C......
R982537VP    .......... .G........ .......... .......... .......G.. ...C......
R5584VP1     .......... .G........ .......... .......... .......G.. ...C......
R982643VP    .......... .G........ .......... .....T.... .......G.. ...C.....G
SABP2VP1     ..A.....A. .G..A..C.. ......A... ..T.....T. .......G.. T..G.....C

V9635VP1     GTGCAAACTA GGCACGTGAT TCAGAAACGT TCACGGTCTG AGTCCACTGT CGAGTCATTC
R5205VP1     .......... .......... .....G...C ..G.....C. .A........ ...A......
R982466VP    .......... .......... .........C ..G.....C. .......... ...A......
R7773VP1     .......... .......... .........C ..G..A..C. ....T..... ...A......
R3628VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R4908VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R6546VP1     .......... ....T..... .........C ..G..A..C. .......... ...A......
R3776VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R4111VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R5009VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R982396VP    .......... .......... .........C ..G..A..C. .......... ...A......
R98239VP     .......... .......... .......... ..G..A..C. .......... ...A......
R982865VP    .......... .......... .........C ..G..A..C. .......... ...A......
R4703VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R6099VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R7283VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R5033VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R982813VP    ........C. .......... .........C ..G..A.... .......... ...A......
R3588VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R982889VP    .......... .......... .........C ..G..A..C. .......... ...A......
R4487VP1     .......... .......... .........C ..G.....C. .......... ...A......
R982537VP    .......... .......... .........C ..G..A..C. .......... ...A......
R5584VP1     .......... .......... .........C ..G..A..C. .......... ...A......
R982643VP    .......... .......... .........C ..G..A..C. .......... ...A......
SABP2VP1     ........GC .C..T..C.. C....G...A A.G..A..A. .......G.. T.........

V9635VP1     TTTGCACGGG GTGCGTGTGT CGCAATAATC GAAGTGGACA ATGACGCACC AACAAAGAGA
R5205VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R982466VP    .......... .C..A..C.. .........T .......... .......... ...T......
R7773VP1     .......... .C..A..C.. .........T .......... .......... ...C......
R3628VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R4908VP1     .......... .C..A..C.. .........T .......... .C........ ...T......
R6546VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R3776VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R4111VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R5009VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R982396VP    .......... .C..A..C.. .........T .......... .......... ...T......
R98239VP     .......... .C..A..C.. .........T .......... .......... ...T...G..
R982865VP    .......... .C..A..C.. .........T .......... .......... ...T......
R4703VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R6099VP1     .......... .C..A..C.. .........T .......... .C........ ...T......
R7283VP1     .......... .C..A..C.. .........T .......... .C........ ...T......
R5033VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R982813VP    ........A. .C..A..C.. .........T .......... .......... ...T......
R3588VP1     .......... .C..A..C.. .........T .......... .......... ...T......
R982889VP    .......... ....A..C.. .........T .......... .......C.. ...T......
R4487VP1     .......... ....A..C.. .........T .......... .......T.. ...T....A.
R982537VP    .......... ....A..C.. .........T .......... .......T.. ...C......
R5584VP1     .......... ....A..C.. .........T .......... .......T.. ...C......
R982643VP    .......... ....A..C.. .........T .......... .......T.. ...C......
SABP2VP1     ......A.A. .G..T..C.. G..T..C..T ..G....... ....T..... G......C.C

V9635VP1     GCCAGCAAAT TATTCTCAGT GTGGAAGATC ACATATAAGG ACACCGTGCA ATTACGCCGC
R5205VP1     ........G. .......... .......... .....C..A. .......... G........T
R982466VP    ........G. .......... .......... .....C..A. .......... G........T
R7773VP1     .......... .G........ .......... ........A. .......... G........T
R3628VP1     .......... .......... .......... ........A. .......... G........T
R4908VP1     .......... .......... .......... ........A. .......... G........T
R6546VP1     .......... .......... .......... ........A. .......... G..G......
R3776VP1     .....T.... .......... .......... ........A. .......... G........T
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R4111VP1    .......... .......... .......... ........A. .......... G........T
R5009VP1    .......... .......... .......... ........A. .......... G........T
R982396VP   .......... .......... .......... ........A. .......... G........T
R98239VP    .......... .......... .......... ........A. .......... G........T
R982865VP   .......... .G........ .......... ..G.....A. .......... G.........
R4703VP1    .......... .G........ .......... ..G.....A. .......... G.........
R6099VP1    .......... .......... .......... ........A. .......... G.........
R7283VP1    .......... .......... .......... ........A. .......... G.........
R5033VP1    .......... .G........ .......... ........A. .......... G.....T..T
R982813VP   .......... .......... .......... ........A. .......... GC.......T
R3588VP1    .......... .......... .......... ........A. .......A.. G........T
R982889VP   .......... .......... .......... ........A. .......... G........A
R4487VP1    .......... .......... .......... ........A. .......... G........A
R982537VP   .......... .......... .......... ........A. .......... GC.......A
R5584VP1    .......... .......... .......... ........A. .......... GC.......A
R982643VP   .......... .......... .......... ........A. .......... GC.......A
SABP2VP1    .......G.. .G..T..G.. T.....A..A ..T..C..A. .T..T..T.. .C.GA.A...

V9635VP1    AAGCTGGAAT TCTTTACATA CTCCAGGTTT GACATGGAAT TCACTTTTGT GGTAACATCA
R5205VP1    ........G. .......... .........C .......... ....C..... ..........
R982466VP   .......... .......... .......... .......... ....C..... ..........
R7773VP1    ...T...... ....C..... .......... .......... ....C..... .........T
R3628VP1    .......... .......... .......... .......... ....C..C.. .........T
R4908VP1    .......... ....C..... .......... .......... ....C..C.. .........T
R6546VP1    .......... ....C..... .......... .......... ....C..... .........T
R3776VP1    .......... .......... .......... .......... ....C..C.. .........T
R4111VP1    .......... .......... .......... .......... ....C..C.. .........T
R5009VP1    .......... .......... .......... .......... ....C..C.. .........T
R982396VP   .....A.... .......... .......... .......... ....C..C.. .........T
R98239VP    .......... .......... .......... .......... ....C..C.. .........C
R982865VP   .......... .......... ...T...... .......... ....C..C.. .........T
R4703VP1    .......... .......... .......... .......... ....C..C.. .........T
R6099VP1    .......... ....C..... .......... .......... ....C..C.. .........T
R7283VP1    .......... .T..C..... .......... .......... ....C..C.. .........T
R5033VP1    .......... .......... .......... .......... ....C..C.. .........T
R982813VP   .......... .......... .......... ..T....... ....C..... .........T
R3588VP1    .......... .......... .......... .......... ....C..C.. .........T
R982889VP   .......... .......... .......... .......... ....C..... .........C
R4487VP1    .......... .......... .......... .......... ....C..... .........T
R982537VP   .......... .......... .......... .......... ....C..... .........T
R5584VP1    .......... .......... .......... .......... ....C..... .........T
R982643VP   .......... .......... .......... .......... ....C..... .........T
SABP2VP1    ..A....... .T..C..... T..G..A... ........G. .......... ...C..C...

V9635VP1    AACTACACAG ATGCCAACAA CGGACACGCT CTGAATCAAG TCTACCAAAT AATGTATATA
R5205VP1    ........G. ....A..... ......T... .......... .T..T..... ..........
R982466VP   .......... .......... ......T... .......... .T..T..... ..........
R7773VP1    .......... .......... ......T... .......... .T........ ..........
R3628VP1    .......... .......... ......T... .......... .T..T..... ......C...
R4908VP1    .......... .......... ......T... .......... .T..T..... ......C...
R6546VP1    .......... .......... ......T... .......... .T..T..... ......C...
R3776VP1    .......... .......... ......T... .......... .T..T..... ..........
R4111VP1    .......... .......... ......T... .......... .T..T..... ..........
R5009VP1    .......... .......... ......T... .......... .T..T..... ..........
R982396VP   .......... .......... ......T... .......... .T..T..... ..........
R98239VP    .......... .......... ......T... .......... .T..T..... ..........
R982865VP   .......... .......... ......T... .......... .T..T..... ......C...
R4703VP1    .......... .......... ......T... .......... .T..T..... ......C...
R6099VP1    .......... .......... ......T... .......... .T..T..... ......C..T
R7283VP1    .......... .......... ......T... .......... .T..T..... ......C..T
R5033VP1    .......... .......... ......T... .......... .T..T..... ..........
R982813VP   .......... .......... ......T... .......... .T..T..... ......C...
R3588VP1    .......... .......... ......T... .......... .T..T..... ..........
R982889VP   .......... .......... ......T... .......... .T..T..... ..........
R4487VP1    .......... .......... ......T... .......... .T..T..... ..........
R982537VP   .......... .......... ......T... .......... .T..T..... ..........
R5584VP1    .......... .......... ......T... .......... .T..T..... ..........
R982643VP   .......... .......... ......T... .......... .T..T..... ..........
SABP2VP1    .......TT. ....A..T.. ......T..A T....C.... .T..T..G.. ..........

V9635VP1    CCTCCAGGAG CACCCATCCC AGGAAAATGG AACGATTACA CATGGCAGAC CTCGTCTAAT
R5205VP1    .......... .T........ ......G... ..T....... .......... ...A.....C
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R982466VP  .......... .T........ ......G... ..T....... .......... ...A.....C
R7773VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R3628VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R4908VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R6546VP1   .......... .......... ......G... ..T....... .......... ...A..C..C
R3776VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R4111VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R5009VP1   .......... .......... ...G..G... ..T....... .......... ...A..C..C
R982396VP  .......... .......... ......G... ..T.....T. .......... ...A.....C
R98239VP   .......... .......T.. ......G... ..T....... .......... ...A.....C
R982865VP  .......... .......... ......G... ..T....... .......... ...A.....C
R4703VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R6099VP1   .....G.... .......... ......G... ..T....... .......... ...A.....C
R7283VP1   .....G.... .......... ......G... ..T....... .......... ...A.....C
R5033VP1   .......... .......... ......G... ..T....... .G........ ...A.....C
R982813VP  .......... .......... ......G... ..T....... .......... ...A.....C
R3588VP1   .......... .......... ......G... ..T.....T. .......... ...A.....C
R982889VP  .......... .......... ......G... ..T....... .......... ...A.....C
R4487VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R982537VP  .......... .......... ......G... ..T....... .......... ...A.....C
R5584VP1   .......... .......... ......G... ..T....... .......... ...A.....C
R982643VP  .......... .......... ......G... ..T....... .......... ...A.....C
SABP2VP1   ..A..C.... ....T..... T..T...... ..T..C..T. .G........ G..C.....C

V9635VP1   CCATCAGTGT TTTACACGTA TGGAGCCCCG CCAGCAAGGA TATCAGTGCC ATACGTAGGA
R5205VP1   ..G....... .......... .........A .......... ....T..... G........T
R982466VP  .......... .......... .........A .......... ....C..... G........T
R7773VP1   .......... .......... .........A .......... .T..C..... G..T.....T
R3628VP1   .......... .......... .........A .......... ....C..... G..T.....T
R4908VP1   .......... .......... .........A .......... ....C..... G..T.....T
R6546VP1   .......... .......... .........A .......... ....C..... G..T.....T
R3776VP1   .......... .......... .........A .......... ....C..... G..T.....T
R4111VP1   .......... .......... .........A .......... ....C..... G..T.....T
R5009VP1   .......... .......... .........A .....G.... ....C..... G..T......
R982396VP  .......... .......... .........A .......... ....C..... G..T.....T
R98239VP   ..G....... .......... .........A .......... ....C..... G..T.....T
R982865VP  .......... .......... .........A .......... ....C..... G..T.....T
R4703VP1   .......... .......... .........A .......... ....C..... ...T.....T
R6099VP1   .......... .......A.. .........A .......... ....C..... G..T.....T
R7283VP1   .......... .......A.. .........A .......... ....C..... G........T
R5033VP1   .......... .......... C.....T..A .......... ....C..... G..T.....T
R982813VP  .......... .......... .........A .......... ....C..... G..T.....T
R3588VP1   .......... .......... .......... .......... ....C..... G..T.....T
R982889VP  .......... .......... .........A .......... ....C..... ...T.....T
R4487VP1   .......... .......... .........A .......... ....C..... ...T.....T
R982537VP  .......... .......... .........A .......... ....C..... ...T.....T
R5584VP1   .......... .......... .........A .......... ....C..... ...T.....T
R982643VP  .......... .......... .........A .......... ....C..... ...T.....T
SABP2VP1   ..G..G.... .......C.. ...G..G..C ........A. .......... C.....G...

V9635VP1   ATTGCCAACG CTTACTCTCA TTTTTATGAT GGGTTTGCCA AGGTCCCATT AGCAGGTCAA
R5205VP1   .......... .C........ .........C .......... .......... G........G
R982466VP  .......... .C........ .........C .......... .......... G........G
R7773VP1   .......... .......... .........C .......... ..A....... G........G
R3628VP1   .......... .......... .........C .......... ..A....... G........G
R4908VP1   .......... .......... .......... .......... ..A....... G........G
R6546VP1   .......... .......... .........C ........T. G.A.T..... G........G
R3776VP1   .......... .......... .........C .......... ..A....... G........G
R4111VP1   .......... .......... .........C .......... ..A....... G........G
R5009VP1   .......... .......... .........C .......... ..A....G.. G........G
R982396VP  .......... .......... .........C .......... ..A....... G........G
R98239VP   ........T. .......... .........C .......... ..A....... G........G
R982865VP  ........T. .......... .........C .....C.... ..A....... G........G
R4703VP1   ........T. .......... .........C .....C.... ..A....... G........G
R6099VP1   .......... .......... .........C ..T....... ..A....... G........G
R7283VP1   .......... .......... .........C ..T....... ..A.T..... G........G
R5033VP1   .......... .......... .........C .......... ..A....... G........G
R982813VP  .......... .......... .........C .......... ..A....... G........G
R3588VP1   .......... .C........ .........C .......... ..A....... G........G
R982889VP  .......... .......... .........C .......... ..A....... G........G
R4487VP1   .......... .......... .........C .......... ..A....... G........G
R982537VP  .......... .......... .........C .......... ..A....... G........G
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R5584VP1     .......... .......... .........C .......... ..A....... G........G
R982643VP    .......... .......... .........C .......... ..A....... G........G
SABP2VP1     .....T..T. .G..T..C.. C......... ........A. .A..A...C. ...G......

V9635VP1     GCATCAACGG AGGGTGACTC TCTTTATGGG GCTGCTTCAC TAAACGATTT TGGGTCCCTC
R5205VP1     ........A. ....C..... .......... ..A....... ....T..... ..........
R982466VP    ........A. ....C..... .......... ..A....... ....T..... ..........
R7773VP1     ........A. .......... .......... .....C.... .......... ..........
R3628VP1     .......... .......... .......... .....C.... .......... .........T
R4908VP1     .......... .......... .......... .....C.... .......... .........T
R6546VP1     ...C...... .......... .......... .....C.... .......... .........T
R3776VP1     .......... .......... .......... .....C.... .......... ..........
R4111VP1     .......... .......... .......... .....C.... .......... ..........
R5009VP1     .......... .......... .......... .....C.... .......... ..........
R982396VP    .......... .......... .......... .....C.... .......... ..........
R98239VP     ........A. .......... .......... .......... .......... ..........
R982865VP    ........A. .......... ......C... .......... .......... ..........
R4703VP1     ........A. .......... ......C... .......... .......... ..........
R6099VP1     ........A. .A........ .......... .......... .......... ..........
R7283VP1     ........A. .A........ .......... .......... .......... ..........
R5033VP1     ........A. .......... .......... .......... .......... ..........
R982813VP    ........A. .......... .......... .......... .......... ..........
R3588VP1     ........A. .......... .......... .......... .......... ..........
R982889VP    ........A. .......... .......... .......... .......... ..........
R4487VP1     ........A. .......... .......... .......... .......... ..........
R982537VP    ........A. .......... .......... .......... .......... ......A...
R5584VP1     ........A. .......... .......... .......... .......... ......A...
R982643VP    ........A. .......... .......... .......... .......... ......A...
SABP2VP1     ..C.....T. .A..C..T.. GT.G..C..T .....C.... .G..T..... ...A..A..G

V9635VP1     GCTGTGCGTG TGGTGAATGA TCACAACCCA ACCAAGCTCA CGTCTAAGAT TAGAGTTTAT
R5205VP1     .......... .......... ...T..T... .......... .......... .........C
R982466VP    .......... .......... ...T..T... .......... .......... .........C
R7773VP1     ........C. .......C.. .......... .......... .A..C..... .........C
R3628VP1     .......... .......C.. .......... .......... .......... .........C
R4908VP1     .......... .......C.. .......... .......... .......... .........C
R6546VP1     ........C. .......C.. .......... .......... .......... .........C
R3776VP1     .......... .......C.. .......... .....A.... .......... .........C
R4111VP1     .......... .......C.. .......... .....A.... .......... .........C
R5009VP1     .......... .......C.. .......... .......... .......... .........C
R982396VP    .......... .......C.. .......... .......... .......... C........C
R98239VP     .......... .......C.. .......... .......... .......... .........C
R982865VP    .......... .......C.. .......... .......... .......... ..........
R4703VP1     .......... .......C.. .......... .......... .......... ..........
R6099VP1     .......... .......C.. .......... .......... .......... ..........
R7283VP1     .......... .......C.. .......... .......... .......... ..........
R5033VP1     .......... .......... .......... .......... .A........ .........C
R982813VP    .......... .......C.. .......... .......... .......... .........C
R3588VP1     .......... .......C.. .......... .......... .......... .........C
R982889VP    .......... .......C.. .......... .......... .......... .........C
R4487VP1     .......... .......C.. .......... .......... .......... .......C..C
R982537VP    .......... .......C.. .......... .......... .......... .........C
R5584VP1     .......... .......C.. .......... .......... .......... .........C
R982643VP    .......... .......C.. .......... .......... .......... .........C
SABP2VP1     .....T..C. ....A..... .........C ..GCG..... .C..C..... C.....G..C

V9635VP1     ATGAAACCCA AACACGTGAG AGTGTGGTGC CCGCGCCCCC CTCGTGCAGT CCCGTACTAT
R5205VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R982466VP    .....G.... .G........ .........T ..A..T..T. .......... ......T...
R7773VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R3628VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R4908VP1     .....G.... .G........ .........T ..A..T.... ......T... ......T...
R6546VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R3776VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R4111VP1     .....G.... .G........ .........T ..A..T.... .......... ......T...
R5009VP1     .....G..T. .G.....A.. .........T ..A..T.... .......... ......T...
R982396VP    .....G.... .G........ .........T ..A..T.... .......... ......T...
R98239VP     .....G.... .G........ .........T ..A.....T. .......... ......T...
R982865VP    .....G.... .G..T..... .........T ..A..T..T. .......... ......T...
R4703VP1     .....G.... .G..T..... .........T ..A..T..T. .......... ......T...
R6099VP1     .....G.... .G........ .........T ..A.....T. .......... ......T...
R7283VP1     .....G.... .G........ .........T ..A.....T. .......... ......T...
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R5033VP1     .....G.... .G..... ..... ..A..T..T. ..... T.....T...
R982813VP    .....G.... .G..... .....T ..A..T.... ..... ......T...
R3588VP1     .....G.... .G..... .....T ..A..T..T. ..... ......T...
R982889VP    .....G..T. .G..... .....T ..A..T..T. ..... ......T...
R4487VP1     .....G..T. .G..... .....T ..A..T..T. ..... ......T...
R982537VP    .....G..T. .G..... .....T ..A..T..T. ..... ......T...
R5584VP1     .....G..T. .G..... .....T ..A..T..T. ..... ......T...
R982643VP    .....G..T. .G..... .....T ..A..T..T. .....G.. ......T...
SABP2VP1     .....G..A. .G..T..C.. ...C..... ..A..A..T. .A..A..... ...A....TC

V9635VP1     GGACCGGGGG TTGACTACAA AGATGGGCTA ACCCCCTTAC CTGAGAAAGG GTTGACTACG
R5205VP1     ..... ..... ..... ..... .....G. .A..... ......C...
R982466VP    ..... ..... ..... ..... .....G. .A..... ......C...
R7773VP1     .....A.... ..... ..... ..... .....G. .A..... A.....A
R3628VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R4908VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R6546VP1     .....A..A. ..... ..... ..... .....G. .A..... .....
R3776VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R4111VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R5009VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R982396VP    .....A.... ..... ..... ..... .....G. .A..... .....
R98239VP     .....A.... ..... ..... ..... .....G. .A..... .....
R982865VP    .....A.... ..... ..... ..... .....G. .A..... ...A..C...
R4703VP1     .....A.... ..... ..... ..... .....G. .A..... ......C...
R6099VP1     .....A.... ..... ...C..... ..... .....G. .A..... ......C..A
R7283VP1     .....A.... ..... ...C..... ..... .....G. .A..... ......C..A
R5033VP1     .....A.... ..... ..... ..... .....G. .A..... .....
R982813VP    .....A.... ....T..... ..... ..... .....G. .A..... .....
R3588VP1     .....A..A. ..... ..... ..... .....G. .A..... .....
R982889VP    ..G..A..A. ..... ..... ..... .....G. .A..... .....
R4487VP1     ..G..A..A. ....T..... ..... ..... .....G. .A..... .....
R982537VP    ..G..A..A. ..... ..... ..... .....G. .A..... .....
R5584VP1     ..G..A..A. ..... ..... ..... .....G. .A..... .....
R982643VP    ..G..A..A. ..... ..... ..... .....G. .A..... .....
SABP2VP1     .....A..T. ....T..T.. .....C ..... .....AC... .A..A..G.. A..A..G..T

V9635VP1     TAC
R5205VP1     ...
R982466VP    ...
R7773VP1     ...
R3628VP1     ...
R4908VP1     ...
R6546VP1     ...
R3776VP1     ...
R4111VP1     ...
R5009VP1     ...
R982396VP    ...
R98239VP     ...
R982865VP    ...
R4703VP1     ...
R6099VP1     ...
R7283VP1     ...
R5033VP1     ...
R982813VP    ...
R3588VP1     ...
R982889VP    ...
R4487VP1     ...
R982537VP    ...
R5584VP1     ...
R982643VP    ...
SABP2VP1     ..T
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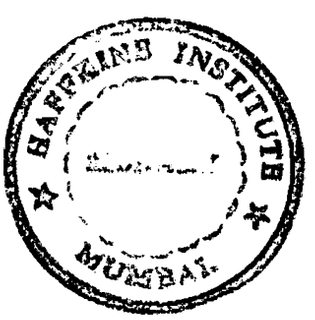

Figure 15: Pairwise genomic distances of complete nucleotide sequence of VP1 of wild poliovirus type 2 using Kimura 2 parameter method.

V9635VP1	0.0000	0.0720	0.0670	0.0796	0.0695	0.0658	0.0737	0.0796
	0.0709	0.0732	0.0734	0.0759	0.0734	0.0734	0.0786	0.0736	0.0709
	0.0771	0.0798	0.0784	0.0786	0.0837	0.0811	0.0708	0.0683	0.0709
	0.0709	0.0709	0.0709	0.0683	0.0796	0.0736	0.0771	0.0670	0.0822
	0.0771	0.0723	0.0787					
R3588VP1	0.0720	0.0000	0.0157	0.0249	0.0134	0.0123	0.0227	0.0249
	0.0226	0.0214	0.0203	0.0214	0.0192	0.0192	0.0379	0.0204	0.0238
	0.0249	0.0272	0.0295	0.0379	0.0331	0.0366	0.0157	0.0146	0.0238
	0.0238	0.0238	0.0238	0.0146	0.0249	0.0204	0.0203	0.0157	0.0249
	0.0203	0.0192	0.0250					
R3628VP1	0.0670	0.0157	0.0000	0.0249	0.0089	0.0056	0.0262	0.0249
	0.0067	0.0146	0.0226	0.0237	0.0214	0.0214	0.0403	0.0238	0.0284
	0.0249	0.0249	0.0157	0.0403	0.0308	0.0296	0.0180	0.0100	0.0284
	0.0284	0.0284	0.0284	0.0100	0.0249	0.0238	0.0203	0.0112	0.0249
	0.0203	0.0226	0.0285					
R3674VP1	0.0796	0.0249	0.0249	0.0000	0.0272	0.0237	0.0332	0.0022
	0.0319	0.0330	0.0296	0.0307	0.0284	0.0284	0.0475	0.0308	0.0355
	0.0022	0.0249	0.0389	0.0475	0.0284	0.0462	0.0249	0.0283	0.0355
	0.0355	0.0355	0.0355	0.0283	0.0022	0.0308	0.0318	0.0272	0.0067
	0.0318	0.0296	0.0355					
R3776VP1	0.0695	0.0134	0.0089	0.0272	0.0000	0.0033	0.0273	0.0272
	0.0157	0.0146	0.0226	0.0237	0.0214	0.0214	0.0403	0.0250	0.0284
	0.0272	0.0272	0.0249	0.0403	0.0331	0.0296	0.0180	0.0100	0.0284
	0.0284	0.0284	0.0284	0.0100	0.0272	0.0250	0.0203	0.0112	0.0272
	0.0203	0.0238	0.0296					
R4111VP1	0.0658	0.0123	0.0056	0.0237	0.0033	0.0000	0.0238	0.0237
	0.0123	0.0112	0.0192	0.0203	0.0180	0.0180	0.0367	0.0215	0.0249
	0.0237	0.0238	0.0214	0.0367	0.0296	0.0284	0.0146	0.0067	0.0249
	0.0249	0.0249	0.0249	0.0067	0.0237	0.0215	0.0191	0.0078	0.0237
	0.0191	0.0203	0.0261					
R4487VP1	0.0737	0.0227	0.0262	0.0332	0.0273	0.0238	0.0000	0.0332
	0.0320	0.0309	0.0321	0.0332	0.0309	0.0309	0.0477	0.0090	0.0333
	0.0332	0.0380	0.0368	0.0477	0.0440	0.0452	0.0273	0.0285	0.0333
	0.0333	0.0333	0.0333	0.0285	0.0332	0.0090	0.0297	0.0285	0.0356
	0.0297	0.0089	0.0135					
R4703VP1	0.0796	0.0249	0.0249	0.0022	0.0272	0.0237	0.0332	0.0000
	0.0319	0.0330	0.0296	0.0307	0.0284	0.0284	0.0475	0.0308	0.0355
	0.0022	0.0272	0.0389	0.0475	0.0308	0.0462	0.0249	0.0283	0.0355
	0.0355	0.0355	0.0355	0.0283	0.0022	0.0308	0.0318	0.0272	0.0067
	0.0318	0.0296	0.0355					
R4908VP1	0.0709	0.0226	0.0067	0.0319	0.0157	0.0123	0.0320	0.0319
	0.0000	0.0215	0.0296	0.0307	0.0284	0.0284	0.0462	0.0308	0.0355
	0.0319	0.0273	0.0180	0.0462	0.0331	0.0343	0.0249	0.0169	0.0355
	0.0355	0.0355	0.0355	0.0169	0.0319	0.0308	0.0272	0.0180	0.0319
	0.0272	0.0296	0.0355					
R5009VP1	0.0732	0.0214	0.0146	0.0330	0.0146	0.0112	0.0309	0.0330
	0.0215	0.0000	0.0284	0.0296	0.0272	0.0272	0.0463	0.0285	0.0343
	0.0330	0.0331	0.0284	0.0463	0.0390	0.0379	0.0237	0.0157	0.0343
	0.0343	0.0343	0.0343	0.0157	0.0330	0.0285	0.0284	0.0146	0.0331
	0.0284	0.0273	0.0332					
R5027VP1	0.0734	0.0203	0.0226	0.0296	0.0226	0.0192	0.0321	0.0296
	0.0296	0.0284	0.0000	0.0033	0.0011	0.0011	0.0451	0.0297	0.0308
	0.0296	0.0343	0.0390	0.0451	0.0403	0.0391	0.0215	0.0238	0.0308
	0.0308	0.0308	0.0308	0.0238	0.0296	0.0297	0.0284	0.0249	0.0296
	0.0284	0.0285	0.0332					
R5028VP1	0.0759	0.0214	0.0237	0.0307	0.0237	0.0203	0.0332	0.0307
	0.0307	0.0296	0.0033	0.0000	0.0022	0.0022	0.0463	0.0308	0.0320
	0.0307	0.0355	0.0401	0.0463	0.0414	0.0402	0.0237	0.0249	0.0320
	0.0320	0.0320	0.0320	0.0249	0.0307	0.0308	0.0295	0.0261	0.0307
	0.0295	0.0296	0.0344					
R5029VP1	0.0734	0.0192	0.0214	0.0284	0.0214	0.0180	0.0309	0.0284
	0.0284	0.0272	0.0011	0.0022	0.0000	0.0000	0.0439	0.0285	0.0296
	0.0284	0.0331	0.0378	0.0439	0.0390	0.0379	0.0214	0.0226	0.0296
	0.0296	0.0296	0.0296	0.0226	0.0284	0.0285	0.0272	0.0237	0.0284
	0.0272	0.0273	0.0320					

R5033VP1	0.0734	0.0192	0.0214	0.0284	0.0214	0.0180	0.0309	0.0284
0.0284	0.0272	0.0011	0.0022	0.0000	0.0000	0.0439	0.0285	0.0296
0.0284	0.0331	0.0378	0.0439	0.0390	0.0379	0.0214	0.0226	0.0296
0.0296	0.0296	0.0296	0.0226	0.0284	0.0285	0.0272	0.0237	0.0284
0.0272	0.0273	0.0320						
R5205VP1	0.0786	0.0379	0.0403	0.0475	0.0403	0.0367	0.0477	0.0475
0.0462	0.0463	0.0451	0.0463	0.0439	0.0439	0.0000	0.0477	0.0135
0.0475	0.0524	0.0534	0.0000	0.0561	0.0598	0.0391	0.0415	0.0135
0.0135	0.0135	0.0135	0.0415	0.0475	0.0477	0.0451	0.0427	0.0499
0.0451	0.0452	0.0525						
R5584VP1	0.0736	0.0204	0.0238	0.0308	0.0250	0.0215	0.0090	0.0308
0.0308	0.0285	0.0297	0.0308	0.0285	0.0285	0.0477	0.0000	0.0332
0.0308	0.0356	0.0344	0.0477	0.0416	0.0404	0.0250	0.0261	0.0332
0.0332	0.0332	0.0332	0.0261	0.0308	0.0000	0.0273	0.0261	0.0332
0.0273	0.0067	0.0044						
R5621VP1	0.0709	0.0238	0.0284	0.0355	0.0284	0.0249	0.0333	0.0355
0.0355	0.0343	0.0308	0.0320	0.0296	0.0296	0.0135	0.0332	0.0000
0.0355	0.0379	0.0426	0.0135	0.0415	0.0475	0.0273	0.0296	0.0000
0.0000	0.0000	0.0000	0.0296	0.0355	0.0332	0.0331	0.0308	0.0355
0.0331	0.0309	0.0380						
R5691VP1	0.0771	0.0249	0.0249	0.0022	0.0272	0.0237	0.0332	0.0022
0.0319	0.0330	0.0296	0.0307	0.0284	0.0284	0.0475	0.0308	0.0355
0.0000	0.0272	0.0389	0.0475	0.0308	0.0438	0.0249	0.0283	0.0355
0.0355	0.0355	0.0355	0.0283	0.0022	0.0308	0.0318	0.0272	0.0067
0.0318	0.0296	0.0355						
R6099VP1	0.0798	0.0272	0.0249	0.0249	0.0272	0.0238	0.0380	0.0272
0.0273	0.0331	0.0343	0.0355	0.0331	0.0331	0.0524	0.0356	0.0379
0.0272	0.0000	0.0366	0.0524	0.0056	0.0439	0.0272	0.0284	0.0379
0.0379	0.0379	0.0379	0.0284	0.0272	0.0356	0.0342	0.0272	0.0273
0.0342	0.0344	0.0403						
R6546VP1	0.0784	0.0295	0.0157	0.0389	0.0249	0.0214	0.0368	0.0389
0.0180	0.0284	0.0390	0.0401	0.0378	0.0378	0.0534	0.0344	0.0426
0.0389	0.0366	0.0000	0.0534	0.0402	0.0390	0.0342	0.0237	0.0426
0.0426	0.0426	0.0426	0.0237	0.0389	0.0344	0.0342	0.0226	0.0390
0.0342	0.0331	0.0391						
R6555VP1	0.0786	0.0379	0.0403	0.0475	0.0403	0.0367	0.0477	0.0475
0.0462	0.0463	0.0451	0.0463	0.0439	0.0439	0.0000	0.0477	0.0135
0.0475	0.0524	0.0534	0.0000	0.0561	0.0598	0.0391	0.0415	0.0135
0.0135	0.0135	0.0135	0.0415	0.0475	0.0477	0.0451	0.0427	0.0499
0.0451	0.0452	0.0525						
R7283VP1	0.0837	0.0331	0.0308	0.0284	0.0331	0.0296	0.0440	0.0308
0.0331	0.0390	0.0403	0.0414	0.0390	0.0390	0.0561	0.0416	0.0415
0.0308	0.0056	0.0402	0.0561	0.0000	0.0499	0.0331	0.0343	0.0415
0.0415	0.0415	0.0415	0.0343	0.0308	0.0416	0.0402	0.0331	0.0308
0.0402	0.0403	0.0464						
R7773VP1	0.0811	0.0366	0.0296	0.0462	0.0296	0.0284	0.0452	0.0462
0.0343	0.0379	0.0391	0.0402	0.0379	0.0379	0.0598	0.0404	0.0475
0.0438	0.0439	0.0390	0.0598	0.0499	0.0000	0.0390	0.0308	0.0475
0.0475	0.0475	0.0475	0.0284	0.0462	0.0404	0.0414	0.0319	0.0462
0.0414	0.0415	0.0451						
R98239VP	0.0708	0.0157	0.0180	0.0249	0.0180	0.0146	0.0273	0.0249
0.0249	0.0237	0.0215	0.0237	0.0214	0.0214	0.0391	0.0250	0.0273
0.0249	0.0272	0.0342	0.0391	0.0331	0.0390	0.0000	0.0191	0.0273
0.0273	0.0273	0.0273	0.0191	0.0249	0.0250	0.0249	0.0203	0.0249
0.0249	0.0215	0.0296						
R982396VP	0.0683	0.0146	0.0100	0.0283	0.0100	0.0067	0.0285	0.0283
0.0169	0.0157	0.0238	0.0249	0.0226	0.0226	0.0415	0.0261	0.0296
0.0283	0.0284	0.0237	0.0415	0.0343	0.0308	0.0191	0.0000	0.0296
0.0296	0.0296	0.0296	0.0022	0.0283	0.0261	0.0237	0.0033	0.0284
0.0237	0.0249	0.0308						
R982464VP	0.0709	0.0238	0.0284	0.0355	0.0284	0.0249	0.0333	0.0355
0.0355	0.0343	0.0308	0.0320	0.0296	0.0296	0.0135	0.0332	0.0000
0.0355	0.0379	0.0426	0.0135	0.0415	0.0475	0.0273	0.0296	0.0000
0.0000	0.0000	0.0000	0.0296	0.0355	0.0332	0.0331	0.0308	0.0355
0.0331	0.0309	0.0380						
R982466VP	0.0709	0.0238	0.0284	0.0355	0.0284	0.0249	0.0333	0.0355
0.0355	0.0343	0.0308	0.0320	0.0296	0.0296	0.0135	0.0332	0.0000
0.0355	0.0379	0.0426	0.0135	0.0415	0.0475	0.0273	0.0296	0.0000
0.0000	0.0000	0.0000	0.0296	0.0355	0.0332	0.0331	0.0308	0.0355
0.0331	0.0309	0.0380						
R982474VP	0.0709	0.0238	0.0284	0.0355	0.0284	0.0249	0.0333	0.0355

0.0355	0.0343	0.0308	0.0320	0.0296	0.0296	0.0135	0.0332	0.0000
0.0355	0.0379	0.0426	0.0135	0.0415	0.0475	0.0273	0.0296	0.0000
0.0000	0.0000	0.0000	0.0296	0.0355	0.0332	0.0331	0.0308	0.0355
0.0331	0.0309	0.0380						
R982489VP	0.0709	0.0238	0.0284	0.0355	0.0284	0.0249	0.0333	0.0355
0.0355	0.0343	0.0308	0.0320	0.0296	0.0296	0.0135	0.0332	0.0000
0.0355	0.0379	0.0426	0.0135	0.0415	0.0475	0.0273	0.0296	0.0000
0.0000	0.0000	0.0000	0.0296	0.0355	0.0332	0.0331	0.0308	0.0355
0.0331	0.0309	0.0380						
R982494VP	0.0683	0.0146	0.0100	0.0283	0.0100	0.0067	0.0285	0.0283
0.0169	0.0157	0.0238	0.0249	0.0226	0.0226	0.0415	0.0261	0.0296
0.0283	0.0284	0.0237	0.0415	0.0343	0.0284	0.0191	0.0022	0.0296
0.0296	0.0296	0.0296	0.0000	0.0283	0.0261	0.0237	0.0033	0.0284
0.0237	0.0249	0.0308						
R982510VP	0.0796	0.0249	0.0249	0.0022	0.0272	0.0237	0.0332	0.0022
0.0319	0.0330	0.0296	0.0307	0.0284	0.0284	0.0475	0.0308	0.0355
0.0022	0.0272	0.0389	0.0475	0.0308	0.0462	0.0249	0.0283	0.0355
0.0355	0.0355	0.0355	0.0283	0.0000	0.0308	0.0318	0.0272	0.0067
0.0318	0.0296	0.0355						
R982537VP	0.0736	0.0204	0.0238	0.0308	0.0250	0.0215	0.0090	0.0308
0.0308	0.0285	0.0297	0.0308	0.0285	0.0285	0.0477	0.0000	0.0332
0.0308	0.0356	0.0344	0.0477	0.0416	0.0404	0.0250	0.0261	0.0332
0.0332	0.0332	0.0332	0.0261	0.0308	0.0000	0.0273	0.0261	0.0332
0.0273	0.0067	0.0044						
R982813VP	0.0771	0.0203	0.0203	0.0318	0.0203	0.0191	0.0297	0.0318
0.0272	0.0284	0.0284	0.0295	0.0272	0.0272	0.0451	0.0273	0.0331
0.0318	0.0342	0.0342	0.0451	0.0402	0.0414	0.0249	0.0237	0.0331
0.0331	0.0331	0.0331	0.0237	0.0318	0.0273	0.0000	0.0249	0.0319
0.0000	0.0284	0.0296						
R982839VP	0.0670	0.0157	0.0112	0.0272	0.0112	0.0078	0.0285	0.0272
0.0180	0.0146	0.0249	0.0261	0.0237	0.0237	0.0427	0.0261	0.0308
0.0272	0.0272	0.0226	0.0427	0.0331	0.0319	0.0203	0.0033	0.0308
0.0308	0.0308	0.0308	0.0033	0.0272	0.0261	0.0249	0.0000	0.0272
0.0249	0.0249	0.0308						
R982865VP	0.0822	0.0249	0.0249	0.0067	0.0272	0.0237	0.0356	0.0067
0.0319	0.0331	0.0296	0.0307	0.0284	0.0284	0.0499	0.0332	0.0355
0.0067	0.0273	0.0390	0.0499	0.0308	0.0462	0.0249	0.0284	0.0355
0.0355	0.0355	0.0355	0.0284	0.0067	0.0332	0.0319	0.0272	0.0000
0.0319	0.0320	0.0379						
R982885VP	0.0771	0.0203	0.0203	0.0318	0.0203	0.0191	0.0297	0.0318
0.0272	0.0284	0.0284	0.0295	0.0272	0.0272	0.0451	0.0273	0.0331
0.0318	0.0342	0.0342	0.0451	0.0402	0.0414	0.0249	0.0237	0.0331
0.0331	0.0331	0.0331	0.0237	0.0318	0.0273	0.0000	0.0249	0.0319
0.0000	0.0284	0.0296						
R982889VP	0.0723	0.0192	0.0226	0.0296	0.0238	0.0203	0.0089	0.0296
0.0296	0.0273	0.0285	0.0296	0.0273	0.0273	0.0452	0.0067	0.0309
0.0296	0.0344	0.0331	0.0452	0.0403	0.0415	0.0215	0.0249	0.0309
0.0309	0.0309	0.0309	0.0249	0.0296	0.0067	0.0284	0.0249	0.0320
0.0284	0.0000	0.0112						
R982643VP	0.0787	0.0250	0.0285	0.0355	0.0296	0.0261	0.0135	0.0355
0.0355	0.0332	0.0332	0.0344	0.0320	0.0320	0.0525	0.0044	0.0380
0.0355	0.0403	0.0391	0.0525	0.0464	0.0451	0.0296	0.0308	0.0380
0.0380	0.0380	0.0380	0.0308	0.0355	0.0044	0.0296	0.0308	0.0379
0.0296	0.0112	0.0000						

Genomic relationship of wild type 2 poliovirus from India

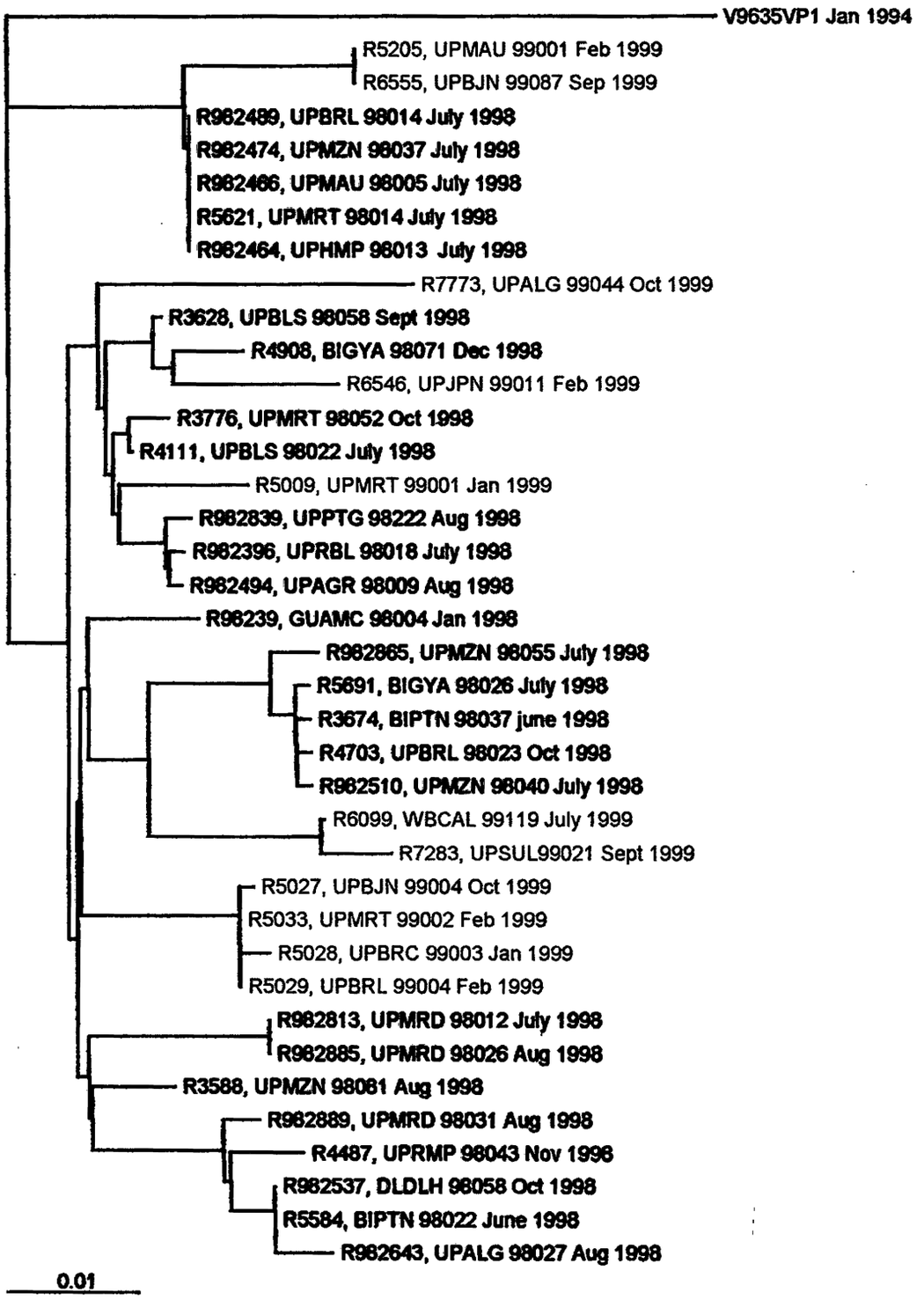

Figure 16: Phylogenetic relationship of wild poliovirus type 2 based on complete VP1 sequences. The dendrogram was drawn using NJ method of package PHYLIP v3.5c.

Figure 17: Amino acid sequences of VP1 protein of wild type 2 poliovirus isolates from India (1998-1999).

```
#V9635VP1  GLGDLLEGVI ESTTKNALVP LASTNNLPDT QPSGPAHSKE IPALTAVE
#R5205VP1  .......... .......... .S........ .......... ........
#R982466VP .......... .......... .T........ .......... ........
#R7773VP1  .......... .......... .TT....... .......... ........
#R3628VP1  .......... .......... .T........ .......... ........
#R4908VP1  ........M. .......... .P........ .......... ........
#R6546VP1  .......... .......... .P........ .......... ........
#R3776VP1  .......... .......... .T........ .......... ........
#R4111VP1  .......... .......... .T........ .......... ........
#R5009VP1  .......... .N........ .T........ .......... ........
#R982839VP .......... .......... .T........ .......... ........
#R98239VP  .......... .......... .T........ .......... ........
#R982865VP .......... .......... MT........ .......... ........
#R4703VP1  .......... .......... .T........ .......... ........
#R6099VP1  .......... .......... .T........ .......... ........
#R7283VP1  .......... .......... .T........ .......... ........
#R5027VP1  .......... .......... .T........ .......... ........
#R982813VP .......... .......... .T..S..... .......... ........
#R3588VP1  .......... .......... .T........ .......... ........
#R982889VP .......... .......... .T........ .......... ........
#R4487VP1  .......... .......I.. .T........ .......... ........
#R982537VP .......... .......... .T........ .......... ........
#R5584VP1  .......... .......... .T........ .......... ........
#R982643VP .......... .......... .T........ .......... ........
#SABP2VP1  .I..MI..AV .GI....... PT...S..GH K......... ........

#V9635VP1  TGATNPLVPS DTVQTRHVIQ KRSRSESTVE SFFARGACVA IIEVDNDA
#R5205VP1  .......... .......... R......... .......... ........
#R982466VP .......... .......... .......... .......... ........
#R7773VP1  .......... .......... .......... .......... ........
#R3628VP1  .......... .......... .......... .......... ........
#R4908VP1  .......... .......... .......... .......... ........
#R6546VP1  .......... .......... .......... .......... ........
#R3776VP1  .......... .......... .......... .......... ........
#R4111VP1  .......... .......... .......... .......... ........
#R5009VP1  .......... .......... .......... .......... ........
#R982839VP .......... .......... .......... .......... ........
#R98239VP  .......... .......... .......... .......... ........
#R982865VP .......... .......... .......... .......... ........
#R4703VP1  .......... .......... .......... .......... ........
#R6099VP1  .......... .......... .......... .......... ........
#R7283VP1  .......... .......... .......... .......... ........
#R5027VP1  .......... .......... .......... .......... ........
#R982813VP .......... .......... .......... .......... ........
#R3588VP1  .......... .......... .......... .......... ........
#R982889VP .......... .......... .......... .......... ........
#R4487VP1  .......... .......... .......... .......... ........
#R982537VP .......... .......... .......... .......... ........
#R5584VP1  .......... .......... .......... .......... ........
#R982643VP .......... .......... .......... .......... ........
#SABP2VP1  .......... .......... R.T....... .......... ........

#V9635VP1  PTKRASKLFS VWKITYKDTV QLRRKLEFFT YSRFDMEFTF VVTSNYTD
#R5205VP1  .......... .......... .......... .......... ........
#R982466VP .......... .......... .......... .......... ........
#R7773VP1  .......... .......... .......... .......... ........
#R3628VP1  .......... .......... .......... .......... ........
#R4908VP1  .......... .......... .......... .......... ........
#R6546VP1  .......... .......... .......... .......... ........
#R3776VP1  .......... .......... .......... .......... ........
#R4111VP1  .......... .......... .......... .......... ........
#R5009VP1  .......... .......... .......... .......... ........
#R982839VP .......... .......... .......... .......... ........
#R98239VP  ...G...... .......... .......... .......... ........
#R982865VP .......... .......... .......... .......... ........
#R4703VP1  .......... .......... .......... .......... ........
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#R6099VP1  .......... .......... .......... .......... ........
#R7283VP1  .......... .......... .......... .......... ........
#R5027VP1  .......... .......... .......... .......... ........
#R982813VP .......... .......... .......... .......... ........
#R3588VP1  .......... .......... .......... .......... ........
#R982889VP .......... .......... .......... .......... ........
#R4487VP1  ...K...... .......... .......... .......... ........
#R982537VP .......... .......... .......... .......... ........
#R5584VP1  .......... .......... .......... .......... ........
#R982643VP .......... .......... .......... .......... ........
#SABP2VP1  ......R... .......... .......... .......... ......I.

#V9635VP1  ANNGHALNQV YQIMYIPPGA PIPGKWNDYT WQTSSNPSVF YTYGAPPA
#R5205VP1  .......... .......... .......... .......... ........
#R982466VP .......... .......... .......... .......... ........
#R7773VP1  .......... .......... .......... .......... ........
#R3628VP1  .......... .......... .......... .......... ........
#R4908VP1  .......... .......... .......... .......... ........
#R6546VP1  .......... .......... .......... .......... ........
#R3776VP1  .......... .......... .......... .......... ........
#R4111VP1  .......... .......... .......... .......... ........
#R5009VP1  .......... .......... .......... .......... ........
#R982839VP .......... .......... .......... .......... ........
#R98239VP  .......... .......... .......... .......... ........
#R982865VP .......... .......... .......... .......... ........
#R4703VP1  .......... .......... .......... .......... ........
#R6099VP1  .......... .......... .......... .......... ........
#R7283VP1  .......... .......... .......... .......... ........
#R5027VP1  .......... .......... .......... .......... ........
#R982813VP .......... .......... .......... .......... ........
#R3588VP1  .......... .......... .......... .......... ........
#R982889VP .......... .......... .......... .......... ........
#R4487VP1  .......... .......... .......... .......... ........
#R982537VP .......... .......... .......... .......... ........
#R5584VP1  .......... .......... .......... .......... ........
#R982643VP .......... .......... .......... .......... ........
#SABP2VP1  .......... .......... .......... .......... ........

#V9635VP1  RISVPYVGIA NAYSHFYDGF AKVPLAGQAS TEGDSLYGAA SLNDFGSL
#R5205VP1  .......... .......... .......... .......... ........
#R982466VP .......... .......... .......... .......... ........
#R7773VP1  .......... .......... ..I....... .......... ........
#R3628VP1  .......... .......... ..I....... .......... ........
#R4908VP1  .......... .......... ..I....... .......... ........
#R6546VP1  .......... .......... .RI......P .......... ........
#R3776VP1  .......... .......... ..I....... .......... ........
#R4111VP1  .......... .......... ..I....... .......... ........
#R5009VP1  .......... .......... ..I....... .......... ........
#R982839VP .......... .......... ..I....... .......... ........
#R98239VP  .......... .......... ..I....... .......... ........
#R982865VP .......... .......... ..I....... .......... ........
#R4703VP1  .......... .......... ..I....... .......... ........
#R6099VP1  .......... .......... ..I....... .......... ........
#R7283VP1  .......... .......... ..I....... .......... ........
#R5027VP1  .......... .......... ..I....... .......... ........
#R982813VP .......... .......... ..I....... .......... ........
#R3588VP1  .......... .......... ..I....... .......... ........
#R982889VP .......... .......... ..I....... .......... ........
#R4487VP1  .......... .......... ..I....... .......... ........
#R982537VP .......... .......... ..I....... .......... ........
#R5584VP1  .......... .......... ..I....... .......... ........
#R982643VP .......... .......... ..I....... .......... ........
#SABP2VP1  .......... .......... ..I....... .......... ........

#V9635VP1  AVRVVNDHNP TKLTSKIRVY MKPKHVRVWC PRPPRAVPYY GPGVDYKD
#R5205VP1  .......... .......... .......... .......... ........
#R982466VP .......... .......... .......... .......... ........
#R7773VP1  .......... .......... .......... .......... ........
#R3628VP1  .......... .......... .......... .......... ........
#R4908VP1  .......... .......... .......... .....V.... ........
#R6546VP1  .......... .......... .......... .......... ........
#R3776VP1  .......... .......... .......... .......... ........
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#R4111VP1  .......... .......... .......... .......... ........
#R5009VP1  .......... .......... .......... .......... ........
#R982839VP .......... .......... .......... .......... ........
#R98239VP  .......... .......... .......... .......... ........
#R982865VP .......... .......... .......... .......... ........
#R4703VP1  .......... .......... .......... .......... ........
#R6099VP1  .......... .......... .......... .......... ........
#R7283VP1  .......... .......... .......... .......... ........
#R5027VP1  .......... .......... .......... .......... ........
#R982813VP .......... .......... .......... .......... ........
#R3588VP1  .......... .......... .......... .......... ........
#R982889VP .......... .......... .......... .......... ........
#R4487VP1  .......... .......... .......... .......... ........
#R982537VP .......... .......... .......... .......... ........
#R5584VP1  .......... .......... .......... .......... ........
#R982643VP .......... .......... .......... .......... ........
#SABP2VP1  .......... .R........ .......... .........F ........

#V9635VP1  GLTPLPEKGL TTY
#R5205VP1  .......... ...
#R982466VP .......... ...
#R7773VP1  .......... ...
#R3628VP1  .......... ...
#R4908VP1  .......... ...
#R6546VP1  .......... ...
#R3776VP1  .......... ...
#R4111VP1  .......... ...
#R5009VP1  .......... ...
#R982839VP .......... ...
#R98239VP  .......... ...
#R982865VP .......... ...
#R4703VP1  .......... ...
#R6099VP1  .......... ...
#R7283VP1  .......... ...
#R5027VP1  .......... ...
#R982813VP .......... ...
#R3588VP1  .......... ...
#R982889VP .......... ...
#R4487VP1  .......... ...
#R982537VP .......... ...
#R5584VP1  .......... ...
#R982643VP .......... ...
#SABP2VP1  .......... ...
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Phylogenetic analysis of wild type 3 poliovirus from Mumbai

A total of 96 wild type 3 poliovirus isolates were sequenced in the VP1\2A junction region of viral RNA to determine the phylogenetic relationship. Out of 96 isolates 30 belonged to the period from 1972 to 1989 (Table 6) and 66 belonged to period between 1992 and 2000 (Table 7). All the sequences were compares with the Sabin type 3 poliovirus vaccine strain for the confirmation of wild character. The aligned sequences are present in Figure 18. Pairwise genetic distances were calculated by Kimura 2 parameter method with transition vs. transversion ration of 10 is shown in Figure 19. Phylogenetic tree was constructed using Neighbor-joining method.

Poliovirus type 3 isolates 1972- 1989:

Phylogenetic relationship of the 30 isolates of years 1971 to 1986 is presented in Figure 20. All the strains grouped into three major clusters with more than 15% sequence divergence, therefore indicating circulation of three genotypes of poliovirus type 3. The genotype A was isolated during 1972 to 1979; the earliest isolate of this group being V55 (S ward, 1972). Genotype B transmission occurred between 1979 and 1986. Isolates V1067 (M ward, 1979) and V1130 (F ward, 1979) provide evidence of co-circulation of genotypes A and B. Genotype C (V5222, N ward, 1985) circulation was observed after 1985. Thus during the mid-1980s also two genotypes co-circulated in Mumbai.

As the wild type 3 poliovirus infections were highly endemic in the city due to low population immunity, each virus isolate could continue transmission over a long period of time and continuously diverged resulting in formation of genetic lineages (> 5% genetic distance). poliomyelitis cases due to viruses of multiple lineages were observed in each genotype.

Poliovirus type 3 isolates 1992- 2000:

Genetic relationship of wild type 3 poliovirus isolated from year 1992 to year 2000 is presented in Figure 21 along with the isolates from 1971 to 1986. out of the poliovirus type 3 isolates available for this period all but 5 were sequenced. As can be seen from Figure 21 the isolates of years 1992 to 2000 represent a single genotype. It was also found that these isolates were related to the previously isolated viruses representing genotype B referred to in Figure 20. within the genotype B the isolates formed clusters of closely similar viruses. Multiple lineages were circulating in certain smaller geographical areas (municipal wards). Three lineages were detected in the year 1992 from P ward (V9193), M ward (V9222) and N ward (V9267).

At least six clusters were identified each having a limited period o transmission between 1992-1995. sequence analysis could define continued presence of individual genetic lineage. Four lineages were detected between 1993-95 from H ward (V9476, 1993), S ward (V9517, 1993), P ward (V9694, 1994) and H ward (V9939, 1995). The virus of the lineage from H ward (1993) spread to different wards resulting in as many as eight paralytic cases . moreover an isolate (V9939) of completely different genetic lineage (9% sequence divergence) was isolated from H ward in the year 1995 inferring thereby its non indigenous transmission. Of particular interest is the lineage that continued to cause paralytic cases from 1992-1996. the lineage was first detected in N ward in the year 1992. In the year 1993 the lineage was detected in F, H, K and R ward and in A, H, P, M and R wards in 1994-95. sequence analysis provided the evidence that the last poliomyelitis case due to indigenous wild poliovirus type 3 in Mumbai was in N ward in the year 1996 (V10177).

However al least three cases of wild poliovirus type 3 etiology were detected in Mumbai in the years 1998-2000. wild poliovirus type 3 was not isolated from any paralytic case in the year 1997. the three isolates belong to three different genetic lineages not detected earlier in the environment

in Mumbai. These results indicate "importation" of wild virus from other endemic areas in the country

As can be observed from the Figure 21, the two isolates from Jamnagar, Gujarat (V10075 and V10076) formed a separate cluster in genotype B. the results show that the genotype B circulated in wide geographic area. The virus diverging to form independent lineages. On the other hand sequence similarity of the virus isolates from the two cases, one each from Dhule district (V10081) and Pune district of Maharashtra (V10087), shows close epidemilogic link with viruses in Mumbai.

Paralytic poliomyelitis cases not only from other districts of Maharashtra but also from other states especially Utter Pradesh is brought to Mumbai for medical treatment. In the year 1997 although there was no case of paralytic poliomyelitis due to poliovirus type 3 infection among residents of Mumbai, at least four cases from non residents were reported from city hospitals. Sequence analysis clearly shows that the two cases from Thane (97-118 and 97-144) were infected by virus of the same genetic make up. This virus lineage caused at least one more case in the district in the year 1998. virus isolates from the two up cases (97-120 and 97-127) were belonged to two separate lineages indicating presence of multiple lineages in Utter Pradesh

In the year 2000 four cases of wild poliovirus type 3 etiology were detected in the Bhiwandi, Thane district. Sequences of these isolates showed more than 98% sequence similarity with isolates from Basti, Utter Pradesh. These data clearly show the value of genetic studies in proving epidemilogic linkage between poliomyelitis cases.

Amino acid sequences of the wild poliovirus type 3 in the VP1/2A region were deduced from the nucleotide sequences and are shown in Figure 22. All wild virus isolates showed four amino acid differences viz. arginine to lysine at 285th , asparagine to aspartic acid at 286th, asparagine to glycine at 287th and aspartic acid to alanine at the 289th positions VP1 compared to Sabin poliovirus type 3 vaccine strain. Nine isolates are

showing phenylalanine to leucine change at the 2nd amino acid in the 2A region and three isolates are showing glycine to glutamic acid change at the 3rd amino acid in the 2A region

Table 6: The wild type 3 isolates from 1971 to 1989 isolated from Mumbai studied in the VP1/2A junction of viral RNA to establish the phylogenetic relationship.

Sr. No.	Virus No.	Month and year if isolation	Ward
1	V55	January 1972	S
2	V335	February 1973	K
3	V445	January 1973	K
4	V512	October 1973	G
5	V583	February 1974	F
6	V787	November 1975	Thane
7	V802	1975	Not known
8	V861	July 1977	H
9	V904	June 1978	G
10	V963	October 1978	S
11	V1007	1979	Not known
12	V1067	January 1979	M
13	V1087	1979	Not known
14	V1130	December 1979	F
15	V1201	1980	Not known
16	V1202	1980	Not known
17	V1725	February 1981	H
18	V1825	1981	Not known
19	V2820	1982	N
20	V2843	1982	R
21	V3104	September 1983	L
22	V3458	1983	Not known
23	V3757	February 1984	G
24	V5222	December 1985	N
25	V5228	December 1985	M
26	V5399	December 1986	H
27	V5402	December 1986	Jonpur, UP
28	V7202	November 1987	L
29	V7210	September 1987	P
30	V8188	1989	Ajmer,

Table 7: Wild type 3 poliovirus isolates from year 1971 to 1989 studied in the VP1/2A region of viral RNA to establish the phylogenetic relationship between these isolates.

Sr.No.	Virus No.	Moth and year of isolation	Ward
1	V9193	June 1992	P
2	V9222	June 1992	M
3	V9264	November 1992	R
4	V9267	November 1992	N
5	V9274	December 1992	F
6	V9275	December 1992	N
7	V9463	January 1993	H
8	V9464	January 1993	N
9	V9467	February 1993	L
10	V9473	March 1993	K
11	V9476	April 1993	H
12	V9477	May 1993	R
13	V9482	June 1993	P
14	V9488	July 1993	K
15	V9492	July 1993	P
16	V9517	August 1993	S
17	V9518	August 1993	P
18	V9622	October 1993	Not known
19	V9647	February 1994	P
20	V9694	June 1994	P
21	V9707	June 1994	F
22	V9708	June 1994	R
23	V9750	July 1994	R
24	V9683	June 1994	K
25	V9736	July 1994	R
26	V9738	July 1994	S
27	V9739	July 1994	G
28	V9742	July 1994	S
29	V9743	July 1994	K
30	V9792	September 1994	M
31	V9939	March 1995	H
32	V9942	March 1995	R
33	V9943	April 1995	M
34	V9944	April 1995	F
35	V9664	April 1995	S
36	V9952	May 1995	E
37	V9961	July 1995	H
38	V9963	July 1995	S
39	V9972	August 1995	P

Sr.No.	Virus No.	Moth and year of isolation	Ward
40	V9973	August 1995	M
41	V9975	August 1995	P
42	V9984	August 1995	A
43	V9983	August 1995	M
44	V10003	August 1995	G
45	V10075	August 1995	Jamnagar
46	V10076	August 1995	Jamnagar
47	V10000	September 1995	M
48	V10081	October 1995	Dhule
49	V10094	November 1995	Surat
50	V10087	November 1995	Pune
51	V10138	December 1995	Not known
52	V10177	January 1996	N
53	97-120	September 1997	UP
54	97-127	September 1997	UP
55	97-118	September 1997	Thane
56	97-144	October 1997	Thane
57	R98-533	January 1998	Thane
58	R98-537	January 1998	Thane
59	R98-543	January 1998	E
60	R98-517	January 1998	Thane
61	R-5755	June 1999	H
62	R-9952	March 2000	Thane
63	R-10151	April 2000	H
64	R10745	August 2000	Thane
65	R10996	October 2000	Thane
66	R11290	November 2000	Thane

Figure 18: Nucleotide sequence alignment of the VP1/2A junction region of wild poliovirus type 3 isolated from Paralytic poliomyelitis cases from Mumbai city during 1971 to 2000.

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V55     CGACCTCCGC GGGCAGTACC GTATTACGGG CCAGGGGTGG ATTACAAGGA TGGGTTGGCA
V335    .......... .A........ .......... .......... .......... ..........
V445    .......... .......G.. .........A .......... .C........ ..........
V512    .......... .......... .........A .......... .......... ..........
V583    .......... .......... .......... .......... .......... ...A...A..
V787    .......... .......G.. .........A .......... .......... .........G
V802    .......... .......... .......... .......... .......... ...A......
V861    ..C....... .A.....G.. .......... .......... .C........ ......A..G
V904    ........A. .A.....G.. .........A .......... .......... .........G
V963    ........A. .A.....G.. .........A .......... .......... .........G
V1007   .......... .......G.. .......... .......... .......... .........G
V1067   .......... .......G.. .......... .......... .......... ......A..G
V1087   .....C.... .......G.. .........A ..G....... .......... .........G
V1130   ..G..C.... .......G.. ...C.....A ..G.....A. .C........ .........G
V1201   .....C..C. .......G.. ...C...... ..G.....A. .C........ ....C....G
V1202   .....C.... .......G.. ...C.....A ..G.....A. .C........ .........G
V1725   .....C.... .......G.. ...C.....A ..G.....A. .C........ .........G
V1825   .....C.... .......G.. A..C..T..A ..G.....A. .C........ .........G
V2820   .....C.... .......G.. A..C.....A ..G.....A. .C........ .........G
V2843   .....C.... .......G.. A..C.....A ..G.....A. .C........ .........G
V3104   .....C.... .......G.. A..C.....A .......... .C........ .........G
V3458   .....C.... .......G.. A..C.....A ..G.....A. .C........ .........G
V3757   .....C...A .......G.. ...C.....A ..G.....A. .C........ .........G
V5222   ..T..C.... .......G.. .........A ..G..A..A. .C.....A.. ..........
V5399   .....C.... .......G.. A..C...... ..G....... .C........ C.........
V5402   ..G..C..A. .A..G..G.. ...C.....A ..G.....A. .C........ ...T.....G
V7202   ..T..C.... .......... ......T..A ..G..A..A. .C.....A.. ..........
V7210   ..G..C.... .A..C..G.. ...C.....A ..G.....A. .C........ ...T......
V8188   ..T..C.... .......G.. .........A ..G..A..A. .C.....A.. ..........
V5228   .....C.... ....T..G.. A..C.....A .......... .C........ ......A..G
V9193   .....C..A. .A.....G.. A..C..T..A ..G.....T. .C........ ...T.....G
V9222   .....C..A. .A........ A..C.....A ..G..A..A. .C........ ...T.....G
V9267   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9274   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9275   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9476   ..G..C..A. .A........ A..C..T..A ..G....... .C........ ...T..A..G
V9477   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9488   .....C..A. .A........ A..C.....A ..G....... .C.....A.. ...T.....G
V9464   ..G..C..A. .A........ A..C..T..A ..G....... .C........ ...T..A..G
V9463   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9482   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9492   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9473   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9517   ..G..C..A. .A........ A..C.....A ..G.....A. .C........ ...T......
V9518   ..G..C..A. .A........ A........A ..G....... .C........ ...T......
V9647   .....C.... .A........ A..C.....A ..G.....A. .......... ...C...A.G
V9694   .....C..A. .A.....G.. A........A ..G....... .C........ C..A.....G
V9683   ..G..C..A. .A........ A..C..T..A ..G....... .C........ ...T..A..G
V9622   .....C..A. .A........ A..C.....A ..G.....A. .C........ ...T.....G
V9750   ..G..C..A. .A........ A..C..T..A ..G....... .C........ ...T..A..G
V9792   .....C..A. .A.....C.. A..C...... ..G....... .C........ C..A..A..G
V9707   ..G..C..A. .A........ A..C..T..A ..G....... .C........ ...T..A..G
V9984   .....C..A. .A........ A..C.....A ..G....... .C........ ...T.....G
V9983   .....C..A. .A.....G.. T..C...... ..G....... .......... ...A.....G
V9939   ..G..C..A. .A........ A..C.....A .......... .C........ ...T..A..G
V9943   .....C..A. .A........ A..C.....A ..G....... .C........ ...T.....G
V9942   .....C..A. .A........ A..C.....A ..G....... .C........ ...T.....G
V9975   .....C..A. .A........ A..C.....A ..G....... .C........ ...C.....G
V9944   .....C..A. .A.....G.. T..C...... ..G....... .......... ...A.....G
V9973   .....C..A. .A........ A..C.....A ..G....... .C........ ...C.....G
V9952   .....C..A. .A.....G.. A..C.....A ..G....... .C........ C..A.....G
V9961   ..G..C..A. .A........ A..C.....A ..G....... .C........ ...T.....G
V9963   .....C..A. .A.....G.. T..C...... ..G....... .......... ...A.....G
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V9972      .....C..A. .A..... A..C.....A ..G..... .C..... ...T.....G
V10081     .....C..A. .A.....G.. A..C.....A ..G..A.... .C..... ...T.....G
V10000     .....C..A. .A.....G.. A..C.....A ..G..... .C..... ..AC....AG
V10003     .....C..A. .A..... A..C.....A ..G..... .C......A. .....G
V10075     ..G..C..A. .T..... A..C..T..A ..G.....A. .C..... ...T.....G
V10138     .....C..A. .A.....G.. T..C..... ..G..... ..... ...A.....G
V10177     .....C..A. .A..... A..C.....A ..G..... .C..... ...T.....G
V10076     ..G..C..A. .T..... A..C..T..A ..G.....A. .C..... ...T.....G
97-144     ..G..C.... .A..... A.....A ..... .C..... ...T..A..G
97-120     ..G.....A. .A..G..G.. A..C.....A ..... .C..... ...T..A..G
97-127     .....C..A. .A.....G.. A.....A .....A. .C..T..... ...A.....
97-118     ..G..C.... .A..... A.....A ..... .C..... ...T..A...
R98533     ..G.....A. .A..G..... A..C.....A ..... .C..... ...T..A..G
R98537     ..G..C.... .A..... A.....A ..... .C..... ...T..A...
R98517     ..G.....A. .A..G..... A..C.....A ..... .C..... ...T..A..G
R5755      ..G..C..A. .A..G..... A..C.....A ..T..... ..... ...C..AC.G
R9952      ..G..C..A. .A..G..G.. A..C.....A ..... .C..... ...T..A..G
RI0151     ..G..C.... .A..G..... A..C.....A ..... ..... ...T..A..G
R10745     ..G..C..A. .A..G..G.. A..C.....A ..... .C..... ...T..A..G
E00111     ..G..C..A. .A..G..G.. A..C.....A ..... .C..... ...A.....G
R10996     ..G..C..A. .A..G..G.. A..C.....A ..... .C..... ...T..A..G
R98543     ..G..C..A. .A..G..G.. A..C.....T ..... .C..... ...T..A..G
SABIN3     A....G.... .C..G..... T.....T..A ..... .C..T.G.A. CAAC....AC

V55        CCTTTGTCAG AGAAGGGACT TACAACCTAT GGGTTTGGAC ACCAGAACAA AGCAGTGTAC
V335       .....  .....  .....  ..A..  .....  .....
V445       .....A....  .....  C..C.....  .....C....  .....  .....
V512       .....A..G.  .....T..  C..T.....  .....C....  .....  G.....
V583       ..CC.....  .....  .....  .....  .....T..  .....
V787       .....  .....  ...C.....  ...C.....  .....  .....
V802       ..CC.....  .....  .....  .....  .....T..  .....
V861       ..C..A....  .A.....  ...T.....  .....  .....  .....
V904       .....A..G.  .....  ...C.....  .....  .....  ......A...
V963       .....A..G.  .....  ...C.....  .....  .....  ......A...
V1007      .....A....  .....  ...C..G...  .....C....  .....  .....
V1067      .....A....  .....  ...C.....  .....C....  .....  .....
V1087      ...C.A..G.  .....  ...C..T...  .....G.  .....  ......A...
V1130      ..C..A..G.  .....T..  ...C..T...  .....  .....T..  .....T
V1201      ..C..A..G.  .....T..  .....T...  .....G.  ....A....  .....T
V1202      ..C..A..G.  .....T..  C..T..T...  .....  .....  .....T
V1725      ..C..A..G.  .....T..  C..T..T...  .....  .....  .....T
V1825      ..C..A....  .....T..  ...C..T..C  .....  .....T..  .....T
V2820      ..C..A..G.  .....T..  ...C.....C  .....  ...G...T..  .....T
V2843      ..CC.A..G.  .....T..  ...C..T..C  .....  .....T..  .....T
V3104      ..CC.A..G.  .....T..  ...C..T..C  .....  .....  .....T
V3458      ..C..A..G.  .....A.T..  ...C..T..C  .....G.  .T.....T..  G.....T
V3757      .....A....  .....T..  ...C..T..C  .....  .....T..  .....T
V5222      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  ...T..C..T
V5399      ..CC.A..G.  .A.....T..  C..C..T..C  .....  ....A..T..  G.....T
V5402      ..C..A..G.  ....A..G..  C..C..T...  ..A.....  .....  G.....T
V7202      ..C..A..G.  .....  ...C..T...  ..A.....  ....A.....  ...T..C..T
V7210      ..C..A..G.  ....A..G..  C..C.....  ..A.....  .T.....  G.....T
V8188      ..C.....G.  .....  C..C..T...  ..A.....  ....A.....  ...T..C...
V5228      ..C..A....  .....G..  C..C..T...  ..A.....  .....  G.....T
V9193      ..C..A..G.  ....A.....  C..C..T...  ..A.....  .....  G.....T
V9222      ..C..A..G.  .....  C..C.....  ..A..C....  .....  .....
V9267      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  ...T.....
V9274      ..C..AG.G.  .....  ...C..T...  ..A.....  ....A.....  ...T.....
V9275      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  ...T.....
V9476      ..C..A....  .A.....  C..C..T...  ..A.....  .....  G.....T
V9477      ..C..A..G.  .....  C..C..T...  ..A.....  ....T.....  ...T.....
V9488      ..C..A..G.  .....  C..C.....  ..A.....  ....A.....  ...T.....
V9464      ..C..A....  .A.....G..  C..C..T...  ..A.....  .....  G.....T
V9463      ..C..AG.G.  .....  ...C..T...  ..A.....  ....A.....  ...T.....
V9482      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  ...T.....
V9492      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  .....
V9473      ..C..A..G.  .....  C..C..T...  ..A.....  ....A.....  ...T.....
V9517      ..C..A..G.  .....G..  C..C..T...  ..A..C..G.  .....  G.....T
V9518      ..C..A..G.  .....G..  ...C..T...  ..A..C..G.  .....  G.....T
V9647      ..C..A..G.  .....  C..T..T...  ..A.....  ....A.....  .....
V9694      ..C..A..G.  .....  C..C..T...  ..AC.....  ....A.....  .....
V9683      ..C..A....  .A.....G..  C..C..T...  ..A.....  .....  G.....T
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V9622       ..C..A..G. .......... C..C..T... ..A....... ....A..... ...T......
V9750       ..C..A.... .A.....G.. C..C..T... ..A....... .......... G........T
V9792       ..C..A..G. .......G.. C..C..T... ..AC...... ....A..... ..........
V9707       ..C..A.... .A.....G.. C..C..T... ..A....... .......... G........T
V9984       ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V9983       ..CC.A..G. .......... C..C..T... ..AC...... ....A..... G.........
V9939       ...C.A.... .A........ C.....T... ..A....... .......... .........T
V9943       ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V9942       ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V9975       ..C..A..G. .......... C..C...... ..A....A.. ....A..... ...T......
V9944       ..CC.A..G. .......... C..C..T... ..AC...... ....A..... G.........
V9973       ..C..A..G. .......... C..C...... .......... ....A..... ...T......
V9952       ..C..A..G. .......... C..C..T... ..AC...... ....A..... ..........
V9961       ..C..A..G. .......... C..C...... ..A.....G. ....A..... ...T......
V9963       ..CC.A..G. .......... C..C..T... ..AC...... ....A..... G.........
V9972       ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V10081      ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V10000      ..C..A..G. .......... C..T...... ..A....... ....A..... ...T......
V10003      ..C..A..G. .......... C..C...... ..A....... ....A..... ...T......
V10075      ..C..A..G. ....A..... C..C..T... ..A....... .......... G........T
V10138      ..CC.A..G. .......... C..C..T... ..AC.C.... ....A..... G.........
V10177      ..C..A..G. .......... C..C...... ..A....... ....A..... ...C......
V10076      ..C..A..G. ....A..... C..C..T... ..A....... .......... G........T
97-144      ...C.A..G. .......... C.....T... ..A....... .......... ...T..C...
97-120      ...C.A.... .......... C.....T... ..AC...... .......T.. .........T
97-127      ..C.....G. .......... C..C..T... ..C....... ....A..... ...G..A...
97-118      ...C.A..G. .......... C.....T... ..A....A.. .......... ...T..C...
R98533      ...C.A.... .......... C.....T... ..A....... .......T.. ..........
R98537      ...C.A..G. .......... C.....T... ..A....A.. .......... ...T..C...
R98517      ...C.A.... .......... C.....T... ..A....... .......T.. ..........
R5755       ...C.A.... .......... C.....T... ..A...A... .T.....T.. ..........
R9952       ...C.A.... .......... C.....T... ..A..C.... .......T.. ..........
R10151      ...C.A.... .......... C.....T... ..A....... .T.....T.. G..G......
R10745      ...C.A.... .......... C.....T... ..A..C.... .......T.. ..........
E00111      ...C.A.... .......... C.....T... ..A..C.... .......T.. ..........
R10996      ...C.A.... .......... C.....T... ..A..C.... .......T.. ..........
R98543      ..CC.A..G. .......... C..G..T... ..A....... .......T.. ..........
SABIN3      ..C..A..T. ....A..TT. G..C..A... ..C.....G. .T.....T.. ...T......

V55         ACAGCTGGCT ACAAGATTTG TAACTATCAC
V335        .......... .......... ..........
V445        ........T. ....A..C.. ......C..T
V512        ........T. .T..A..C.. C.....C..T
V583        ........T. ....A..C.. C.....C...
V787        .......... .T..A..C.. C.........
V802        .......... ....A..... ..........
V861        .......... ....A..C.. ......C..T
V904        .......... ....A..C.. ..........
V963        .......... ....A..... ..........
V1007       ........T. ....A..... C.....C...
V1067       ........T. ....A..... C.....C...
V1087       ..T.....G. .T.....A.. C..T..C...
V1130       ........G. .T........ C..T..C...
V1201       ........G. .T.....A.. C..T..C...
V1202       ........G. .T........ C..T..C...
V1725       ........G. .T........ C..T..C...
V1825       ........G. .T........ C..T..C...
V2820       ........G. .T........ C..T..C...
V2843       ........G. .......C.. C..T..C...
V3104       ........G. .......C.. C..T..C...
V3458       ........G. .T........ C..T..C...
V3757       ........G. TT........ C..T..C...
V5222       .....C..T. ....A..C.. ......C...
V5399       .....C..G. .T........ C..T..C...
V5402       ........G. .T........ C.....C...
V7202       ........T. T...A..C.. ......C...
V7210       GT......G. .T........ C.....C...
V8188       .....C..G. ....A..C.. ......C...
V5228       ........G. .......... C..T.....T
V9193       ........G. ....A..C.. C.........
V9222       ........G. .......... C..T..C...
V9267       ........G. .......... C..T..C...
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V9274        ........A. .......... C..T......
V9275        ........G. .......... C..T......
V9476        ........G. ....A..C.. C.....C...
V9477        ........G. .......C.. C.........
V9488        ........G. .......... C..T......
V9464        ........G. ....A..C.. C.....C...
V9463        ........A. .......... C..T......
V9482        ........G. .......C.. C.........
V9492        ........G. .......... ...T..C...
V9473        ........G. .......C.. C.........
V9517        ..G.....G. .......... ......C...
V9518        ..G.....G. .......... ......C...
V9647        ........G. .......... C..T..C...
V9694        ........G. .......... C..T..C...
V9683        ........G. ....A..C.. C.....C...
V9622        ........G. .......... C..T......
V9750        ........G. ....A..C.. C.....C...
V9792        ........G. .......... C..T..C...
V9707        ........G. ....A..C.. C.....C...
V9984        ........G. .......... C..T......
V9983        ........G. .......... C..T..C...
V9939        .....C..G. .......... ......C...
V9943        ..G.....G. .......... C..T......
V9942        .....C..G. .......... C..T......
V9975        ........G. .......... C..T......
V9944        ........G. .......... C..T..C...
V9973        ........G. ....A..... ...T......
V9952        ........G. .......C.. C..T..C...
V9961        ........G. .......... C..T......
V9963        ..G.....G. .......... C..T..C...
V9972        ..G.....G. .......... C..T......
V10081       ........G. .......... C..T......
V10000       ........G. .......... C..T..C...
V10003       ........G. .......... C..T......
V10075       ..G.....G. ....A..C.. C.....C..T
V10138       ........G. .......... C..T..C...
V10177       ........G. .......... C..T......
V10076       ..G.....G. ....A..C.. C.....C..T
97-144       .....G..A. ....A..A.. C.........
97-120       .....C..G. .T..A..... ......C...
97-127       .....C..G. ....A..C.. C.....C...
97-118       .....A..A. .T..A..A.. ......C...
R98533       .....C..G. ....A..... ......C...
R98537       .....A..A. ....A..A.. ..........
R98517       .....C..G. ....A..... ..........
R5755        .....C..G. ....A..... ......C...
R9952        .....C..G. .T..A..... ......C...
R10151       .....C..T. ....A..... ......C...
R10745       .....C..G. .T..A..... ......C...
E00111       .....C..G. .T..A..... ......C..T
R10996       .....C..G. .T..A..... ......C...
R98543       .....C..G. ....A..C.. C..T..C...
SABIN3       ..T.....T. .......C.. C.....C..T
```

Figure 19: Genetic distance calculated using Kimura 2 Parameter algorithm of 96 strains isolated in years 1971-2000 from paralytic cases from Mumbai

V55	0.0000	0.0135	0.0864	0.1108	0.0706	0.0636	0.0343	0.1028
0.0787	0.0711	0.0716	0.0711	0.1463	0.1716	0.1821	0.1627	0.1627
0.1807	0.1807	0.1899	0.1627	0.2183	0.1743	0.2008	0.2183	0.2280
0.1930	0.2300	0.1836	0.1716	0.2201	0.1807	0.1914	0.1836	0.1821
0.2183	0.1836	0.1730	0.2280	0.1836	0.1821	0.1716	0.1821	0.2086
0.1807	0.1716	0.1883	0.2280	0.1821	0.2280	0.2086	0.2280	0.1640
0.1899	0.1614	0.1730	0.1730	0.1730	0.1899	0.1551	0.2069	0.1821
0.1992	0.1730	0.1821	0.2025	0.1627	0.2501	0.1992	0.1640	0.2501
0.1758	0.1976	0.1992	0.1851	0.1614	0.1666	0.1527	0.2008	0.1976
0.1778	0.1976	0.1960	0.1976	0.2379	0.3674			
V335	0.0135	0.0000	0.1020	0.1272	0.0858	0.0787	0.0486	0.1028
0.0787	0.0711	0.0870	0.0864	0.1640	0.1899	0.2008	0.1807	0.1807
0.1992	0.1992	0.2086	0.1807	0.2379	0.1930	0.2008	0.2379	0.2086
0.1930	0.2104	0.1836	0.1716	0.2008	0.1627	0.1730	0.1652	0.1640
0.1992	0.1652	0.1551	0.2086	0.1652	0.1640	0.1539	0.1640	0.1899
0.1627	0.1539	0.1703	0.2086	0.1640	0.2086	0.1899	0.2086	0.1463
0.1716	0.1441	0.1551	0.1551	0.1551	0.1716	0.1551	0.1883	0.1640
0.1807	0.1551	0.1640	0.1836	0.1452	0.2400	0.1807	0.1463	0.2400
0.1575	0.1792	0.1899	0.1666	0.1441	0.1486	0.1356	0.1821	0.1792
0.1602	0.1792	0.1778	0.1792	0.2183	0.3674			
V445	0.0864	0.1020	0.0000	0.0562	0.1020	0.0782	0.1100	0.0787
0.0782	0.0858	0.0562	0.0558	0.1452	0.1614	0.2025	0.1441	0.1441
0.1703	0.1703	0.1614	0.1356	0.2069	0.1640	0.1282	0.2069	0.1976
0.1551	0.1992	0.1376	0.1441	0.1716	0.1527	0.1807	0.2104	0.1899
0.1703	0.1730	0.1807	0.1792	0.2104	0.1716	0.1614	0.1716	0.1792
0.1703	0.1883	0.1602	0.1792	0.1899	0.1792	0.2069	0.1792	0.1716
0.1976	0.1716	0.1807	0.1807	0.1807	0.1976	0.1452	0.1602	0.1899
0.2069	0.1807	0.1716	0.1821	0.1703	0.1807	0.1883	0.1716	0.1807
0.1772	0.1716	0.1527	0.1679	0.1539	0.1679	0.1627	0.2122	0.1539
0.1792	0.1539	0.1356	0.1539	0.1914	0.2989			
V512	0.1108	0.1272	0.0562	0.0000	0.1108	0.0942	0.1356	0.1272
0.1100	0.1181	0.0870	0.0864	0.1463	0.1602	0.1914	0.1263	0.1263
0.1869	0.1690	0.1778	0.1516	0.1869	0.1807	0.1821	0.1869	0.1899
0.1930	0.1914	0.1930	0.1716	0.1914	0.1716	0.2008	0.2320	0.2104
0.1899	0.1930	0.2008	0.1899	0.2320	0.1914	0.1992	0.1914	0.1899
0.1807	0.1716	0.1976	0.1899	0.2104	0.1899	0.2280	0.1899	0.1914
0.1992	0.2201	0.2008	0.2008	0.2008	0.1992	0.1821	0.1976	0.2104
0.2086	0.2008	0.2104	0.2025	0.1899	0.1821	0.1899	0.1914	0.1821
0.1882	0.2201	0.1899	0.1787	0.2008	0.1979	0.2104	0.2443	0.2008
0.1899	0.2008	0.1807	0.2008	0.2220	0.3079			
V583	0.0706	0.0858	0.1020	0.1108	0.0000	0.0942	0.0343	0.1190
0.1263	0.1346	0.0870	0.0864	0.1640	0.1807	0.2008	0.1899	0.1899
0.1899	0.1899	0.1627	0.1539	0.2280	0.2025	0.2008	0.2086	0.2479
0.1930	0.2501	0.1930	0.2183	0.2201	0.1992	0.2104	0.2220	0.2201
0.1992	0.2025	0.2104	0.2086	0.2220	0.2008	0.2086	0.2008	0.2479
0.2183	0.1716	0.1976	0.2086	0.2201	0.2086	0.2183	0.2086	0.2008
0.1807	0.1976	0.2104	0.2104	0.2104	0.1807	0.1914	0.1976	0.2201
0.1899	0.2104	0.2201	0.2220	0.2086	0.2300	0.1899	0.2008	0.2300
0.1851	0.1976	0.1716	0.1946	0.1614	0.1946	0.1703	0.1914	0.1976
0.1690	0.1976	0.1869	0.1976	0.1807	0.3274			
V787	0.0636	0.0787	0.0782	0.0942	0.0942	0.0000	0.0864	0.1020
0.0558	0.0632	0.0636	0.0632	0.1116	0.1441	0.1836	0.1441	0.1441
0.1527	0.1527	0.1614	0.1356	0.1883	0.1463	0.1899	0.2261	0.1976
0.2008	0.2183	0.1730	0.1614	0.1716	0.1883	0.1992	0.1914	0.1899
0.1883	0.1730	0.1807	0.1976	0.1914	0.1716	0.1976	0.1716	0.2559
0.2261	0.1883	0.1602	0.1976	0.1899	0.1976	0.2069	0.1976	0.1716
0.1792	0.2086	0.1807	0.1807	0.1807	0.1792	0.1627	0.1602	0.1899
0.1883	0.1807	0.1716	0.2008	0.1703	0.2183	0.1883	0.1716	0.2183
0.1772	0.1716	0.1703	0.2059	0.1899	0.2059	0.1807	0.2320	0.1899
0.2069	0.1899	0.1883	0.1899	0.2104	0.3631			
V802	0.0343	0.0486	0.1100	0.1356	0.0343	0.0864	0.0000	0.1108
0.1020	0.0942	0.0948	0.0942	0.1730	0.1807	0.2104	0.1899	0.1899
0.1899	0.1899	0.1807	0.1716	0.2280	0.2025	0.2104	0.2086	0.2479
0.2025	0.2501	0.1930	0.1992	0.2201	0.1992	0.2104	0.2025	0.2008

0.2183 0.2025 0.1914 0.2280 0.2025 0.2008 0.1899 0.2008 0.2280
0.1992 0.1899 0.1976 0.2280 0.2008 0.2280 0.2183 0.2280 0.1821
0.1807 0.1792 0.1914 0.1914 0.1914 0.1807 0.1551 0.2164 0.2008
0.1899 0.1914 0.2008 0.2220 0.1899 0.2501 0.1899 0.1821 0.2501
0.1758 0.1792 0.1899 0.1851 0.1441 0.1666 0.1356 0.1821 0.1792
0.1602 0.1792 0.1690 0.1792 0.1992 0.3674

V861 0.1028 0.1028 0.0787 0.1272 0.1190 0.1020 0.1108 0.0000
0.0864 0.0942 0.0948 0.0787 0.1821 0.1716 0.1946 0.1627 0.1627
0.1899 0.1899 0.1807 0.1539 0.2280 0.2025 0.1792 0.2280 0.2086
0.1899 0.2300 0.1807 0.1627 0.1914 0.1899 0.2008 0.2122 0.2104
0.1452 0.1930 0.2008 0.1539 0.2122 0.1914 0.1807 0.1914 0.2280
0.1992 0.1899 0.1792 0.1539 0.2104 0.1539 0.1899 0.1539 0.1914
0.1992 0.1551 0.2008 0.2008 0.2008 0.1992 0.1640 0.1792 0.2008
0.2086 0.2008 0.1914 0.1836 0.1899 0.2008 0.2086 0.1914 0.2008
0.1787 0.1730 0.1899 0.1882 0.1551 0.1882 0.1640 0.2140 0.1730
0.1899 0.1730 0.1716 0.1730 0.1652 0.3538

V904 0.0787 0.0787 0.0782 0.1100 0.1263 0.0558 0.1020 0.0864
0.0000 0.0067 0.0787 0.0782 0.1116 0.1614 0.1930 0.1614 0.1614
0.1883 0.1703 0.1614 0.1356 0.2069 0.1821 0.1627 0.2458 0.1792
0.1730 0.2183 0.1640 0.1792 0.1366 0.1527 0.1627 0.1551 0.1539
0.1703 0.1376 0.1452 0.1792 0.1551 0.1366 0.1441 0.1366 0.1976
0.1703 0.1703 0.1430 0.1792 0.1539 0.1792 0.1883 0.1792 0.1366
0.1614 0.1716 0.1452 0.1452 0.1452 0.1614 0.1116 0.1430 0.1539
0.1703 0.1452 0.1366 0.1640 0.1356 0.1899 0.1703 0.1366 0.1899
0.1497 0.1716 0.1356 0.1772 0.1539 0.1587 0.1452 0.1930 0.1716
0.1883 0.1716 0.1703 0.1716 0.1730 0.3631

V963 0.0711 0.0711 0.0858 0.1181 0.1346 0.0632 0.0942 0.0942
0.0067 0.0000 0.0711 0.0706 0.1116 0.1527 0.1930 0.1527 0.1527
0.1792 0.1614 0.1703 0.1441 0.1976 0.1730 0.1716 0.2359 0.1703
0.1821 0.2086 0.1730 0.1703 0.1452 0.1441 0.1539 0.1463 0.1452
0.1792 0.1463 0.1366 0.1883 0.1463 0.1452 0.1356 0.1452 0.1883
0.1614 0.1614 0.1346 0.1883 0.1452 0.1883 0.1792 0.1883 0.1282
0.1527 0.1627 0.1366 0.1366 0.1366 0.1527 0.1035 0.1516 0.1452
0.1614 0.1366 0.1282 0.1551 0.1272 0.1992 0.1614 0.1282 0.1992
0.1497 0.1627 0.1441 0.1772 0.1452 0.1587 0.1366 0.1836 0.1627
0.1792 0.1627 0.1614 0.1627 0.1821 0.3768

V1007 0.0716 0.0870 0.0562 0.0870 0.0870 0.0636 0.0948 0.0948
0.0787 0.0711 0.0000 0.0136 0.1124 0.1366 0.1666 0.1366 0.1366
0.1452 0.1539 0.1539 0.1282 0.1807 0.1387 0.1730 0.1992 0.1899
0.1836 0.2201 0.1836 0.1539 0.1821 0.1539 0.1730 0.1836 0.1821
0.1807 0.1836 0.1821 0.1899 0.1836 0.1821 0.1716 0.1821 0.2086
0.1807 0.1627 0.1527 0.1899 0.1821 0.1899 0.1807 0.1899 0.1730
0.1539 0.1821 0.1821 0.1821 0.1821 0.1539 0.1640 0.1703 0.1914
0.1627 0.1821 0.1730 0.1836 0.1716 0.2104 0.1452 0.1730 0.2104
0.1787 0.1821 0.1807 0.2077 0.1640 0.2077 0.1730 0.2042 0.1640
0.1716 0.1640 0.1627 0.1640 0.1930 0.3329

V1067 0.0711 0.0864 0.0558 0.0864 0.0864 0.0632 0.0942 0.0787
0.0782 0.0706 0.0136 0.0000 0.1198 0.1441 0.1743 0.1441 0.1441
0.1527 0.1527 0.1614 0.1356 0.1883 0.1463 0.1807 0.2069 0.1976
0.1914 0.2183 0.1914 0.1441 0.1899 0.1527 0.1807 0.1914 0.1899
0.1703 0.1914 0.1807 0.1792 0.1914 0.1899 0.1792 0.1899 0.2164
0.1883 0.1703 0.1602 0.1792 0.1899 0.1792 0.1703 0.1792 0.1716
0.1614 0.1716 0.1807 0.1807 0.1807 0.1614 0.1627 0.1778 0.1899
0.1703 0.1807 0.1716 0.1821 0.1703 0.2183 0.1527 0.1716 0.2183
0.1679 0.1716 0.1883 0.1962 0.1539 0.1962 0.1627 0.1930 0.1539
0.1614 0.1539 0.1703 0.1539 0.1821 0.3497

V1087 0.1463 0.1640 0.1452 0.1463 0.1640 0.1116 0.1730 0.1821
0.1116 0.1116 0.1124 0.1198 0.0000 0.0955 0.1042 0.0955 0.0955
0.1198 0.1198 0.1035 0.0955 0.1198 0.1132 0.1851 0.1539 0.1627
0.1962 0.2008 0.1743 0.1627 0.1821 0.1539 0.1463 0.1652 0.1551
0.1992 0.1652 0.1640 0.2086 0.1652 0.1640 0.1452 0.1640 0.1899
0.1627 0.1366 0.1272 0.2086 0.1551 0.2086 0.1716 0.2086 0.1551
0.1282 0.1914 0.1551 0.1640 0.1640 0.1282 0.1640 0.1356 0.1551
0.1282 0.1551 0.1551 0.1652 0.1539 0.2008 0.1366 0.1551 0.2008
0.1666 0.2104 0.1807 0.1758 0.2104 0.1946 0.2201 0.2239 0.1914
0.2220 0.1914 0.1899 0.1914 0.1836 0.3538

V1130 0.1716 0.1899 0.1614 0.1602 0.1807 0.1441 0.1807 0.1716
0.1614 0.1527 0.1366 0.1441 0.0955 0.0000 0.0645 0.0273 0.0273
0.0343 0.0343 0.0414 0.0632 0.0486 0.0419 0.1474 0.0935 0.0792
0.1575 0.1116 0.1551 0.1108 0.1366 0.1108 0.1035 0.1207 0.1116
0.1441 0.1291 0.1366 0.1441 0.1207 0.1282 0.1028 0.1282 0.1272
0.1356 0.1108 0.1181 0.1441 0.1116 0.1441 0.1356 0.1441 0.1282

0.1356 0.1627 0.1366 0.1366 0.1366 0.1356 0.1366 0.1181 0.1282
0.1441 0.1366 0.1282 0.1376 0.1272 0.1366 0.1441 0.1282 0.1366
0.2059 0.1627 0.1792 0.2158 0.1807 0.2361 0.1899 0.2122 0.1627
0.2104 0.1627 0.1614 0.1627 0.1463 0.3497
V1201 0.1821 0.2008 0.2025 0.1914 0.2008 0.1836 0.2104 0.1946
0.1930 0.1930 0.1666 0.1743 0.1042 0.0645 0.0000 0.0570 0.0570
0.0876 0.0876 0.0876 0.0955 0.0876 0.0969 0.1787 0.1198 0.1301
0.1898 0.1758 0.1866 0.1563 0.1666 0.1474 0.1225 0.1408 0.1311
0.1930 0.1497 0.1575 0.1930 0.1408 0.1398 0.1216 0.1398 0.1652
0.1743 0.1301 0.1376 0.1930 0.1311 0.1930 0.1387 0.1930 0.1486
0.1387 0.2008 0.1575 0.1575 0.1575 0.1387 0.1575 0.1291 0.1486
0.1474 0.1575 0.1486 0.1497 0.1474 0.1851 0.1474 0.1486 0.1851
0.2340 0.2201 0.1930 0.2443 0.2400 0.2653 0.2501 0.2760 0.2201
0.2843 0.2201 0.2183 0.2201 0.1930 0.4212
V1202 0.1627 0.1807 0.1441 0.1263 0.1899 0.1441 0.1899 0.1627
0.1614 0.1527 0.1366 0.1441 0.0955 0.0273 0.0570 0.0000 0.0000
0.0486 0.0486 0.0558 0.0632 0.0632 0.0566 0.1387 0.0935 0.0792
0.1666 0.1116 0.1463 0.0948 0.1198 0.0948 0.0876 0.1207 0.0955
0.1441 0.1124 0.1198 0.1441 0.1207 0.1116 0.0870 0.1116 0.1272
0.1527 0.0792 0.1020 0.1441 0.0955 0.1441 0.1190 0.1441 0.1116
0.1190 0.1539 0.1198 0.1198 0.1198 0.1190 0.1198 0.1020 0.1282
0.1272 0.1198 0.1116 0.1042 0.1108 0.1366 0.1272 0.1116 0.1366
0.1962 0.1716 0.1614 0.2059 0.1899 0.2259 0.1992 0.2220 0.1716
0.2201 0.1716 0.1703 0.1716 0.1551 0.3631
V1725 0.1627 0.1807 0.1441 0.1263 0.1899 0.1441 0.1899 0.1627
0.1614 0.1527 0.1366 0.1441 0.0955 0.0273 0.0570 0.0000 0.0000
0.0486 0.0486 0.0558 0.0632 0.0632 0.0566 0.1387 0.0935 0.0792
0.1666 0.1116 0.1463 0.0948 0.1198 0.0948 0.0876 0.1207 0.0955
0.1441 0.1124 0.1198 0.1441 0.1207 0.1116 0.0870 0.1116 0.1272
0.1527 0.0792 0.1020 0.1441 0.0955 0.1441 0.1190 0.1441 0.1116
0.1190 0.1539 0.1198 0.1198 0.1198 0.1190 0.1198 0.1020 0.1282
0.1272 0.1198 0.1116 0.1042 0.1108 0.1366 0.1272 0.1116 0.1366
0.1962 0.1716 0.1614 0.2059 0.1899 0.2259 0.1992 0.2220 0.1716
0.2201 0.1716 0.1703 0.1716 0.1551 0.3631
V1825 0.1807 0.1992 0.1703 0.1869 0.1899 0.1527 0.1899 0.1899
0.1883 0.1792 0.1452 0.1527 0.1198 0.0343 0.0876 0.0486 0.0486
0.0000 0.0273 0.0343 0.0558 0.0414 0.0347 0.1836 0.0858 0.1190
0.1758 0.1539 0.1914 0.1028 0.1282 0.1190 0.1116 0.1291 0.1198
0.1356 0.1376 0.1452 0.1356 0.1291 0.1366 0.1108 0.1366 0.1527
0.1614 0.1190 0.1263 0.1356 0.1198 0.1356 0.1441 0.1356 0.1366
0.1527 0.1716 0.1452 0.1452 0.1452 0.1527 0.1452 0.1263 0.1539
0.1614 0.1452 0.1366 0.1463 0.1356 0.1452 0.1614 0.1366 0.1452
0.2361 0.1716 0.1883 0.2465 0.1899 0.2677 0.1992 0.2220 0.1716
0.2201 0.1716 0.1703 0.1716 0.1730 0.3366
V2820 0.1807 0.1992 0.1703 0.1690 0.1899 0.1527 0.1899 0.1899
0.1703 0.1614 0.1539 0.1527 0.1198 0.0343 0.0876 0.0486 0.0486
0.0273 0.0000 0.0343 0.0558 0.0414 0.0492 0.1836 0.0858 0.1190
0.1946 0.1366 0.1914 0.1190 0.1452 0.1028 0.1116 0.1291 0.1198
0.1703 0.1376 0.1282 0.1703 0.1291 0.1366 0.1108 0.1366 0.1527
0.1614 0.1190 0.1263 0.1703 0.1198 0.1703 0.1441 0.1703 0.1198
0.1527 0.1899 0.1282 0.1282 0.1282 0.1527 0.1282 0.1263 0.1366
0.1614 0.1282 0.1198 0.1291 0.1190 0.1627 0.1614 0.1198 0.1627
0.2361 0.1899 0.1883 0.2465 0.2086 0.2677 0.2183 0.2421 0.1899
0.2400 0.1899 0.1883 0.1899 0.1730 0.3631
V2843 0.1899 0.2086 0.1614 0.1778 0.1627 0.1614 0.1807 0.1807
0.1614 0.1703 0.1539 0.1614 0.1035 0.0414 0.0876 0.0558 0.0558
0.0343 0.0343 0.0000 0.0203 0.0486 0.0566 0.1563 0.0782 0.1272
0.1666 0.1627 0.1640 0.1108 0.1198 0.1108 0.1035 0.1207 0.1116
0.1441 0.1124 0.1366 0.1441 0.1207 0.1116 0.1028 0.1116 0.1441
0.1527 0.1108 0.1181 0.1441 0.1116 0.1441 0.1356 0.1441 0.1282
0.1272 0.1627 0.1366 0.1366 0.1366 0.1272 0.1366 0.1020 0.1452
0.1356 0.1366 0.1282 0.1376 0.1272 0.1366 0.1356 0.1282 0.1366
0.1962 0.1807 0.1614 0.2259 0.1807 0.2259 0.1899 0.2122 0.1807
0.2104 0.1807 0.1792 0.1807 0.1291 0.3366
V3104 0.1627 0.1807 0.1356 0.1516 0.1539 0.1356 0.1716 0.1539
0.1356 0.1441 0.1282 0.1356 0.0955 0.0632 0.0955 0.0632 0.0632
0.0558 0.0558 0.0203 0.0000 0.0706 0.0792 0.1652 0.0858 0.1356
0.1758 0.1716 0.1730 0.0870 0.1198 0.1190 0.1116 0.1291 0.1198
0.1356 0.1207 0.1282 0.1356 0.1291 0.1198 0.1108 0.1198 0.1527
0.1441 0.1190 0.1100 0.1356 0.1198 0.1356 0.1272 0.1356 0.1198
0.1190 0.1366 0.1282 0.1282 0.1282 0.1190 0.1282 0.0942 0.1366
0.1272 0.1282 0.1198 0.1291 0.1190 0.1452 0.1272 0.1198 0.1452

0.1679 0.1716 0.1527 0.1962 0.1716 0.1962 0.1807 0.2122 0.1716
0.2008 0.1716 0.1703 0.1716 0.1207 0.3238
V3458 0.2183 0.2379 0.2069 0.1869 0.2280 0.1883 0.2280 0.2280
0.2069 0.1976 0.1807 0.1883 0.1198 0.0486 0.0876 0.0632 0.0632
0.0414 0.0414 0.0486 0.0706 0.0000 0.0640 0.2025 0.0858 0.1190
0.2140 0.1366 0.2104 0.1190 0.1452 0.1356 0.1282 0.1463 0.1366
0.1703 0.1551 0.1627 0.1703 0.1463 0.1539 0.1272 0.1539 0.1356
0.1441 0.1356 0.1430 0.1703 0.1366 0.1703 0.1614 0.1703 0.1539
0.1527 0.2086 0.1627 0.1627 0.1627 0.1527 0.1627 0.1430 0.1539
0.1614 0.1627 0.1539 0.1640 0.1527 0.1627 0.1614 0.1539 0.1627
0.2570 0.2086 0.2069 0.2677 0.2280 0.2898 0.2379 0.2421 0.2086
0.2201 0.2086 0.2069 0.2086 0.1914 0.3497
V3757 0.1743 0.1930 0.1640 0.1807 0.2025 0.1463 0.2025 0.2025
0.1821 0.1730 0.1387 0.1463 0.1132 0.0419 0.0969 0.0566 0.0566
0.0347 0.0492 0.0566 0.0792 0.0640 0.0000 0.1962 0.1108 0.1291
0.1851 0.1652 0.2042 0.1291 0.1743 0.1463 0.1387 0.1575 0.1474
0.1821 0.1666 0.1743 0.1821 0.1575 0.1652 0.1376 0.1652 0.1821
0.1914 0.1463 0.1539 0.1821 0.1474 0.1821 0.1730 0.1821 0.1652
0.1730 0.1836 0.1743 0.1743 0.1743 0.1730 0.1743 0.1539 0.1836
0.1821 0.1743 0.1652 0.1758 0.1640 0.1930 0.1821 0.1652 0.1930
0.2510 0.1836 0.2201 0.2619 0.2025 0.2841 0.2122 0.2361 0.1836
0.2340 0.1836 0.1821 0.1836 0.2042 0.4007
V5222 0.2008 0.2008 0.1282 0.1821 0.2008 0.1899 0.2104 0.1792
0.1627 0.1716 0.1730 0.1807 0.1851 0.1474 0.1787 0.1387 0.1387
0.1836 0.1836 0.1563 0.1652 0.2025 0.1962 0.0000 0.1836 0.1652
0.0345 0.1851 0.0205 0.1930 0.1575 0.1652 0.1376 0.1652 0.1463
0.1743 0.1387 0.1551 0.1836 0.1652 0.1291 0.1387 0.1291 0.1652
0.1743 0.1652 0.1551 0.1836 0.1463 0.1836 0.2025 0.1836 0.1640
0.1930 0.1946 0.1730 0.1551 0.1730 0.1930 0.1551 0.1551 0.1730
0.2025 0.1730 0.1463 0.1743 0.1627 0.1666 0.2025 0.1730 0.1666
0.1666 0.2140 0.1216 0.1575 0.2140 0.1575 0.2239 0.2487 0.2140
0.2122 0.2140 0.2122 0.2140 0.1962 0.3956
V5399 0.2183 0.2379 0.2069 0.1869 0.2086 0.2261 0.2086 0.2280
0.2458 0.2359 0.1992 0.2069 0.1539 0.0935 0.1198 0.0935 0.0935
0.0858 0.0858 0.0782 0.0858 0.0858 0.1108 0.1836 0.0000 0.1527
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0.1272 0.1627 0.1539 0.1640 0.1527 0.1992 0.1272 0.1539 0.1992
0.2465 0.1899 0.1883 0.2361 0.2086 0.2570 0.2183 0.2421 0.1899
0.2201 0.1899 0.1883 0.1899 0.1640 0.4051
V5402 0.2280 0.2086 0.1976 0.1899 0.2479 0.1976 0.2479 0.2086
0.1792 0.1703 0.1899 0.1976 0.1627 0.0792 0.1301 0.0792 0.0792
0.1190 0.1190 0.1272 0.1356 0.1190 0.1291 0.1652 0.1527 0.0000
0.1946 0.0489 0.1730 0.1108 0.0711 0.0935 0.0864 0.1190 0.0942
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0.0935 0.1173 0.1100 0.0858 0.0942 0.0858 0.1190 0.0858 0.1100
0.1108 0.1263 0.1181 0.1181 0.1263 0.1108 0.1430 0.1100 0.1100
0.1190 0.1181 0.1100 0.1272 0.1272 0.0787 0.1190 0.1100 0.0787
0.1836 0.1263 0.1614 0.1930 0.1430 0.2122 0.1516 0.1807 0.1263
0.1703 0.1263 0.1356 0.1263 0.1272 0.3631
V7202 0.1930 0.1930 0.1551 0.1930 0.1930 0.2008 0.2025 0.1899
0.1730 0.1821 0.1836 0.1914 0.1962 0.1575 0.1898 0.1666 0.1666
0.1758 0.1946 0.1666 0.1758 0.2140 0.1851 0.0345 0.2340 0.1946
0.0000 0.2158 0.0566 0.2239 0.1679 0.1758 0.1474 0.1575 0.1563
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0.2239 0.2259 0.1836 0.1836 0.1836 0.2239 0.1652 0.1836 0.1836
0.2340 0.1836 0.1743 0.2042 0.1730 0.1587 0.2340 0.1836 0.1587
0.1866 0.2677 0.1666 0.1772 0.2465 0.1772 0.2570 0.2841 0.2677
0.2443 0.2677 0.2653 0.2677 0.2487 0.3719
V7210 0.2300 0.2104 0.1992 0.1914 0.2501 0.2183 0.2501 0.2300
0.2183 0.2086 0.2201 0.2183 0.2008 0.1116 0.1758 0.1116 0.1116
0.1539 0.1366 0.1627 0.1716 0.1366 0.1652 0.1851 0.1716 0.0489
0.2158 0.0000 0.1930 0.1430 0.1190 0.1263 0.1356 0.1716 0.1441
0.1430 0.1452 0.1527 0.1346 0.1716 0.1441 0.1346 0.1441 0.1020
0.1263 0.1516 0.1614 0.1346 0.1441 0.1346 0.1716 0.1346 0.1441
0.1627 0.1792 0.1527 0.1527 0.1614 0.1627 0.1792 0.1614 0.1441
0.1716 0.1527 0.1441 0.1627 0.1627 0.1272 0.1716 0.1441 0.1272
0.2239 0.1883 0.1992 0.2140 0.2069 0.2340 0.2164 0.2300 0.1883
0.1992 0.1883 0.1992 0.1883 0.1899 0.3814

V8188 0.1836 0.1836 0.1376 0.1930 0.1930 0.1730 0.1930 0.1807
0.1640 0.1730 0.1836 0.1914 0.1743 0.1551 0.1866 0.1463 0.1463
0.1914 0.1914 0.1640 0.1730 0.2104 0.2042 0.0205 0.1914 0.1730
0.0566 0.1930 0.0000 0.2008 0.1652 0.1551 0.1282 0.1640 0.1366
0.1821 0.1291 0.1452 0.1914 0.1640 0.1198 0.1291 0.1198 0.1730
0.1821 0.1551 0.1452 0.1914 0.1366 0.1914 0.1914 0.1914 0.1539
0.1821 0.2025 0.1627 0.1452 0.1627 0.1821 0.1452 0.1452 0.1627
0.1914 0.1627 0.1366 0.1640 0.1527 0.1743 0.1914 0.1627 0.1743
0.1652 0.2220 0.0962 0.1563 0.2025 0.1563 0.2122 0.2361 0.2025
0.2239 0.2025 0.2008 0.2025 0.1851 0.4156
V5228 0.1716 0.1716 0.1441 0.1716 0.2183 0.1614 0.1992 0.1627
0.1792 0.1703 0.1539 0.1441 0.1627 0.1108 0.1563 0.0948 0.0948
0.1028 0.1190 0.1108 0.0870 0.1190 0.1291 0.1930 0.1356 0.1108
0.2239 0.1430 0.2008 0.0000 0.1116 0.1272 0.1198 0.1376 0.1116
0.1108 0.1291 0.1198 0.1028 0.1376 0.1282 0.1190 0.1282 0.1356
0.1441 0.1272 0.1181 0.1028 0.1116 0.1028 0.1108 0.1028 0.1116
0.1272 0.1282 0.1198 0.1198 0.1198 0.1272 0.1366 0.1181 0.1282
0.1356 0.1198 0.1116 0.1376 0.1108 0.1366 0.1356 0.1116 0.1366
0.1679 0.1539 0.1883 0.2158 0.1539 0.1962 0.1452 0.1930 0.1539
0.1640 0.1539 0.1527 0.1539 0.1376 0.3580
V9193 0.2201 0.2008 0.1716 0.1914 0.2201 0.1716 0.2201 0.1914
0.1366 0.1452 0.1821 0.1899 0.1821 0.1366 0.1666 0.1198 0.1198
0.1282 0.1452 0.1198 0.1198 0.1452 0.1743 0.1575 0.1716 0.0711
0.1679 0.1190 0.1652 0.1116 0.0000 0.0942 0.0870 0.1035 0.0792
0.0562 0.0645 0.0948 0.0636 0.1035 0.0640 0.0864 0.0640 0.1020
0.1181 0.1181 0.1028 0.0636 0.0792 0.0636 0.1282 0.0636 0.0870
0.1116 0.1356 0.0948 0.0948 0.1028 0.1116 0.1028 0.0870 0.1028
0.1198 0.0948 0.0870 0.1198 0.1035 0.0492 0.1198 0.0870 0.0492
0.1486 0.1527 0.1282 0.1946 0.1527 0.1758 0.1441 0.1914 0.1527
0.1807 0.1527 0.1627 0.1527 0.1198 0.3538
V9222 0.1807 0.1627 0.1527 0.1716 0.1992 0.1883 0.1992 0.1899
0.1527 0.1441 0.1539 0.1527 0.1539 0.1108 0.1474 0.0948 0.0948
0.1190 0.1028 0.1108 0.1190 0.1356 0.1463 0.1652 0.1703 0.0935
0.1758 0.1263 0.1551 0.1272 0.0942 0.0000 0.0345 0.0640 0.0417
0.1013 0.0566 0.0489 0.1093 0.0640 0.0562 0.0343 0.0562 0.0782
0.1013 0.0632 0.0711 0.1093 0.0417 0.1093 0.0870 0.1093 0.0417
0.0948 0.1181 0.0489 0.0489 0.0562 0.0948 0.0711 0.0711 0.0562
0.1028 0.0489 0.0417 0.0716 0.0566 0.1020 0.0870 0.0417 0.1020
0.1563 0.1690 0.1356 0.1836 0.1346 0.1836 0.1430 0.1716 0.1346
0.1792 0.1346 0.1441 0.1346 0.1190 0.3631
V9267 0.1914 0.1730 0.1807 0.2008 0.2104 0.1992 0.2104 0.2008
0.1627 0.1539 0.1730 0.1807 0.1463 0.1035 0.1225 0.0876 0.0876
0.1116 0.1116 0.1035 0.1116 0.1282 0.1387 0.1376 0.1452 0.0864
0.1474 0.1356 0.1282 0.1198 0.0870 0.0345 0.0000 0.0275 0.0067
0.0942 0.0275 0.0273 0.1020 0.0275 0.0203 0.0136 0.0203 0.0864
0.1100 0.0417 0.0492 0.1020 0.0067 0.1020 0.0645 0.1020 0.0203
0.0721 0.1108 0.0273 0.0273 0.0343 0.0721 0.0486 0.0492 0.0343
0.0798 0.0273 0.0343 0.0489 0.0345 0.0948 0.0798 0.0273 0.0948
0.1291 0.1614 0.1035 0.1551 0.1272 0.1551 0.1356 0.1640 0.1441
0.1627 0.1441 0.1539 0.1441 0.1116 0.3329
V9274 0.1836 0.1652 0.2104 0.2320 0.2220 0.1914 0.2025 0.2122
0.1551 0.1463 0.1836 0.1914 0.1652 0.1207 0.1408 0.1207 0.1207
0.1291 0.1291 0.1207 0.1291 0.1463 0.1575 0.1652 0.1821 0.1190
0.1575 0.1716 0.1640 0.1376 0.1035 0.0640 0.0275 0.0000 0.0205
0.1272 0.0419 0.0417 0.1356 0.0000 0.0345 0.0419 0.0345 0.1190
0.1272 0.0716 0.0798 0.1356 0.0205 0.1356 0.0962 0.1356 0.0345
0.1042 0.1452 0.0417 0.0417 0.0489 0.1042 0.0636 0.0798 0.0489
0.1124 0.0417 0.0489 0.0792 0.0492 0.1282 0.1124 0.0417 0.1282
0.1301 0.1992 0.1376 0.1743 0.1627 0.1563 0.1539 0.2025 0.1807
0.1914 0.1807 0.1914 0.1807 0.1463 0.3631
V9275 0.1821 0.1640 0.1899 0.2104 0.2201 0.1899 0.2008 0.2104
0.1539 0.1452 0.1821 0.1899 0.1551 0.1116 0.1311 0.0955 0.0955
0.1198 0.1198 0.1116 0.1198 0.1366 0.1474 0.1463 0.1539 0.0942
0.1563 0.1441 0.1366 0.1116 0.0792 0.0417 0.0067 0.0205 0.0000
0.1020 0.0205 0.0203 0.1100 0.0205 0.0135 0.0205 0.0135 0.0942
0.1181 0.0489 0.0566 0.1100 0.0000 0.1100 0.0721 0.1100 0.0135
0.0798 0.1190 0.0203 0.0203 0.0273 0.0798 0.0414 0.0566 0.0273
0.0876 0.0203 0.0273 0.0562 0.0275 0.1028 0.0876 0.0203 0.1028
0.1207 0.1703 0.1116 0.1640 0.1356 0.1463 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3457
V9476 0.2183 0.1992 0.1703 0.1899 0.1992 0.1883 0.2183 0.1452
0.1703 0.1792 0.1807 0.1703 0.1992 0.1441 0.1930 0.1441 0.1441

0.1356 0.1703 0.1441 0.1356 0.1703 0.1821 0.1743 0.1703 0.0935
0.1666 0.1430 0.1821 0.1108 0.0562 0.1013 0.0942 0.1272 0.1020
0.0000 0.0870 0.1100 0.0067 0.1272 0.0864 0.0935 0.0864 0.0935
0.1013 0.1254 0.1181 0.0067 0.1020 0.0067 0.1190 0.0067 0.1020
0.1272 0.0711 0.1100 0.1100 0.1181 0.1272 0.1181 0.1020 0.1020
0.1356 0.1100 0.1181 0.1356 0.1190 0.0562 0.1356 0.1020 0.0562
0.1301 0.1181 0.1527 0.1563 0.1020 0.1563 0.1100 0.1366 0.1181
0.1272 0.1181 0.1441 0.1181 0.1028 0.3631

V9477 0.1836 0.1652 0.1730 0.1930 0.2025 0.1730 0.2025 0.1930
0.1376 0.1463 0.1836 0.1914 0.1652 0.1291 0.1497 0.1124 0.1124
0.1376 0.1376 0.1124 0.1207 0.1551 0.1666 0.1387 0.1821 0.0948
0.1486 0.1452 0.1291 0.1291 0.0645 0.0566 0.0275 0.0419 0.0205
0.0870 0.0000 0.0417 0.0948 0.0419 0.0067 0.0419 0.0067 0.0948
0.1190 0.0716 0.0798 0.0948 0.0205 0.0948 0.0962 0.0948 0.0345
0.1042 0.1198 0.0417 0.0417 0.0489 0.1042 0.0636 0.0645 0.0489
0.1124 0.0417 0.0489 0.0792 0.0492 0.0876 0.1124 0.0417 0.0876
0.1132 0.1716 0.1042 0.1563 0.1366 0.1387 0.1282 0.1743 0.1539
0.1730 0.1539 0.1640 0.1539 0.1207 0.3238

V9488 0.1730 0.1551 0.1807 0.2008 0.2104 0.1807 0.1914 0.2008
0.1452 0.1366 0.1821 0.1807 0.1640 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1551 0.1627 0.1181
0.1652 0.1527 0.1452 0.1198 0.0948 0.0489 0.0273 0.0417 0.0203
0.1100 0.0417 0.0000 0.1181 0.0417 0.0343 0.0417 0.0343 0.1181
0.1263 0.0711 0.0640 0.1181 0.0203 0.1181 0.0798 0.1181 0.0067
0.0876 0.1272 0.0135 0.0135 0.0203 0.0876 0.0343 0.0640 0.0203
0.0955 0.0135 0.0203 0.0489 0.0205 0.1272 0.0955 0.0135 0.1272
0.1291 0.1792 0.1366 0.1730 0.1441 0.1551 0.1356 0.1821 0.1614
0.1807 0.1614 0.1716 0.1614 0.1282 0.3457

V9464 0.2280 0.2086 0.1792 0.1899 0.2086 0.1976 0.2280 0.1539
0.1792 0.1883 0.1899 0.1792 0.2086 0.1441 0.1930 0.1441 0.1441
0.1356 0.1703 0.1441 0.1356 0.1703 0.1821 0.1836 0.1703 0.0858
0.1758 0.1346 0.1914 0.1028 0.0636 0.1093 0.1020 0.1356 0.1100
0.0067 0.0948 0.1181 0.0000 0.1356 0.0942 0.1013 0.0942 0.0858
0.0935 0.1336 0.1263 0.0000 0.1100 0.0000 0.1108 0.0000 0.1100
0.1356 0.0787 0.1181 0.1181 0.1263 0.1356 0.1263 0.1100 0.1100
0.1441 0.1181 0.1263 0.1441 0.1272 0.0636 0.1441 0.1100 0.0636
0.1387 0.1263 0.1614 0.1652 0.1100 0.1652 0.1181 0.1452 0.1263
0.1356 0.1263 0.1527 0.1263 0.1108 0.3631

V9463 0.1836 0.1652 0.2104 0.2320 0.2220 0.1914 0.2025 0.2122
0.1551 0.1463 0.1836 0.1914 0.1652 0.1207 0.1408 0.1207 0.1207
0.1291 0.1291 0.1207 0.1291 0.1463 0.1575 0.1652 0.1821 0.1190
0.1575 0.1716 0.1640 0.1376 0.1035 0.0640 0.0275 0.0000 0.0205
0.1272 0.0419 0.0417 0.1356 0.0000 0.0345 0.0419 0.0345 0.1190
0.1272 0.0716 0.0798 0.1356 0.0205 0.1356 0.0962 0.1356 0.0345
0.1042 0.1452 0.0417 0.0417 0.0489 0.1042 0.0636 0.0798 0.0489
0.1124 0.0417 0.0489 0.0792 0.0492 0.1282 0.1124 0.0417 0.1282
0.1301 0.1992 0.1376 0.1743 0.1627 0.1563 0.1539 0.2025 0.1807
0.1914 0.1807 0.1914 0.1807 0.1463 0.3631

V9482 0.1821 0.1640 0.1716 0.1914 0.2008 0.1716 0.2008 0.1914
0.1366 0.1452 0.1821 0.1899 0.1640 0.1282 0.1398 0.1116 0.1116
0.1366 0.1366 0.1116 0.1198 0.1539 0.1652 0.1291 0.1716 0.0942
0.1387 0.1441 0.1198 0.1282 0.0640 0.0562 0.0203 0.0345 0.0135
0.0864 0.0067 0.0343 0.0942 0.0345 0.0000 0.0345 0.0000 0.0942
0.1181 0.0636 0.0716 0.0942 0.0135 0.0942 0.0876 0.0942 0.0273
0.0955 0.1190 0.0343 0.0343 0.0414 0.0955 0.0558 0.0566 0.0414
0.1035 0.0343 0.0414 0.0711 0.0417 0.0870 0.1035 0.0343 0.0870
0.1124 0.1703 0.0955 0.1551 0.1356 0.1376 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3202

V9492 0.1716 0.1539 0.1614 0.1992 0.2086 0.1976 0.1899 0.1807
0.1441 0.1356 0.1716 0.1792 0.1452 0.1028 0.1216 0.0870 0.0870
0.1108 0.1108 0.1028 0.1108 0.1272 0.1376 0.1387 0.1441 0.0858
0.1486 0.1346 0.1291 0.1190 0.0864 0.0343 0.0136 0.0419 0.0205
0.0935 0.0419 0.0417 0.1013 0.0419 0.0345 0.0000 0.0345 0.0706
0.0935 0.0414 0.0489 0.1013 0.0205 0.1013 0.0640 0.1013 0.0345
0.0716 0.0942 0.0417 0.0417 0.0489 0.0716 0.0489 0.0489 0.0489
0.0792 0.0417 0.0489 0.0640 0.0492 0.0942 0.0792 0.0345 0.0942
0.1474 0.1430 0.1108 0.1563 0.1100 0.1563 0.1181 0.1452 0.1263
0.1527 0.1263 0.1356 0.1263 0.1108 0.3631

V9473 0.1821 0.1640 0.1716 0.1914 0.2008 0.1716 0.2008 0.1914
0.1366 0.1452 0.1821 0.1899 0.1640 0.1282 0.1398 0.1116 0.1116
0.1366 0.1366 0.1116 0.1198 0.1539 0.1652 0.1291 0.1716 0.0942
0.1387 0.1441 0.1198 0.1282 0.0640 0.0562 0.0203 0.0345 0.0135

0.0864 0.0067 0.0343 0.0942 0.0345 0.0000 0.0345 0.0000 0.0942
0.1181 0.0636 0.0716 0.0942 0.0135 0.0942 0.0876 0.0942 0.0273
0.0955 0.1190 0.0343 0.0343 0.0414 0.0955 0.0558 0.0566 0.0414
0.1035 0.0343 0.0414 0.0711 0.0417 0.0870 0.1035 0.0343 0.0870
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0.1716 0.1527 0.1627 0.1527 0.1198 0.3202

V9517 0.2086 0.1899 0.1792 0.1899 0.2479 0.2559 0.2280 0.2280
0.1976 0.1883 0.2086 0.2164 0.1899 0.1272 0.1652 0.1272 0.1272
0.1527 0.1527 0.1441 0.1527 0.1356 0.1821 0.1652 0.1703 0.0706
0.1758 0.1020 0.1730 0.1356 0.1020 0.0782 0.0864 0.1190 0.0942
0.0935 0.0948 0.1181 0.0858 0.1190 0.0942 0.0706 0.0942 0.0000
0.0203 0.1173 0.1263 0.0858 0.0942 0.0858 0.1272 0.0858 0.1100
0.1356 0.1100 0.1020 0.1181 0.1263 0.1356 0.1263 0.1263 0.0942
0.1272 0.1020 0.1263 0.1441 0.1272 0.0787 0.1272 0.1100 0.0787
0.1836 0.1602 0.1614 0.1743 0.1430 0.1743 0.1516 0.1807 0.1430
0.1703 0.1430 0.1527 0.1430 0.1614 0.3768

V9518 0.1807 0.1627 0.1703 0.1807 0.2183 0.2261 0.1992 0.1992
0.1703 0.1614 0.1807 0.1883 0.1627 0.1356 0.1743 0.1527 0.1527
0.1614 0.1614 0.1527 0.1441 0.1441 0.1914 0.1743 0.1792 0.0935
0.1666 0.1263 0.1821 0.1441 0.1181 0.1013 0.1100 0.1272 0.1181
0.1013 0.1190 0.1263 0.0935 0.1272 0.1181 0.0935 0.1181 0.0203
0.0000 0.1420 0.1181 0.0935 0.1181 0.0935 0.1356 0.0935 0.1181
0.1441 0.1181 0.1100 0.1263 0.1346 0.1441 0.1346 0.1346 0.1020
0.1356 0.1100 0.1346 0.1527 0.1356 0.1020 0.1356 0.1181 0.1020
0.1743 0.1690 0.1703 0.1652 0.1516 0.1652 0.1602 0.1899 0.1516
0.1792 0.1516 0.1614 0.1516 0.1703 0.3497

V9647 0.1716 0.1539 0.1883 0.1716 0.1716 0.1883 0.1899 0.1899
0.1703 0.1614 0.1627 0.1703 0.1366 0.1108 0.1301 0.0792 0.0792
0.1190 0.1190 0.1108 0.1190 0.1356 0.1463 0.1652 0.1527 0.1173
0.1758 0.1516 0.1551 0.1272 0.1181 0.0632 0.0417 0.0716 0.0489
0.1254 0.0716 0.0711 0.1336 0.0716 0.0636 0.0414 0.0636 0.1173
0.1420 0.0000 0.0711 0.1336 0.0489 0.1336 0.0870 0.1336 0.0636
0.0792 0.1346 0.0711 0.0711 0.0636 0.0792 0.0787 0.0711 0.0787
0.0870 0.0711 0.0787 0.0640 0.0716 0.1263 0.0870 0.0636 0.1263
0.1563 0.1869 0.1356 0.1836 0.1516 0.1836 0.1602 0.1452 0.1690
0.1614 0.1690 0.1703 0.1690 0.1356 0.3631

V9694 0.1883 0.1703 0.1602 0.1976 0.1976 0.1602 0.1976 0.1792
0.1430 0.1346 0.1527 0.1602 0.1272 0.1181 0.1376 0.1020 0.1020
0.1263 0.1263 0.1181 0.1100 0.1430 0.1539 0.1551 0.1263 0.1100
0.1836 0.1614 0.1452 0.1181 0.1028 0.0711 0.0492 0.0798 0.0566
0.1181 0.0798 0.0640 0.1263 0.0798 0.0716 0.0489 0.0716 0.1263
0.1181 0.0711 0.0000 0.1263 0.0566 0.1263 0.0345 0.1263 0.0566
0.0489 0.1356 0.0640 0.0640 0.0640 0.0489 0.0792 0.0135 0.0716
0.0562 0.0640 0.0566 0.0645 0.0636 0.1356 0.0562 0.0566 0.1356
0.1575 0.1527 0.1020 0.1851 0.1527 0.1851 0.1614 0.1821 0.1527
0.1992 0.1527 0.1430 0.1527 0.1198 0.3404

V9683 0.2280 0.2086 0.1792 0.1899 0.2086 0.1976 0.2280 0.1539
0.1792 0.1883 0.1899 0.1792 0.2086 0.1441 0.1930 0.1441 0.1441
0.1356 0.1703 0.1441 0.1356 0.1703 0.1821 0.1836 0.1703 0.0858
0.1758 0.1346 0.1914 0.1028 0.0636 0.1093 0.1020 0.1356 0.1100
0.0067 0.0948 0.1181 0.0000 0.1356 0.0942 0.1013 0.0942 0.0858
0.0935 0.1336 0.1263 0.0000 0.1100 0.0000 0.1108 0.0000 0.1100
0.1356 0.0787 0.1181 0.1181 0.1263 0.1356 0.1263 0.1100 0.1100
0.1441 0.1181 0.1263 0.1441 0.1272 0.0636 0.1441 0.1100 0.0636
0.1387 0.1263 0.1614 0.1652 0.1100 0.1652 0.1181 0.1452 0.1263
0.1356 0.1263 0.1527 0.1263 0.1108 0.3631

V9622 0.1821 0.1640 0.1899 0.2104 0.2201 0.1899 0.2008 0.2104
0.1539 0.1452 0.1821 0.1899 0.1551 0.1116 0.1311 0.0955 0.0955
0.1198 0.1198 0.1116 0.1198 0.1366 0.1474 0.1463 0.1539 0.0942
0.1563 0.1441 0.1366 0.1116 0.0792 0.0417 0.0067 0.0205 0.0000
0.1020 0.0205 0.0203 0.1100 0.0205 0.0135 0.0205 0.0135 0.0942
0.1181 0.0489 0.0566 0.1100 0.0000 0.1100 0.0721 0.1100 0.0135
0.0798 0.1190 0.0203 0.0203 0.0273 0.0798 0.0414 0.0566 0.0273
0.0876 0.0203 0.0273 0.0562 0.0275 0.1028 0.0876 0.0203 0.1028
0.1207 0.1703 0.1116 0.1640 0.1356 0.1463 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3457

V9750 0.2280 0.2086 0.1792 0.1899 0.2086 0.1976 0.2280 0.1539
0.1792 0.1883 0.1899 0.1792 0.2086 0.1441 0.1930 0.1441 0.1441
0.1356 0.1703 0.1441 0.1356 0.1703 0.1821 0.1836 0.1703 0.0858
0.1758 0.1346 0.1914 0.1028 0.0636 0.1093 0.1020 0.1356 0.1100
0.0067 0.0948 0.1181 0.0000 0.1356 0.0942 0.1013 0.0942 0.0858
0.0935 0.1336 0.1263 0.0000 0.1100 0.0000 0.1108 0.0000 0.1100

0.1356 0.0787 0.1181 0.1181 0.1263 0.1356 0.1263 0.1100 0.1100
0.1441 0.1181 0.1263 0.1441 0.1272 0.0636 0.1441 0.1100 0.0636
0.1387 0.1263 0.1614 0.1652 0.1100 0.1652 0.1181 0.1452 0.1263
0.1356 0.1263 0.1527 0.1263 0.1108 0.3631

V9792 0.2086 0.1899 0.2069 0.2280 0.2183 0.2069 0.2183 0.1899
0.1883 0.1792 0.1807 0.1703 0.1716 0.1356 0.1387 0.1190 0.1190
0.1441 0.1441 0.1356 0.1272 0.1614 0.1730 0.2025 0.1272 0.1190
0.2239 0.1716 0.1914 0.1108 0.1282 0.0870 0.0645 0.0962 0.0721
0.1190 0.0962 0.0798 0.1108 0.0962 0.0876 0.0640 0.0876 0.1272
0.1356 0.0870 0.0345 0.1108 0.0721 0.1108 0.0000 0.1108 0.0721
0.0566 0.1366 0.0798 0.0798 0.0798 0.0566 0.0955 0.0345 0.0876
0.0640 0.0798 0.0798 0.0882 0.0792 0.1539 0.0640 0.0721 0.1539
0.1772 0.1627 0.1441 0.2059 0.1539 0.2059 0.1627 0.1836 0.1627
0.2008 0.1627 0.1703 0.1627 0.1207 0.3861

V9707 0.2280 0.2086 0.1792 0.1899 0.2086 0.1976 0.2280 0.1539
0.1792 0.1883 0.1899 0.1792 0.2086 0.1441 0.1930 0.1441 0.1441
0.1356 0.1703 0.1441 0.1356 0.1703 0.1821 0.1836 0.1703 0.0858
0.1758 0.1346 0.1914 0.1028 0.0636 0.1093 0.1020 0.1356 0.1100
0.0067 0.0948 0.1181 0.0000 0.1356 0.0942 0.1013 0.0942 0.0858
0.0935 0.1336 0.1263 0.0000 0.1100 0.0000 0.1108 0.0000 0.1100
0.1356 0.0787 0.1181 0.1181 0.1263 0.1356 0.1263 0.1100 0.1100
0.1441 0.1181 0.1263 0.1441 0.1272 0.0636 0.1441 0.1100 0.0636
0.1387 0.1263 0.1614 0.1652 0.1100 0.1652 0.1181 0.1452 0.1263
0.1356 0.1263 0.1527 0.1263 0.1108 0.3631

V9984 0.1640 0.1463 0.1716 0.1914 0.2008 0.1716 0.1821 0.1914
0.1366 0.1282 0.1730 0.1716 0.1551 0.1282 0.1486 0.1116 0.1116
0.1366 0.1198 0.1282 0.1198 0.1539 0.1652 0.1640 0.1539 0.1100
0.1743 0.1441 0.1539 0.1116 0.0870 0.0417 0.0203 0.0345 0.0135
0.1020 0.0345 0.0067 0.1100 0.0345 0.0273 0.0345 0.0273 0.1100
0.1181 0.0636 0.0566 0.1100 0.0135 0.1100 0.0721 0.1100 0.0000
0.0798 0.1190 0.0067 0.0067 0.0135 0.0798 0.0273 0.0566 0.0135
0.0876 0.0067 0.0135 0.0417 0.0136 0.1190 0.0876 0.0067 0.1190
0.1207 0.1703 0.1282 0.1640 0.1356 0.1463 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3329

V9983 0.1899 0.1716 0.1976 0.1992 0.1807 0.1792 0.1807 0.1992
0.1614 0.1527 0.1539 0.1614 0.1282 0.1356 0.1387 0.1190 0.1190
0.1527 0.1527 0.1272 0.1190 0.1527 0.1730 0.1930 0.1190 0.1108
0.2239 0.1627 0.1821 0.1272 0.1116 0.0948 0.0721 0.1042 0.0798
0.1272 0.1042 0.0876 0.1356 0.1042 0.0955 0.0716 0.0955 0.1356
0.1441 0.0792 0.0489 0.1356 0.0798 0.1356 0.0566 0.1356 0.0798
0.0000 0.1452 0.0876 0.0876 0.0876 0.0000 0.1035 0.0489 0.0955
0.0067 0.0876 0.0798 0.0882 0.0870 0.1452 0.0067 0.0798 0.1452
0.1866 0.1627 0.1441 0.2158 0.1627 0.2158 0.1716 0.1743 0.1627
0.1730 0.1627 0.1527 0.1627 0.1207 0.4051

V9939 0.1614 0.1441 0.1716 0.2201 0.1976 0.2086 0.1792 0.1551
0.1716 0.1627 0.1821 0.1716 0.1914 0.1627 0.2008 0.1539 0.1539
0.1716 0.1899 0.1627 0.1366 0.2086 0.1836 0.1946 0.1716 0.1263
0.2259 0.1792 0.2025 0.1282 0.1356 0.1181 0.1108 0.1452 0.1190
0.0711 0.1198 0.1272 0.0787 0.1452 0.1190 0.0942 0.1190 0.1100
0.1181 0.1346 0.1356 0.0787 0.1190 0.0787 0.1366 0.0787 0.1190
0.1452 0.0000 0.1272 0.1108 0.1356 0.1452 0.1356 0.1356 0.1190
0.1539 0.1272 0.1356 0.1452 0.1366 0.1356 0.1539 0.1190 0.1356
0.0962 0.0558 0.1716 0.1042 0.0414 0.1042 0.0486 0.0792 0.0558
0.0787 0.0558 0.0787 0.0558 0.0942 0.4252

V9943 0.1730 0.1551 0.1807 0.2008 0.2104 0.1807 0.1914 0.2008
0.1452 0.1366 0.1821 0.1807 0.1551 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1730 0.1627 0.1181
0.1836 0.1527 0.1627 0.1198 0.0948 0.0489 0.0273 0.0417 0.0203
0.1100 0.0417 0.0135 0.1181 0.0417 0.0343 0.0417 0.0343 0.1020
0.1100 0.0711 0.0640 0.1181 0.0203 0.1181 0.0798 0.1181 0.0067
0.0876 0.1272 0.0000 0.0135 0.0203 0.0876 0.0343 0.0640 0.0203
0.0798 0.0000 0.0203 0.0489 0.0205 0.1108 0.0955 0.0135 0.1108
0.1291 0.1792 0.1366 0.1730 0.1441 0.1551 0.1356 0.1821 0.1614
0.1807 0.1614 0.1716 0.1614 0.1282 0.3329

V9942 0.1730 0.1551 0.1807 0.2008 0.2104 0.1807 0.1914 0.2008
0.1452 0.1366 0.1821 0.1807 0.1640 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1551 0.1452 0.1181
0.1836 0.1527 0.1452 0.1198 0.0948 0.0489 0.0273 0.0417 0.0203
0.1100 0.0417 0.0135 0.1181 0.0417 0.0343 0.0417 0.0343 0.1181
0.1263 0.0711 0.0640 0.1181 0.0203 0.1181 0.0798 0.1181 0.0067
0.0876 0.1108 0.0135 0.0000 0.0203 0.0876 0.0343 0.0640 0.0203
0.0955 0.0135 0.0203 0.0489 0.0205 0.1272 0.0955 0.0135 0.1272

0.1207 0.1614 0.1198 0.1640 0.1272 0.1463 0.1190 0.1640 0.1441
0.1627 0.1441 0.1539 0.1441 0.1116 0.3457
V9975 0.1730 0.1551 0.1807 0.2008 0.2104 0.1807 0.1914 0.2008
0.1452 0.1366 0.1821 0.1807 0.1640 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1730 0.1627 0.1263
0.1836 0.1614 0.1627 0.1198 0.1028 0.0562 0.0343 0.0489 0.0273
0.1181 0.0489 0.0203 0.1263 0.0489 0.0414 0.0489 0.0414 0.1263
0.1346 0.0636 0.0640 0.1263 0.0273 0.1263 0.0798 0.1263 0.0135
0.0876 0.1356 0.0203 0.0203 0.0000 0.0876 0.0273 0.0640 0.0273
0.0955 0.0203 0.0273 0.0417 0.0205 0.1356 0.0955 0.0203 0.1356
0.1376 0.1883 0.1366 0.1640 0.1527 0.1463 0.1441 0.1730 0.1703
0.1899 0.1703 0.1716 0.1703 0.1366 0.3329
V9944 0.1899 0.1716 0.1976 0.1992 0.1807 0.1792 0.1807 0.1992
0.1614 0.1527 0.1539 0.1614 0.1282 0.1356 0.1387 0.1190 0.1190
0.1527 0.1527 0.1272 0.1190 0.1527 0.1730 0.1930 0.1190 0.1108
0.2239 0.1627 0.1821 0.1272 0.1116 0.0948 0.0721 0.1042 0.0798
0.1272 0.1042 0.0876 0.1356 0.1042 0.0955 0.0716 0.0955 0.1356
0.1441 0.0792 0.0489 0.1356 0.0798 0.1356 0.0566 0.1356 0.0798
0.0000 0.1452 0.0876 0.0876 0.0876 0.0000 0.1035 0.0489 0.0955
0.0067 0.0876 0.0798 0.0882 0.0870 0.1452 0.0067 0.0798 0.1452
0.1866 0.1627 0.1441 0.2158 0.1627 0.2158 0.1716 0.1743 0.1627
0.1730 0.1627 0.1527 0.1627 0.1207 0.4051
V9973 0.1551 0.1551 0.1452 0.1821 0.1914 0.1627 0.1551 0.1640
0.1116 0.1035 0.1640 0.1627 0.1640 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1551 0.1627 0.1430
0.1652 0.1792 0.1452 0.1366 0.1028 0.0711 0.0486 0.0636 0.0414
0.1181 0.0636 0.0343 0.1263 0.0636 0.0558 0.0489 0.0558 0.1263
0.1346 0.0787 0.0792 0.1263 0.0414 0.1263 0.0955 0.1263 0.0273
0.1035 0.1356 0.0343 0.0343 0.0273 0.1035 0.0000 0.0792 0.0414
0.1116 0.0343 0.0414 0.0562 0.0345 0.1356 0.1116 0.0343 0.1356
0.1376 0.1703 0.1282 0.1640 0.1356 0.1463 0.1272 0.1551 0.1527
0.1716 0.1527 0.1539 0.1527 0.1366 0.3457
V9952 0.2069 0.1883 0.1602 0.1976 0.1976 0.1602 0.2164 0.1792
0.1430 0.1516 0.1703 0.1778 0.1356 0.1181 0.1291 0.1020 0.1020
0.1263 0.1263 0.1020 0.0942 0.1430 0.1539 0.1551 0.1263 0.1100
0.1836 0.1614 0.1452 0.1181 0.0870 0.0711 0.0492 0.0798 0.0566
0.1020 0.0645 0.0640 0.1100 0.0798 0.0566 0.0489 0.0566 0.1263
0.1346 0.0711 0.0135 0.1100 0.0566 0.1100 0.0345 0.1100 0.0566
0.0489 0.1356 0.0640 0.0640 0.0640 0.0489 0.0792 0.0000 0.0716
0.0562 0.0640 0.0566 0.0645 0.0636 0.1190 0.0562 0.0566 0.1190
0.1666 0.1527 0.1020 0.1946 0.1527 0.1946 0.1614 0.1821 0.1527
0.1992 0.1527 0.1430 0.1527 0.1035 0.3404
V9961 0.1821 0.1640 0.1899 0.2104 0.2201 0.1899 0.2008 0.2008
0.1539 0.1452 0.1914 0.1899 0.1551 0.1282 0.1486 0.1282 0.1282
0.1539 0.1366 0.1452 0.1366 0.1539 0.1836 0.1730 0.1716 0.1100
0.1836 0.1441 0.1627 0.1282 0.1028 0.0562 0.0343 0.0489 0.0273
0.1020 0.0489 0.0203 0.1100 0.0489 0.0414 0.0489 0.0414 0.0942
0.1020 0.0787 0.0716 0.1100 0.0273 0.1100 0.0876 0.1100 0.0135
0.0955 0.1190 0.0203 0.0203 0.0273 0.0955 0.0414 0.0716 0.0000
0.1035 0.0203 0.0273 0.0562 0.0275 0.1190 0.1035 0.0203 0.1190
0.1207 0.1703 0.1452 0.1640 0.1356 0.1463 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3329
V9963 0.1992 0.1807 0.2069 0.2086 0.1899 0.1883 0.1899 0.2086
0.1703 0.1614 0.1627 0.1703 0.1282 0.1441 0.1474 0.1272 0.1272
0.1614 0.1614 0.1356 0.1272 0.1614 0.1821 0.2025 0.1272 0.1190
0.2340 0.1716 0.1914 0.1356 0.1198 0.1028 0.0798 0.1124 0.0876
0.1356 0.1124 0.0955 0.1441 0.1124 0.1035 0.0792 0.1035 0.1272
0.1356 0.0870 0.0562 0.1441 0.0876 0.1441 0.0640 0.1441 0.0876
0.0067 0.1539 0.0798 0.0955 0.0955 0.0067 0.1116 0.0562 0.1035
0.0000 0.0798 0.0876 0.0962 0.0948 0.1366 0.0135 0.0876 0.1366
0.1962 0.1716 0.1527 0.2259 0.1716 0.2259 0.1807 0.1836 0.1716
0.1821 0.1716 0.1614 0.1716 0.1291 0.4051
V9972 0.1730 0.1551 0.1807 0.2008 0.2104 0.1807 0.1914 0.2008
0.1452 0.1366 0.1821 0.1807 0.1551 0.1366 0.1575 0.1198 0.1198
0.1452 0.1282 0.1366 0.1282 0.1627 0.1743 0.1730 0.1627 0.1181
0.1836 0.1527 0.1627 0.1198 0.0948 0.0489 0.0273 0.0417 0.0203
0.1100 0.0417 0.0135 0.1181 0.0417 0.0343 0.0417 0.0343 0.1020
0.1100 0.0711 0.0640 0.1181 0.0203 0.1181 0.0798 0.1181 0.0067
0.0876 0.1272 0.0000 0.0135 0.0203 0.0876 0.0343 0.0640 0.0203
0.0798 0.0000 0.0203 0.0489 0.0205 0.1108 0.0955 0.0135 0.1108
0.1291 0.1792 0.1366 0.1730 0.1441 0.1551 0.1356 0.1821 0.1614
0.1807 0.1614 0.1716 0.1614 0.1282 0.3329

V10081 0.1821 0.1640 0.1716 0.2104 0.2201 0.1716 0.2008 0.1914
0.1366 0.1282 0.1730 0.1716 0.1551 0.1282 0.1486 0.1116 0.1116
0.1366 0.1198 0.1282 0.1198 0.1539 0.1652 0.1463 0.1539 0.1100
0.1743 0.1441 0.1366 0.1116 0.0870 0.0417 0.0343 0.0489 0.0273
0.1181 0.0489 0.0203 0.1263 0.0489 0.0414 0.0489 0.0414 0.1263
0.1346 0.0787 0.0566 0.1263 0.0273 0.1263 0.0798 0.1263 0.0135
0.0798 0.1356 0.0203 0.0203 0.0273 0.0798 0.0414 0.0566 0.0273
0.0876 0.0203 0.0000 0.0417 0.0275 0.1356 0.0876 0.0203 0.1356
0.1376 0.1703 0.1282 0.1821 0.1527 0.1640 0.1441 0.1914 0.1527
0.1899 0.1527 0.1627 0.1527 0.1198 0.3589
V10000 0.2025 0.1836 0.1821 0.2025 0.2220 0.2008 0.2220 0.1836
0.1640 0.1551 0.1836 0.1821 0.1652 0.1376 0.1497 0.1042 0.1042
0.1463 0.1291 0.1376 0.1291 0.1640 0.1758 0.1743 0.1640 0.1272
0.2042 0.1627 0.1640 0.1376 0.1198 0.0716 0.0489 0.0792 0.0562
0.1356 0.0792 0.0489 0.1441 0.0792 0.0711 0.0640 0.0711 0.1441
0.1527 0.0640 0.0645 0.1441 0.0562 0.1441 0.0882 0.1441 0.0417
0.0882 0.1452 0.0489 0.0489 0.0417 0.0882 0.0562 0.0645 0.0562
0.0962 0.0489 0.0417 0.0000 0.0492 0.1539 0.0962 0.0489 0.1539
0.1652 0.1807 0.1376 0.1930 0.1627 0.1930 0.1716 0.1836 0.1627
0.2008 0.1627 0.1640 0.1627 0.1291 0.3047
V10003 0.1627 0.1452 0.1703 0.1899 0.2086 0.1703 0.1899 0.1899
0.1356 0.1272 0.1716 0.1703 0.1539 0.1272 0.1474 0.1108 0.1108
0.1356 0.1190 0.1272 0.1190 0.1527 0.1640 0.1627 0.1527 0.1272
0.1730 0.1627 0.1527 0.1108 0.1035 0.0566 0.0345 0.0492 0.0275
0.1190 0.0492 0.0205 0.1272 0.0492 0.0417 0.0492 0.0417 0.1272
0.1356 0.0716 0.0636 0.1272 0.0275 0.1272 0.0792 0.1272 0.0136
0.0870 0.1366 0.0205 0.0205 0.0205 0.0870 0.0345 0.0636 0.0275
0.0948 0.0205 0.0275 0.0492 0.0000 0.1366 0.0948 0.0205 0.1366
0.1387 0.1899 0.1356 0.1836 0.1539 0.1652 0.1452 0.1836 0.1716
0.1914 0.1716 0.1703 0.1716 0.1376 0.3238
V10075 0.2501 0.2400 0.1807 0.1821 0.2300 0.2183 0.2501 0.2008
0.1899 0.1992 0.2104 0.2183 0.2008 0.1366 0.1851 0.1366 0.1366
0.1452 0.1627 0.1366 0.1452 0.1627 0.1930 0.1666 0.1992 0.0787
0.1587 0.1272 0.1743 0.1366 0.0492 0.1020 0.0948 0.1282 0.1028
0.0562 0.0876 0.1272 0.0636 0.1282 0.0870 0.0942 0.0870 0.0787
0.1020 0.1263 0.1356 0.0636 0.1028 0.0636 0.1539 0.0636 0.1190
0.1452 0.1356 0.1108 0.1272 0.1356 0.1452 0.1356 0.1190 0.1190
0.1366 0.1108 0.1356 0.1539 0.1366 0.0000 0.1539 0.1190 0.0000
0.1666 0.1703 0.1539 0.1946 0.1527 0.1946 0.1614 0.1914 0.1703
0.1807 0.1703 0.1627 0.1703 0.1366 0.3202
V10138 0.1992 0.1807 0.1883 0.1899 0.1899 0.1883 0.1899 0.2086
0.1703 0.1614 0.1452 0.1527 0.1366 0.1441 0.1474 0.1272 0.1272
0.1614 0.1614 0.1356 0.1272 0.1614 0.1821 0.2025 0.1272 0.1190
0.2340 0.1716 0.1914 0.1356 0.1198 0.0870 0.0798 0.1124 0.0876
0.1356 0.1124 0.0955 0.1441 0.1124 0.1035 0.0792 0.1035 0.1272
0.1356 0.0870 0.0562 0.1441 0.0876 0.1441 0.0640 0.1441 0.0876
0.0067 0.1539 0.0955 0.0955 0.0955 0.0067 0.1116 0.0562 0.1035
0.0135 0.0955 0.0876 0.0962 0.0948 0.1539 0.0000 0.0876 0.1539
0.1962 0.1716 0.1527 0.2259 0.1716 0.2259 0.1807 0.1836 0.1539
0.1821 0.1539 0.1441 0.1539 0.1291 0.4197
V10177 0.1640 0.1463 0.1716 0.1914 0.2008 0.1716 0.1821 0.1914
0.1366 0.1282 0.1730 0.1716 0.1551 0.1282 0.1486 0.1116 0.1116
0.1366 0.1198 0.1282 0.1198 0.1539 0.1652 0.1730 0.1539 0.1100
0.1836 0.1441 0.1627 0.1116 0.0870 0.0417 0.0273 0.0417 0.0203
0.1020 0.0417 0.0135 0.1100 0.0417 0.0343 0.0345 0.0343 0.1100
0.1181 0.0636 0.0566 0.1100 0.0203 0.1100 0.0721 0.1100 0.0067
0.0798 0.1190 0.0135 0.0135 0.0203 0.0798 0.0343 0.0566 0.0203
0.0876 0.0135 0.0203 0.0489 0.0205 0.1190 0.0876 0.0000 0.1190
0.1291 0.1703 0.1282 0.1730 0.1356 0.1551 0.1272 0.1730 0.1527
0.1716 0.1527 0.1627 0.1527 0.1198 0.3457
V10076 0.2501 0.2400 0.1807 0.1821 0.2300 0.2183 0.2501 0.2008
0.1899 0.1992 0.2104 0.2183 0.2008 0.1366 0.1851 0.1366 0.1366
0.1452 0.1627 0.1366 0.1452 0.1627 0.1930 0.1666 0.1992 0.0787
0.1587 0.1272 0.1743 0.1366 0.0492 0.1020 0.0948 0.1282 0.1028
0.0562 0.0876 0.1272 0.0636 0.1282 0.0870 0.0942 0.0870 0.0787
0.1020 0.1263 0.1356 0.0636 0.1028 0.0636 0.1539 0.0636 0.1190
0.1452 0.1356 0.1108 0.1272 0.1356 0.1452 0.1356 0.1190 0.1190
0.1366 0.1108 0.1356 0.1539 0.1366 0.0000 0.1539 0.1190 0.0000
0.1666 0.1703 0.1539 0.1946 0.1527 0.1946 0.1614 0.1914 0.1703
0.1807 0.1703 0.1627 0.1703 0.1366 0.3202
97-144 0.1758 0.1575 0.1772 0.1882 0.1851 0.1772 0.1758 0.1787
0.1497 0.1497 0.1787 0.1679 0.1666 0.2059 0.2340 0.1962 0.1962

0.2361 0.2361 0.1962 0.1679 0.2570 0.2510 0.1666 0.2465 0.1836
0.1866 0.2239 0.1652 0.1679 0.1486 0.1563 0.1291 0.1301 0.1207
0.1301 0.1132 0.1291 0.1387 0.1301 0.1124 0.1474 0.1124 0.1836
0.1743 0.1563 0.1575 0.1387 0.1207 0.1387 0.1772 0.1387 0.1207
0.1866 0.0962 0.1291 0.1207 0.1376 0.1866 0.1376 0.1666 0.1207
0.1962 0.1291 0.1376 0.1652 0.1387 0.1666 0.1962 0.1291 0.1666
0.0000 0.1291 0.1587 0.0414 0.0962 0.0273 0.0882 0.1398 0.1124
0.1050 0.1124 0.1387 0.1124 0.1132 0.3910

97-120 0.1976 0.1792 0.1716 0.2201 0.1976 0.1716 0.1792 0.1730
0.1716 0.1627 0.1821 0.1716 0.2104 0.1627 0.2201 0.1716 0.1716
0.1716 0.1899 0.1807 0.1716 0.2086 0.1836 0.2140 0.1899 0.1263
0.2677 0.1883 0.2220 0.1539 0.1527 0.1690 0.1614 0.1992 0.1703
0.1181 0.1716 0.1792 0.1263 0.1992 0.1703 0.1430 0.1703 0.1602
0.1690 0.1869 0.1527 0.1263 0.1703 0.1263 0.1627 0.1263 0.1703
0.1627 0.0558 0.1792 0.1614 0.1883 0.1627 0.1703 0.1527 0.1703
0.1716 0.1792 0.1703 0.1807 0.1899 0.1703 0.1716 0.1703 0.1703
0.1291 0.0000 0.1899 0.1207 0.0273 0.1376 0.0343 0.0792 0.0273
0.0787 0.0273 0.0489 0.0273 0.0787 0.4406

97-127 0.1992 0.1899 0.1527 0.1899 0.1716 0.1703 0.1899 0.1899
0.1356 0.1441 0.1807 0.1883 0.1807 0.1792 0.1930 0.1614 0.1614
0.1883 0.1883 0.1614 0.1527 0.2069 0.2201 0.1216 0.1883 0.1614
0.1666 0.1992 0.0962 0.1883 0.1282 0.1356 0.1035 0.1376 0.1116
0.1527 0.1042 0.1366 0.1614 0.1376 0.0955 0.1108 0.0955 0.1614
0.1703 0.1356 0.1020 0.1614 0.1116 0.1614 0.1441 0.1614 0.1282
0.1441 0.1716 0.1366 0.1198 0.1366 0.1441 0.1282 0.1020 0.1452
0.1527 0.1366 0.1282 0.1376 0.1356 0.1539 0.1527 0.1282 0.1539
0.1587 0.1899 0.0000 0.1679 0.1716 0.1679 0.1807 0.2122 0.1716
0.2008 0.1716 0.1614 0.1716 0.1376 0.3366

97-118 0.1851 0.1666 0.1679 0.1787 0.1946 0.2059 0.1851 0.1882
0.1772 0.1772 0.2077 0.1962 0.1758 0.2158 0.2443 0.2059 0.2059
0.2465 0.2465 0.2259 0.1962 0.2677 0.2619 0.1575 0.2361 0.1930
0.1772 0.2140 0.1563 0.2158 0.1946 0.1836 0.1551 0.1743 0.1640
0.1563 0.1563 0.1730 0.1652 0.1743 0.1551 0.1563 0.1551 0.1743
0.1652 0.1836 0.1851 0.1652 0.1640 0.1652 0.2059 0.1652 0.1640
0.2158 0.1042 0.1730 0.1640 0.1640 0.2158 0.1640 0.1946 0.1640
0.2259 0.1730 0.1821 0.1930 0.1836 0.1946 0.2259 0.1730 0.1946
0.0414 0.1207 0.1679 0.0000 0.1042 0.0135 0.1124 0.1486 0.1042
0.1132 0.1042 0.1301 0.1042 0.1387 0.4212

R98533 0.1614 0.1441 0.1539 0.2008 0.1614 0.1899 0.1441 0.1551
0.1539 0.1452 0.1640 0.1539 0.2104 0.1807 0.2400 0.1899 0.1899
0.1899 0.2086 0.1807 0.1716 0.2280 0.2025 0.2140 0.2086 0.1430
0.2465 0.2069 0.2025 0.1539 0.1527 0.1346 0.1272 0.1627 0.1356
0.1020 0.1366 0.1441 0.1100 0.1627 0.1356 0.1100 0.1356 0.1430
0.1516 0.1516 0.1527 0.1100 0.1356 0.1100 0.1539 0.1100 0.1356
0.1627 0.0414 0.1441 0.1272 0.1527 0.1627 0.1356 0.1527 0.1356
0.1716 0.1441 0.1527 0.1627 0.1539 0.1527 0.1716 0.1356 0.1527
0.0962 0.0273 0.1716 0.1042 0.0000 0.1042 0.0067 0.0492 0.0273
0.0489 0.0273 0.0489 0.0273 0.0636 0.3814

R98537 0.1666 0.1486 0.1679 0.1979 0.1946 0.2059 0.1666 0.1882
0.1587 0.1587 0.2077 0.1962 0.1946 0.2361 0.2653 0.2259 0.2259
0.2677 0.2677 0.2259 0.1962 0.2898 0.2841 0.1575 0.2570 0.2122
0.1772 0.2340 0.1563 0.1962 0.1758 0.1836 0.1551 0.1563 0.1463
0.1563 0.1387 0.1551 0.1652 0.1563 0.1376 0.1563 0.1376 0.1743
0.1652 0.1836 0.1851 0.1652 0.1463 0.1652 0.2059 0.1652 0.1463
0.2158 0.1042 0.1551 0.1463 0.1463 0.2158 0.1463 0.1946 0.1463
0.2259 0.1551 0.1640 0.1930 0.1652 0.1946 0.2259 0.1551 0.1946
0.0273 0.1376 0.1679 0.0135 0.1042 0.0000 0.0962 0.1486 0.1207
0.1132 0.1207 0.1474 0.1207 0.1387 0.4212

R98517 0.1527 0.1356 0.1627 0.2104 0.1703 0.1807 0.1356 0.1640
0.1452 0.1366 0.1730 0.1627 0.2201 0.1899 0.2501 0.1992 0.1992
0.1992 0.2183 0.1899 0.1807 0.2379 0.2122 0.2239 0.2183 0.1516
0.2570 0.2164 0.2122 0.1452 0.1441 0.1430 0.1356 0.1539 0.1272
0.1100 0.1282 0.1356 0.1181 0.1539 0.1272 0.1181 0.1272 0.1516
0.1602 0.1602 0.1614 0.1181 0.1272 0.1181 0.1627 0.1181 0.1272
0.1716 0.0486 0.1356 0.1190 0.1441 0.1716 0.1272 0.1614 0.1272
0.1807 0.1356 0.1441 0.1716 0.1452 0.1614 0.1807 0.1272 0.1614
0.0882 0.0343 0.1807 0.1124 0.0067 0.0962 0.0000 0.0566 0.0343
0.0562 0.0343 0.0562 0.0343 0.0711 0.3956

R5755 0.2008 0.1821 0.2122 0.2443 0.1914 0.2320 0.1821 0.2140
0.1930 0.1836 0.2042 0.1930 0.2239 0.2122 0.2760 0.2220 0.2220
0.2220 0.2421 0.2122 0.2122 0.2421 0.2361 0.2487 0.2421 0.1807
0.2841 0.2300 0.2361 0.1930 0.1914 0.1716 0.1640 0.2025 0.1730

0.1366 0.1743 0.1821 0.1452 0.2025 0.1730 0.1452 0.1730 0.1807
0.1899 0.1452 0.1821 0.1452 0.1730 0.1452 0.1836 0.1452 0.1730
0.1743 0.0792 0.1821 0.1640 0.1730 0.1743 0.1551 0.1821 0.1730
0.1836 0.1821 0.1914 0.1836 0.1836 0.1914 0.1836 0.1730 0.1914
0.1398 0.0792 0.2122 0.1486 0.0492 0.1486 0.0566 0.0000 0.0640
0.0570 0.0640 0.0798 0.0640 0.1035 0.4212

R9952 0.1976 0.1792 0.1539 0.2008 0.1976 0.1899 0.1792 0.1730
0.1716 0.1627 0.1640 0.1539 0.1914 0.1627 0.2201 0.1716 0.1716
0.1716 0.1899 0.1807 0.1716 0.2086 0.1836 0.2140 0.1899 0.1263
0.2677 0.1883 0.2025 0.1539 0.1527 0.1346 0.1441 0.1807 0.1527
0.1181 0.1539 0.1614 0.1263 0.1807 0.1527 0.1263 0.1527 0.1430
0.1516 0.1690 0.1527 0.1263 0.1527 0.1263 0.1627 0.1263 0.1527
0.1627 0.0558 0.1614 0.1441 0.1703 0.1627 0.1527 0.1527 0.1527
0.1716 0.1614 0.1527 0.1627 0.1716 0.1703 0.1539 0.1527 0.1703
0.1124 0.0273 0.1716 0.1042 0.0273 0.1207 0.0343 0.0640 0.0000
0.0636 0.0000 0.0205 0.0000 0.0636 0.4252

R10151 0.1778 0.1602 0.1792 0.1899 0.1690 0.2069 0.1602 0.1899
0.1883 0.1792 0.1716 0.1614 0.2220 0.2104 0.2843 0.2201 0.2201
0.2201 0.2400 0.2104 0.2008 0.2201 0.2340 0.2122 0.2201 0.1703
0.2443 0.1992 0.2239 0.1640 0.1807 0.1792 0.1627 0.1914 0.1716
0.1272 0.1730 0.1807 0.1356 0.1914 0.1716 0.1527 0.1716 0.1703
0.1792 0.1614 0.1992 0.1356 0.1716 0.1356 0.2008 0.1356 0.1716
0.1730 0.0787 0.1807 0.1627 0.1899 0.1730 0.1716 0.1992 0.1716
0.1821 0.1807 0.1899 0.2008 0.1914 0.1807 0.1821 0.1716 0.1807
0.1050 0.0787 0.2008 0.1132 0.0489 0.1132 0.0562 0.0570 0.0636
0.0000 0.0636 0.0870 0.0636 0.1028 0.3631

R10745 0.1976 0.1792 0.1539 0.2008 0.1976 0.1899 0.1792 0.1730
0.1716 0.1627 0.1640 0.1539 0.1914 0.1627 0.2201 0.1716 0.1716
0.1716 0.1899 0.1807 0.1716 0.2086 0.1836 0.2140 0.1899 0.1263
0.2677 0.1883 0.2025 0.1539 0.1527 0.1346 0.1441 0.1807 0.1527
0.1181 0.1539 0.1614 0.1263 0.1807 0.1527 0.1263 0.1527 0.1430
0.1516 0.1690 0.1527 0.1263 0.1527 0.1263 0.1627 0.1263 0.1527
0.1627 0.0558 0.1614 0.1441 0.1703 0.1627 0.1527 0.1527 0.1527
0.1716 0.1614 0.1527 0.1627 0.1716 0.1703 0.1539 0.1527 0.1703
0.1124 0.0273 0.1716 0.1042 0.0273 0.1207 0.0343 0.0640 0.0000
0.0636 0.0000 0.0205 0.0000 0.0636 0.4252

E00111 0.1960 0.1778 0.1356 0.1807 0.1869 0.1883 0.1690 0.1716
0.1703 0.1614 0.1627 0.1703 0.1899 0.1614 0.2183 0.1703 0.1703
0.1703 0.1883 0.1792 0.1703 0.2069 0.1821 0.2122 0.1883 0.1356
0.2653 0.1992 0.2008 0.1527 0.1627 0.1441 0.1539 0.1914 0.1627
0.1441 0.1640 0.1716 0.1527 0.1914 0.1627 0.1356 0.1627 0.1527
0.1614 0.1703 0.1430 0.1527 0.1627 0.1527 0.1703 0.1527 0.1627
0.1527 0.0787 0.1716 0.1539 0.1716 0.1527 0.1539 0.1430 0.1627
0.1614 0.1716 0.1627 0.1640 0.1703 0.1627 0.1441 0.1627 0.1627
0.1387 0.0489 0.1614 0.1301 0.0489 0.1474 0.0562 0.0798 0.0205
0.0870 0.0205 0.0000 0.0205 0.0870 0.4007

R10996 0.1976 0.1792 0.1539 0.2008 0.1976 0.1899 0.1792 0.1730
0.1716 0.1627 0.1640 0.1539 0.1914 0.1627 0.2201 0.1716 0.1716
0.1716 0.1899 0.1807 0.1716 0.2086 0.1836 0.2140 0.1899 0.1263
0.2677 0.1883 0.2025 0.1539 0.1527 0.1346 0.1441 0.1807 0.1527
0.1181 0.1539 0.1614 0.1263 0.1807 0.1527 0.1263 0.1527 0.1430
0.1516 0.1690 0.1527 0.1263 0.1527 0.1263 0.1627 0.1263 0.1527
0.1627 0.0558 0.1614 0.1441 0.1703 0.1627 0.1527 0.1527 0.1527
0.1716 0.1614 0.1527 0.1627 0.1716 0.1703 0.1539 0.1527 0.1703
0.1124 0.0273 0.1716 0.1042 0.0273 0.1207 0.0343 0.0640 0.0000
0.0636 0.0000 0.0205 0.0000 0.0636 0.4252

R98543 0.2379 0.2183 0.1914 0.2220 0.1807 0.2104 0.1992 0.1652
0.1730 0.1821 0.1930 0.1821 0.1836 0.1463 0.1930 0.1551 0.1551
0.1730 0.1730 0.1291 0.1207 0.1914 0.2042 0.1962 0.1640 0.1272
0.2487 0.1899 0.1851 0.1376 0.1198 0.1190 0.1116 0.1463 0.1198
0.1028 0.1207 0.1282 0.1108 0.1463 0.1198 0.1108 0.1198 0.1614
0.1703 0.1356 0.1198 0.1108 0.1198 0.1108 0.1207 0.1108 0.1198
0.1207 0.0942 0.1282 0.1116 0.1366 0.1207 0.1366 0.1035 0.1198
0.1291 0.1282 0.1198 0.1291 0.1376 0.1366 0.1291 0.1198 0.1366
0.1132 0.0787 0.1376 0.1387 0.0636 0.1387 0.0711 0.1035 0.0636
0.1028 0.0636 0.0870 0.0636 0.0000 0.3861

SABIN3 0.3674 0.3674 0.2989 0.3079 0.3274 0.3631 0.3674 0.3538
0.3631 0.3768 0.3329 0.3497 0.3538 0.3497 0.4212 0.3631 0.3631
0.3366 0.3631 0.3366 0.3238 0.3497 0.4007 0.3956 0.4051 0.3631
0.3719 0.3814 0.4156 0.3580 0.3538 0.3631 0.3329 0.3631 0.3457
0.3631 0.3238 0.3457 0.3631 0.3631 0.3202 0.3631 0.3202 0.3768
0.3497 0.3631 0.3404 0.3631 0.3457 0.3631 0.3861 0.3631 0.3329

```
0.4051  0.4252  0.3329  0.3457  0.3329  0.4051  0.3457  0.3404  0.3329
0.4051  0.3329  0.3589  0.3047  0.3238  0.3202  0.4197  0.3457  0.3202
0.3910  0.4406  0.3366  0.4212  0.3814  0.4212  0.3956  0.4212  0.4252
0.3631  0.4252  0.4007  0.4252  0.3861  0.0000
```

Figure 22: Amino acid sequence of VP1/2A region of wild poliovirus type 3 isolated from year 1971 to 2000.

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#SABIN3 RPPRAVPYYG PGVDYRNNLD PLSEKGLTTY GFGHQNKAVY TAGYKICN
#V55     .......... .....KDG.A .......... .......... ........
#V335    .......... .....KDG.A .......... .......... ........
#V445    .......... .....KDG.A .......... .......... ........
#V512    .......... .....KDG.A .......... .......... ........
#V583    .......... .....KDG.T .......... .......... ........
#V787    .......... .....KDG.A .......... .L........ ........
#V802    .......... .....KDG.A .......... .......... ........
#V861    .......... .....KDG.A .......... .......... ........
#V904    .......... .....KDG.A .......... .......... ........
#V963    .......... .....KDG.A .......... .......... ........
#V1007   .......... .....KDG.A .......... .......... ........
#V1067   .......... .....KDG.A .......... .......... ........
#V1087   .......... .....KDG.A .......... .......... ........
#V1130   .......... .....KDG.A .......... .......... ........
#V1201   .......... .....KDG.A .......... .......... ........
#V1202   .......... .....KDG.A .......... .......... ........
#V1725   .......... .....KDG.A .......... .......... ........
#V1825   .......... .....KDG.A .......... .......... ........
#V2820   .......... .....KDG.A .......... ....R..... ........
#V2843   .......... .....KDG.A .......... .......... ........
#V3104   .......... .....KDG.A .......... .......... ........
#V3458   .......... .....KDG.A .....S.... .......... ........
#V3757   .......... .....KDG.A .......... .......... ...F....
#V5222   .......... .....KDG.A .......... .......... ........
#V5399   .......... .....KDG.A .......... .......... ........
#V5402   .......... .....KDG.A .......... .......... ........
#V7202   .......... .....KDG.A .......... .......... ...F....
#V7210   .......... .....KDG.A .......... .......... V.......
#V8188   .......... .....KDG.A .......... .......... ........
#V5228   .......... .....KDG.A .......... .......... ........
#V9193   .......... .....KDG.A .......... .......... ........
#V9222   .......... .....KDG.A .......... .......... ........
#V9267   .......... .....KDG.A .......... .......... ........
#V9274   .......... .....KDG.A ..A....... .......... ........
#V9275   .......... .....KDG.A .......... .......... ........
#V9476   .......... .....KDG.A .......... .......... ........
#V9477   .......... .....KDG.A .......... ....H..... ........
#V9488   .......... .....KDG.A .......... .......... ........
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#V9647   .......... .....KDG.T .......... .......... ........
#V9694   .......... .....KDG.A .......... .L........ ........
#V9683   .......... .....KDG.A .......... .......... ........
#V9622   .......... .....KDG.A .......... .......... ........
#V9750   .......... .....KDG.A .......... .......... ........
#V9792   .......... .....KDG.A .......... .L........ ........
#V9707   .......... .....KDG.A .......... .......... ........
```

```
#V9984  .......... .....KDG.A .......... .......... ........
#V9983  .......... .....KDG.A .......... .L........ ........
#V9939  .......... .....KDG.A .......... .......... ........
#V9943  .......... .....KDG.A .......... .......... ........
#V9942  .......... .....KDG.A .......... .......... ........
#V9975  .......... .....KDG.A .......... ..E....... ........
#V9944  .......... .....KDG.A .......... .L........ ........
#V9973  .......... .....KDG.A .......... .......... ........
#V9952  .......... .....KDG.A .......... .L........ ........
#V9961  .......... .....KDG.A .......... .......... ........
#V9963  .......... .....KDG.A .......... .L........ ........
#V9972  .......... .....KDG.A .......... .......... ........
#V10081 .......... .....KDG.A .......... .......... ........
#V10000 .......... .....KDD.E .......... .......... ........
#V10003 .......... .....K.G.A .......... .......... ........
#V10075 .......... .....KDG.A .......... .......... ........
#V10138 .......... .....KDG.A .......... .L........ ........
#V10177 .......... .....KDG.A .......... .......... ........
#V10076 .......... .....KDG.A .......... .......... ........
#97-144 .......... .....KDG.A .......... .......... ........
#97-120 .......... .....KDG.A .......... .L........ ........
#97-127 .......... .....KDG.A .......... .......... ........
#97-118 .......... .....KDG.A .......... ..E....... ........
#R98533 .......... .....KDG.A .......... .......... ........
#R98537 .......... .....KDG.A .......... ..E....... ........
#R98517 .......... .....KDG.A .......... .......... ........
#R5755  .......... .....KDG.P .......... ..R....... ........
#R9952  .......... .....KDG.A .......... .......... ........
#R10151 .......... .....KDG.A .......... .......... ........
#R10745 .......... .....KDG.A .......... .......... ........
#E00111 .......... .....KDG.A .......... .......... ........
#R10996 .......... .....KDG.A .......... .......... ........
#R98543 .......... .....KDG.A .......... .......... ........

#SABIN3 YH
#V55    ..
#V335   ..
#V445   ..
#V512   ..
#V583   ..
#V787   ..
#V802   ..
#V861   ..
#V904   ..
#V963   ..
#V1007  ..
#V1067  ..
#V1087  ..
#V1130  ..
#V1201  ..
#V1202  ..
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#V7210  ..
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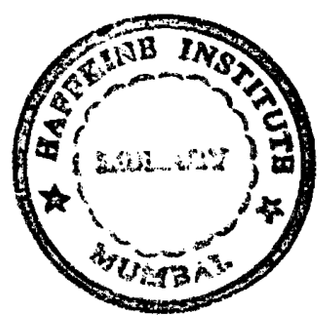

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#V9274 ..
#V9275 ..
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#V9952 ..
#V9961 ..
#V9963 ..
#V9972 ..
#V10081 ..
#V10000 ..
#V10003 ..
#V10075 ..
#V10138 ..
#V10177 ..
#V10076 ..
#97-144 ..
#97-120 ..
#97-127 ..
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#R98533 ..
#R98537 ..
#R98517 ..
#R5755 ..
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#R10745 ..
#E00111 ..
#R10996 ..
#R98543 ..

Genomic relationship of Wild poliovirus type 3 from Mumbai (1971-89)

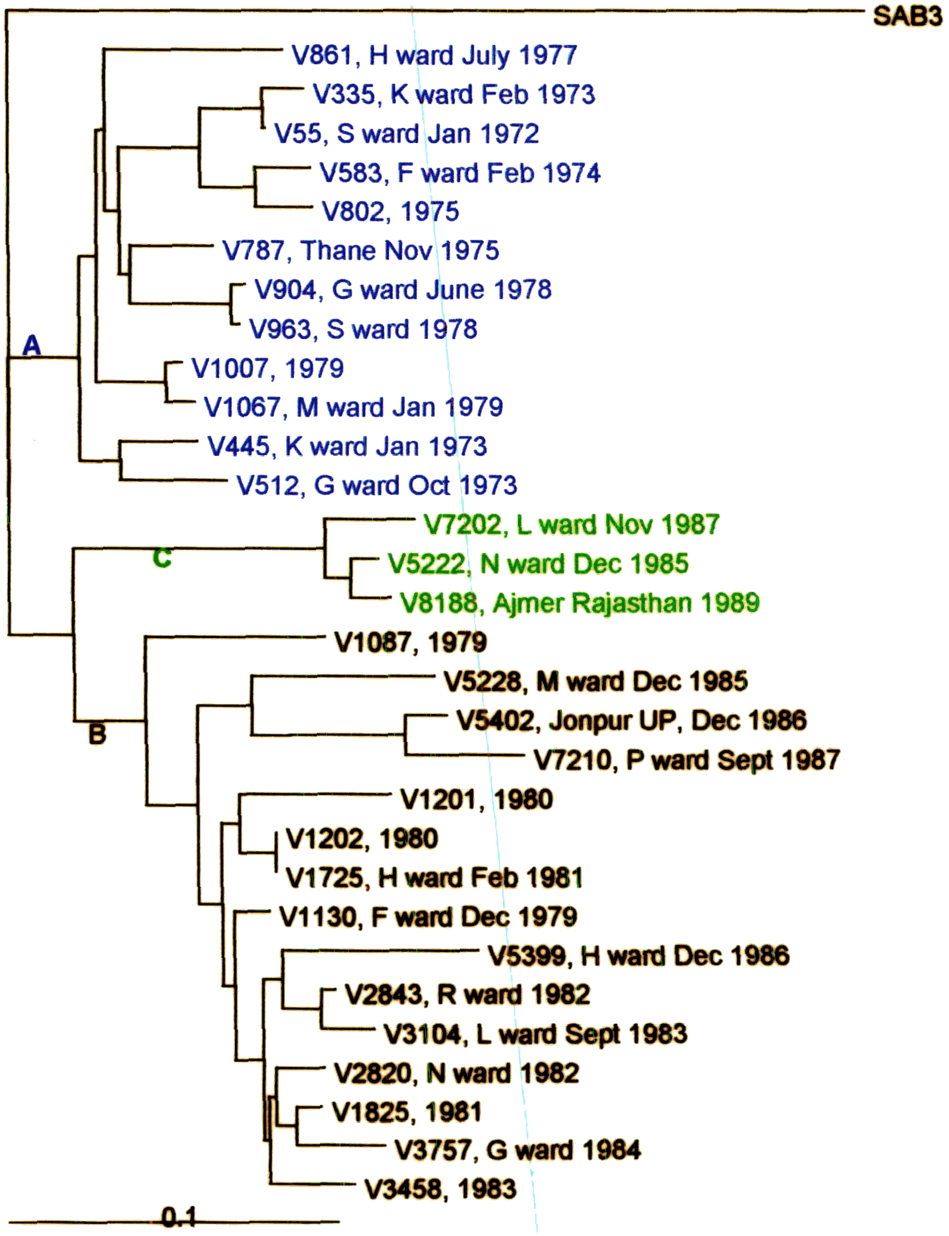

Figure 20: Phylogenetic relationship of wild poliovirus type 3 based on VP1/2A junction sequence data. The relationship was determined by drawing dendrogram by using NJ method of pakage PHYLIP v3.5c.

Genomic relationship of Wild poliovirus type 3 from Mumbai (1972-2000)

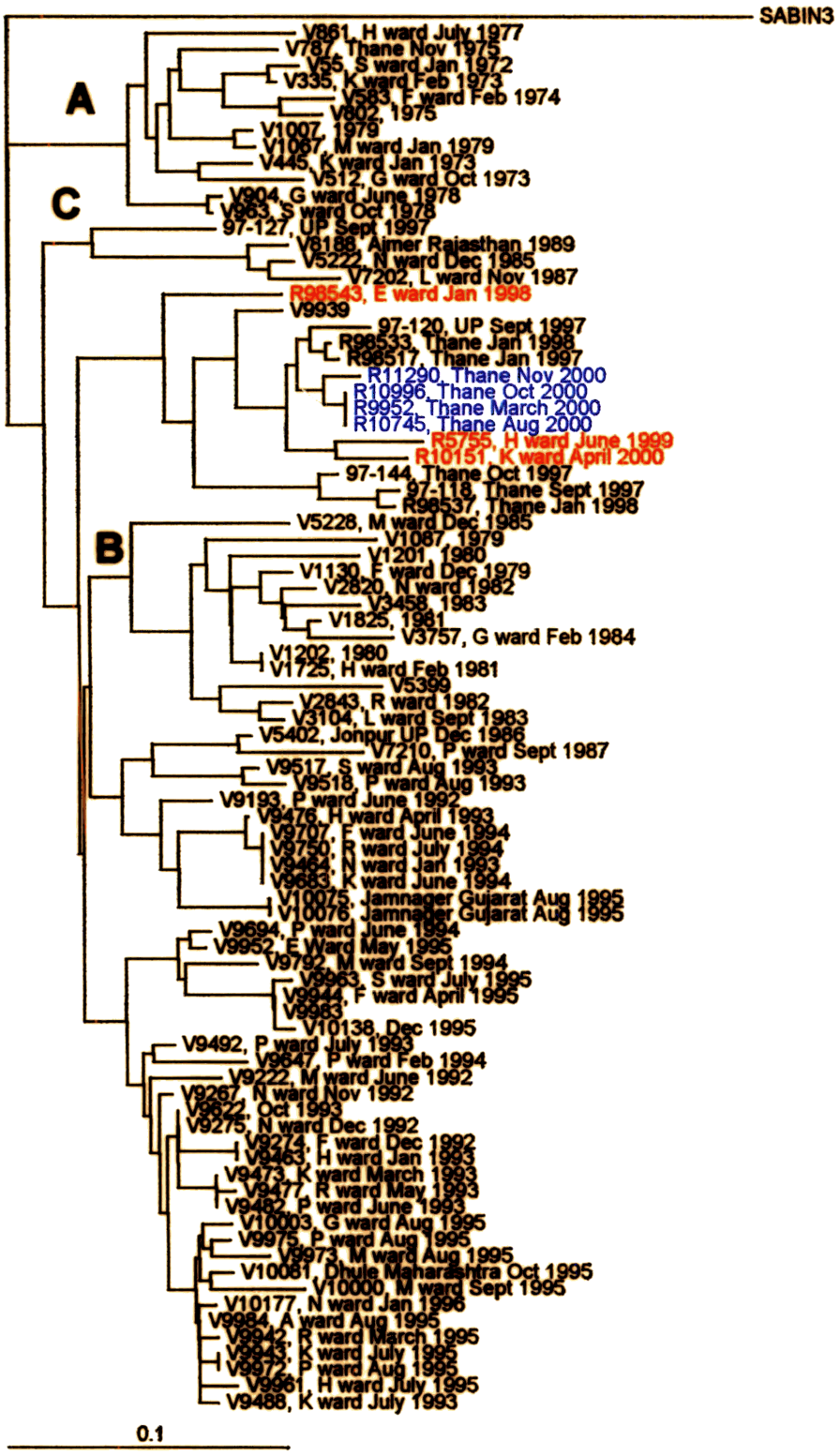

Figure 21: Phylogenetic relationship of wild type 3 poliovirus based on the VP1/2A junction sequence data. The dendrogram was drawn using NJ method of package PHYLIP v3.5c.

Phylogenetic relationship of wild poliovirus type 3 from different parts of India based on VP1/2A sequence data:

VP1/2A junction region of a total of 134 wild poliovirus type 3 isolated during the years 1997-2001 was sequenced to establish phylogenetic relationship. The details of these isolates are given in Table 8. All the isolates from the low polio burden areas isolated between 1997 and 2001 were sequenced whereas selective strains from the high polio burden states were sequenced for the phylogenetic analysis. All the virus isolates were compared with the Sabin type 3 polio vaccine strain to confirm wild origin. Figure 23 represents the sequence alignment of the isolates. Genetic distance calculated using Kimura 2 Parameter algorithm is presented in Figure 24. Phylogenetic relationship in the form of dendrogram is shown in Figure 25 and 26.

Sequence data shows that all the wild type 3 poliovirus grouped into three clusters with more than 15% sequence divergence indicating the circulation of three genotype 1, 2 and 3.

Genotype 1 was detected from the Chitradurga, Karnataka in the year 1997 represented by virus numbered as R98-010. This is the only isolate representing genotype 1, as the earlier isolates were not available for the study because of the fact that AFP surveillance was initiated in the year 1997.

Virus isolates belonging to the genotype 2 were detected in Utter Pradesh (R10829), Karnataka (R98-2207) and West Bengal (R5118, R5253). The earliest representative of the genotype 2 in the database was represented by the isolate 97-127 from a case of paralytic poliomyelitis from Uttar Pradesh that was brought to Mumbai for medical treatment. The sequence diversity between the isolates suggests presence of three independent lineages circulating in the different states. Genotype 2 transmission was detected during the period 1997-2000, after which the genotype has not been detected

Genotype 3 was the major genotype of poliovirus type 3 in India. The widespread transmission of the viruses of this genotype in the country resulted in development of several lineages. Wild poliovirus type 3 infections were endemic in India and resulted in development of transmission chains. At least six lineages with more than 5% sequence divergence were circulating within genotype 3 in different states. The virus of the lineage 3a was isolated from states of Maharashtra (R98-342 and R98-537), Karnataka (R98-019 and R98-891) and three cases of Gujarat (R98-1513, R98-1694 and R98-1722). Lineage 3b was shared between Madhya Pradesh (R981616 and R981620) and West Bengal (R98-2809 and R98-3302). West Bengal has its own lineage circulated in 1998 (R98-613, R98-958, and R98-613) and the virus of the same lineage was detected from one case of Tamil Nadu (R98-492). In Utter Pradesh the 6 viruses sequenced in the year 1998 clustered in one lineage named as 3c. Three orphan lineages were also detected which were isolated from Tamil Nadu (R98-702), Maharashtra (R98-543) and Bihar (R4083).

Sequence analysis was useful in tracking wild virus transmission. More than 98% sequence similarity was found for the following virus isolates, (R98-891, R98-019, R98-537, R98-1722, R98-1513). High sequence similarly was found when the cases are epidemiologically linked. The data therefore shows transmission of virus between the three states viz. Karnataka, Maharashtra and Gujarat. It was also found that transmission of the viruses of this lineage was eliminated from Maharashtra and Karnataka by the end of 1998. however, the virus continued circulation in Junagarh, Gujarat causing at least one case of paralytic poliomyelitis (R4398). The dendrogram also shows that one of the poliovirus type 3 lineage was shared between Karnataka and Andhra Pradesh. Sequence data shows that the four cases in Andhra Pradesh (1999) were epidemiologically linked.

More than 600 cases of wild type 3 etiology were reported from infection occurred in Utter Pradesh with more than 600 cases. This

represented a large outbreak of polio type 3 in India. The sequence data shows that the outbreak was due to a single lineage. The epidemic virus was isolated from polio cases from far and wide geographical areas including Punjab, Maharashtra, Karnataka, Gujarat, Rajasthan and West Bengal. The outbreak virus replaced all the other previously circulating lineages from the country. The lineage from the outbreak virus continued in the year 2000

Karnataka eliminated transmission of the 1998 lineage (R98-2165). However it had already established infections in Andhra Pradesh. It is interesting to note that the epidemic virus from Utter Pradesh gained entry in Karnataka in 1999 (R8223, R8227), continued to circulate and caused paralytic disease in the year 2000 (R12055).

Six polio cases due to wild poliovirus type 3 infections were reported from Maharashtra in 2000. Five cases were due to the outbreak virus, which was previously detected in the state in the year 1999. The origin of the virus responsible for the paralytic case in Mumbai (R10151) could not be ascertained. Sequence data showed that the virus was not similar to the UP outbreak virus. The genetically most closely related isolates were from Andhra Pradesh. But the genetic distance between Andhra Pradesh viruses and Mumbai isolate highlights surveillance gap.

Another interesting information generated through sequence studies relates to importation and subsequent establishment of indigenous transmission of the outbreak virus from Utter Pradesh in Thane district in Maharashtra (R9952, R10745, R10996, R11290). All the viruses show very close genetic distance with the virus from Basti, Utter Pradesh and diverging sufficiently in the area to become transmission chain. The year 2000 transmission chain from the Basti district continued in the year 2001 and caused two infections (R12275, R12276), which are related to each other.

Figure 27 represents the amino acid sequences of the wild poliovirus type 3 in the VP1/2A junction. All the wild poliovirus shows four

major amino acid changes when compare to the Sabin poliovirus type 3 vaccine strain in the VP1 region. At 286th amino acid an arginine to lysine, at 287th asparagine to aspartic acid , at 288th asparagine to glycine and at 290th aspartic acid to alanine change was observed.

Phylogenetic relationship of wild poliovirus type 3 from different parts of India based on VP1 sequence data:

Entire VP1 protein of the selected wild type 3 poliovirus isolates from the year 1998 to 2001 isolated from different parts of India were sequenced for high resolution molecular epidemiology. The details of the isolates sequenced are present in Table 9. Figure 28 shows the aligned sequences of the isolate. The genomic distances are shown in Figure 29.

Phylogenetic relationship of wild poliovirus type 3 using complete VP1 sequence is shown in Figure 30. The viruses includes isolates from the states of Andhra Pradesh, Bihar, Delhi, Gujarat, Haryana, Karnataka, Maharashtra, Madhya Pradesh, Tamil Nadu, Utter Pradesh and West Bengal. All the virus isolates between this periods shows less than 15% sequence divergence indicating the circulation of one genotype throughout India.

The last wild type 3 poliovirus from Tamil Nadu was isolated from 1998. We have sequenced all the wild type 3 poliovirus from Karnataka from the year 1999 and 2000 and all the year 1999 isolates from Andhra Pradesh. It can be seen from the dendrogram that the isolates from Utter Pradesh, West Bengal, Andhra Pradesh, Tamil Nadu and Maharashtra of the year 1998-99 show a very large diversity of the sequences. This information indicates circulation of different lineages in each of these geographical areas. The data also shows that at least four lineages of wild poliovirus type 3 existed in West Bengal in the year 1999. Furthermore, more than one lineage was observed in Murshidabad district. In the year 1999 more than three lineages were detected in the state of Utter Pradesh. In case of Utter Pradesh, however we have not been able to

sequence majority of the isolates because of the fact that there were more than 600 cases in that year.

As can be seen from the Figure 30 the four cases from the state of Andhra Pradesh in the year 1999 two each from East Godavari and West Godavari belong to one lineage and distance indicates a rapid transmission of the parental virus through this cases. No case due to wild type 3 infection was isolated after these four cases and so this was supposed to be the last cases due to wild type 3 etiology in this state.

As mentioned earlier in this thesis, an outbreak of wild poliovirus type 3 occurred in the state of Utter Pradesh in the year 1999. The dendrogram in Figure 26 (VP1/2A) shows that the outbreak strain was a single major lineage, as can be jugged from isolation of genetically closely related viruses from different districts of Utter Pradesh. It is most interesting to note from the dendrogram that the outbreak strain spread to Bihar, Delhi, Haryana, Punjab, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Karnataka and West Bengal. This study also shows the spread of virus in Karnataka in the year 1999, survival of the strain in the year 2000 and causing paralytic disease in the Raichure district. No wild type 3 poliovirus was isolated after this in Karnataka.

The dendrogram also very elegantly shows the establishment of indigenous circulation of wild type 3 poliovirus in Thane district in Maharashtra, once the virus was introduced from Utter Pradesh. The data also shows that the virus, which came in Maharashtra, was most likely from The Basti district, Utter Pradesh. This thinking is supported by the fact that closely similar virus was isolated from the Basti district in the year 2001.

The virus transmission continued in the eastern Utter Pradesh in the year 2001-2002. Poliovirus being RNA virus has a higher mutation rate because of the absence of proof reading mechanism. The lineage, which showed high sequence similarity during the spread within Utter Pradesh and outside, the state in the year 1999 continued to diverged through

nucleotide substitution (mutation) by the year 2001. the outbreak virus has diverged to show as individual lineages or clusters. At least four lineages can now be identified in the year 2001-02.. this can be identified in the dendrogram as virus circulation in Moradabad, Meerut, Bareiley, Bijnor, Basti, Ambedkar Nagar, Gonda and Behrampur region.

The successful programmatic action could be documented from the dendrogram as the virus circulation in West Bengal, Maharashtra, Karnataka, Gujarat, Rajasthan, and Haryana etc by the end of the year 2000. However the virus transmission remains in circulation in the western parts of Utter Pradesh. It can also be seen from virus isolates from Badaun district, the year 2000-01 chain has been broken but the virus re-introduced from Moradabad region causing at least three cases in 2002. Another interesting finding is the documentation of silent transmission (? Surveillance gap) in Gonda region. The viruses isolated from Behrampur region (UP BRP 02011, 012,and 022) were genetically of UP GND 01153 origin. These two districts are adjacent districts in Utter Pradesh. The time lapsed from UP GND 01153 (isolated in January 2001) to the Behrampur cases are about 18 months. No cases were reported from Behrampue, Gonda, silent transmission (?), and surveillance gap (?) of wild poliovirus type 3 during these 18 months period.

Thus multiple transmission chain, high sequence bio diversity, spread of epidemic virus, effects of control measures, as well as failure of polio eradication program in some places has been documented.

Figure 31 represents the amino acid sequences of wild type 3 poliovirus deduced from the nucleotide sequences. The wild type 3 and Sabin type 3 vaccine strain differ in as many as 26 amino acid. Majority of the amino acid changes were at the 5' end of the VP1 protein. The 89-100th amino acids are the major antigenic sites in all the three poliovirus serotypes. The strain (WB MBD 99 007) showed threonine to serine change at the 97th amino acid from VP1. At the 98th amino acid from the

VP1 region three strains (GU AMC 00 039, WB MBD 99 011, and WB CAL 00 077) shows arginine to lysine change.

Table 8: List of wild poliovirus type 3 isolated from different parts of India sequenced in the VP1/2A junction region

Sr. No.	Epid No.	R No.	Month and year of isolation
1	AP EGV 99 008	R-5799	May 1999
2	AP EGV 99 012	R-6053	June 1999
3	AP WGV 99 025	R-6774	September 1999
4	AP WGV 99 026	R-7586	September 1999
5	BI CPE 98 043	R98 2953	August 1998
6	BI KHA 98 023	R-4083	November 1998
7	BI MBD 99 015	R-8682	November 1998
8	BI PRN 99 006	R-8719	November 1999
9	BI CPE 99 050	R-9082	December 1999
10	BI BJR 00 001	R-9524	February 2000
11	BI CPE 00 003	R-9374	January 2000
12	BI DBG 00 015	R-10175	May 2000
13	BI GYA 00 004	R-9320	January 2000
14	BI MBD 00 003	R-9167	January 2000
15	BI MBD 00 004	R-9309	January 2000
16	BI MBD 00 008	R-9681	March 2000
17	BI MZF 00 001	R-9117	January 2000
18	BI MZF 00 002	R-9136	January 2000
19	BI PRN 00 001	R-9108	January 2000
20	BI PTN 00 002	R-9523	February 2000
21	BI PTN 99 129	R-9101	December 1999
22	BI RTS 00 001	R-9187	January 2000
23	BI SAM 00 001	R-9324	January 2000
24	BI SIT 00 001	R-9376	January 2000
25	CH CHD 99 037	R-6700	August 1999
26	DL DLH 99 402	R-8988	December 1999
27	DL DLH 99 410	R- 8994	December 1999
28	DL DLH 00 004	R-9190	January 2000
29	GU AMC 98 008	R98-1513	February 1998
30	GU BVC 98 002	R98-1694	July 1998
31	GU BVN 98 055	R98-1722	July 1998
32	GU BVN 99 013	R-8958	December 1999
33	GU JUN 99 001	R-4398	January 1999
34	GU AMC 00 039	R-10443	July 2000
35	HA FTB 99 009	R-8129	November 1999

Sr. No.	Epid No.	R No.	Month and year of isolation
36	HA KKR 99 006	R-6698	October 1999
37	HA KTL 99 006	R-7674	October 1999
38	HA PKL 99 006	R-6692	August 1999
39	KA CDG 97 001	R98-10	Year 1997
40	KA GBG 98 004	R98-019	January 1998
41	KA BDR 98 010	R98-2165	September 1998
42	KA GBG 98 018	R98-891	March 1998
43	KA GBG 98 042	R98-2202	October 1998
44	KA DKN 99 018	R-7603	October 1998
45	KA GBG 99 021	R-8223	November 1999
46	KA GBG 99 023	R-8227	November 1999
47	KA RCR 00 016	R-12055	December 2000
48	MH ANG 98 048	R98-2809	Year 1998
49	MH BMC 98 008	R98-533	January 1998
50	MH BMC 98 025	R98-537	January 1998
51	MH BMC 98 052	R98-543	March 1998
52	MH DHL 98 004	R98-342	February 1998
53	MH STR 98 004	R98-528	March 1998
54	MH THN 98 001	R98-517	January 1998
55	MH BLD 99 011	R-9075	December 1999
56	MH BMC 99 052	R-5755	March 1999
57	MH PNA 99 020	R-5714	June 1999
58	MH RTG 99 008	R-8483	December 1999
59	MH BMC 00 019	R-9952	March 2000
60	MH BMC 00 037	R-10151	April 2000
61	MH BMC 00 084	R-10745	August 2000
62	MH BMC 00 113	R-11290	October 2000
63	MH BLD 00 001	R-9402	January 2000O
64	MH THN 00 015	R-10996	October 2000
65	MP TKM 98 002	R98-1620	July 1998
66	MP GLR 98 010	R98-1616	July 1998
67	MP CTP 99 016	R-8564	December 1999
68	MP DUR 99 008	R-6597	March 1999
69	MP RGH 99 024	R-7896	July 1999
70	MPJBA 99 004	R-8048	November 1999
71	MPJBA 00 001	R-9252	January 2000
72	PB PTN 99 016	R-8800	Year 1999
73	RJ ALW 99 016	R-8513	November 1999
74	RJ BTP 99 011	R-7960	October 1999
75	RJ BTP 99 012	R-8509	November 1999
76	RJ JPR 99 045	R-7018	September 1999
77	RJ JPR 99 074	R-9242	December 1999
78	TN CBE 98-006	R98-702	March 1998

Sr. No.	Epid No.	R No.	Month and year of isolation
79	TN CNI 98-014	R98-492	April 1998
80	UP BRC 98 009	R98-1439	July 1998
81	UP MRD 98 017	R98-2542	July 1998
82	UP RMP 98 021	R98-2872	August 1998
83	UP RMP 98 029	R98-2720	September 1998
84	UP RMP 98 033	R98-3081	September 1998
85	UP UDS 98 019	R98-3088	August 1998
86	UP AGR 99 005	R-5764	June 1999
87	UP BST 99 023	R-5889	July 1999
88	UP BST 99 046	R-7801	October 1999
89	UP JAL 99 018	R-7550	October 1999
90	UP STP 99 032	R-7522	October 1999
91	UP ALG 00 001	R-9114	January 2000
92	UP ABN 00 006	R-9332	January 2000
93	UP ABN 00 014	R-9914	August 2000
94	UP BLS 00 001	R-9180	January 2000
95	UP BLS 00 002	R-9182	January 2000
96	UP BRC 00 001	R-9144	January 2000
97	UP BRL 00 001	R-9102	January 2000
98	UP BST 00 030	R-11859	November 2000
99	UP BST 00 031	R-12051	December 2000
100	UP ETA 00 001	R-9112	January 2000
101	UP ETA 00 024	R-10653	August 2000
102	UP FAI 00 018	R-10699	August 2000
103	UP FER 00 001	R-9527	February 2000
104	UP GND 00 166	R-10829	August 2000
105	UP LNO 00 047	R-10647	June 2000
106	UP KPD 00 001	R-9175	January 2000
107	UP KRI 00 001	R-9104	January 2000
108	UP KRI 00 002	R-9165	January 2000
109	UP MZF 00 002	R-9515	February 2000
110	UP PIL 00 001	R-9110	January 2000
111	UP RMP 00 020	R-10706	June 2000
112	UP STP 00 001	R-9372	January 2000
113	UP BST 01 002	R-12275	January 2001
114	UP BST 01 003	R-12276	February 2001
115	UP BAD 01 001	R-12122	January 2001
116	UP GND 01 153	R-12234	January 2001
117	UP MRD 01 014	R-12787	April 2001
118	WB CAL 98 036	R98-958	April 1998
119	WB CAL 98 037	R98-963	May 1998
120	WB CAL 98 070	R98-2077	September 1998
121	WB CAL 98 088	R98-3300	October 1998

Sr. No.	Epid No.	R No.	Month and year of isolation
122	WB CAL 98 089	R98-3302	October 1998
123	WB TML 98 002	R98-613	March 1998
124	WB BBM 99 002	R-5132	March 1999
125	WB HRA 99 013	R-5914	July 1999
126	WB MBD 99 007	R-5118	February 1999
127	WB MBD 99 011	R-5246	March 1999
128	WB MBD 99 014	R-5253	March 1999
129	WB MLD 99 002	R-4332	January 1999
130	WB UDJ 99 039	R-9054	December 1999
131	WB CAL 00 077	R-9721	March 2000
132	WB CAL 00 165	R-11260	October 2000
133	WB HRA 00 001	R-9196	January 2000
134	WB SPG 00 001	R-9444	February 2000

Table 9: List of wild poliovirus type 3 isolated from different parts of India sequenced in the VP1 region.

Sr. No.	Epid No.	R No.	Month and year of isolation
1	AP EGV 99 008	R-5799	May 1999
2	AP EGV 99 012	R-6053	June 1999
3	AP WGV 99 025	R-6774	September 1999
4	AP WGV 99 026	R-7586	September 1999
5	BI DBG 00 015	R-10175	May 2000
6	BI MBD 00 008	R-9681	March 2000
7	BI MZF 00 002	R-9136	January 2000
8	DL DLH 01 170	R-14453	October 2001
9	DL DLH 02 100	R-18509	July 2002
10	DL DLH 02 108	R-19288	July 2002
11	GU AMC 00 039	R-10443	July 2000
12	HA GUR 00006	R-9989	April 2000
13	KA DKN 99 018	R-7603	October 1999
14	KA GBG 99 021	R-8223	November 1999
15	KA GBG 99 023	R-8227	November 1999
16	KA RCR 00 016	R-12055	December 2000
17	MH BLD 99 011	R-9075	December 1999
18	MH BMC 00 019	R-9952	March 2000
19	MH BMC 00 037	R-10151	April 2000
20	MH BMC 00 084	R-10745	August 2000
21	MH BMC 00 113	R-11290	October 2000
22	MH BLD 00 001	R-9402	January 2000O
23	MH THN 00 015	R-10996	October 2000
24	MP JBA 00 001	R-9525	January 2000
25	TN CBE 98 006	R98-492	March 1998
26	UP BST 99 023	R-5889	July 1999
27	UP BST 99 046	R-7801	October 1999
28	UP ABN 00 014	R-9914	August 2000
29	UP ALG 00 001	R-9114	January 2000
30	UP BAD 00 015	R-10713	August 2000
31	UP BLS 00 003	R-9182	January 2000
32	UP BRL 00 001	R-9104	January 2000
33	UP BST 00 004	R-9519	January 2000
34	UP BST 00 030	R-11859	November 2000
35	UP BST 00 031	R-12051	December 2000
36	UP ETA 00 024	R-10653	August 2000
37	UP FAI 00 018	R-10699	August 2000
38	UP GND 00 152	R-10178	May 2000
39	UP GND 00 166	R-10829	August 2000

Sr. No.	Epid No.	R No.	Month and year of isolation
40	UP KRI 00 002	R-9165	January 2000
41	UP LNO 00 047	R-10647	August 2000
42	UP PIL 00 001	R-9110	January 2000
43	UP RMP 00 020	R-10706	August 2000
44	UP ABN 01 014	R-15580	December 2000
45	UP BAD 01 001	R-12122	January 2001
46	UP BJN 01 017	R-14588	October 2001
47	UP BRL 01 024	R-13392	July 2001
48	UP BRL 01 086	R-15794	December 2001
49	UP BRL 01 026	R-13411	July 2001
50	UP BRL 01 034	R-13661	August 2001
51	UP BRL 01 039	R-13810	August 2001
52	UP BST 01 002	R-12275	January 2001
53	UP BST 01 003	R-12276	February 2001
54	UP GND 01 153	R-12234	January 2001
55	UP MRD 01 039	R-13315	July 2001
56	UP MRD 01 099	R-14374	October 2001
57	UP MRD 01 107	R-14688	October 2001
58	UP MRT 01 047	R-15922	December 2001
59	UP ABN 02 002	R-16560	March 2002
60	UP ABN 02 017	R-17962	June 2002
61	UP BAD 02 008	R-16492	February 2002
62	UP BAD 02 014	R-17027	April 2002
63	UP BAD 02 027	R-17747	June 2002
64	UP BAD 02 052	R-19666	July 2002
65	UP BRP 02 011	R-18416	June 2002
66	UP BRP 02 012	R-18631	July 2002
67	UP BRP 02 022	R-20819	September 2002
68	UP MRD 02 003	R-16201	January 2002
69	UP MRT 02 026	R-17346	May 2002
70	UP MRT 02 033	R-18302	July 2002
71	WB BBM 99 002	R-5132	March 1999
72	WB HRA 99 013	R-5914	July 1999
73	WB MBD 99 007	R-5118	February 1999
74	WB MBD 99 011	R-5246	March 1999
75	WB MBD 99 014	R-5253	March 1999
76	WB CAL 00 077	R-9721	March 2000
77	WB CAL 00 165	R-11260	October 2000
78	WB MBD 00 006	R-9925	April 2000
79	WB TML 00 007	R-9931	March 2000

Figure 23: Nucleotide sequence alignment of the wild type 3 poliovirus in the VP1\2A junction region (1997-2001) isolated from different parts of India.

```
R98543      CGGCCCCCAC GAGCGGTGCC ATACTACGGT CCAGGGGTGG ACTACAAGGA TGGTTTAGCG
97-127      ..A..... ....A..... ...T.....A ........A. ....T..... ...A..G..A
R982202     ..A..... ....A..A.. ...T.....A ..G..... .......... ......G...
R5118       ..A..... ....A..... .........A ..G..... ....T..... ......G...
R4083       .......... .......A.. ...T.....C .......... .T........ ......G..A
R8513       .......... .......... .........A .......... .......... C.........
R9144       .......... .......A.. ...T.....A ........A. .......... ..........
R98010      .......... .G..A..A.. G........A ..G.....A. .......... ...C..G..A
R98492      .......... .......A.. ..........A .......... .......... .........A
R98958      .......... ...T...A.. .........A .......... .......... .........A
R98702      .....T.... .......... G......... .......... .T........ ..........
97-120      .....T.... .......... .........A .......... .......... ..........
R5914       .......... .......A.. .........A .......... ....T..... ......G..A
R982077     .......... .G.....A.. .........A .......... .T........ ..........
R98533      .....T.... .......A.. .........A .......... .......... ..........
R9721       .......... .......A.. .........A .......... .......... ..........
R982165     .......... .......A.. .........A .......... .T........ ..........
R10151      ........G. .......A.. .........A .......... .T........ ..........
R5799       .......... .......A.. T........A .......... .T........ ..........
R9252       .......... .......A.. ...T.....A .......... .......... ..........
R10647      .......... .......... .........A .......... .......... ..........
R6698       .......... .......A.. .........A .......... ....T..... ..........
R981439     .......... ....A..A.. ...T.....A .......... .......... ..........
R98537      ........G. ....A..A.. ...T.....A .......... .......... .........A
R7801       ........G. ....A..A.. ...T.....A .......... .......... ......G...
R981620     ........G. ....A..A.. ...T.....A .......... .......... ..........
SAB3        A.A..G..G. .C.....A.. T..T..T..A .......... ....T.G.A. CAAC..G.AC

R98543      CCCCTATCGG AGAAGGGACT CACGACTTAT GGATTTGGAC ACCAGAATAA AGCAGTGTAC
97-127      ...T.G.... .......... ...C...... ..C....... ....A..C.. ...G..A...
R982202     ...T....T. ....A..... T..C..C... .......... ....A..C.. ...T......
R5118       ...T....A. .......T.. ...C..C... ..G....... ....A..... ...G.....T
R4083       ..T....... .......... ...A.....C ..G....... .......C.. .........T
R8513       ..T.....A. .......... ...A..C..C ..T.....C. .T.....C.. ...C..C...
R9144       ..T.....A. ....A..... ...A...... ..T....... .......... G........T
R98010      ...T...... .......... ...C...... ..T..C.... .......... G.....A..T
R98492      ..T.....A. .......... ...A...... .......... .......... .........T
R98958      ..T.....A. .......... ...A...... .......... .......... .........T
R98702      ..T....... .......... ...A...... .......... .......... ......A..T
97-120      ..T.....A. .......... ...A...... ...C...... .......... .........T
R5914       ..T....... ....A..... ...A...... .......... .......... .........T
R982077     ..T.....A. .......... ...A...... .......... .......... ..........
R98533      ..T.....A. .......... ...A...... .......... .......... ..........
R9721       ..T.....A. .......... ...A...... .......... .......... ..........
R982165     ..T.....A. .......... ...A...... .......... .T........ G.........
R10151      ..T.....A. .......... ...A...... .......... .T........ G..G......
R5799       ..T.....A. .......... ...A...... .......... .T........ G.........
R9252       ..T.....A. .......... ...A...... .....C.... .......... ..........
R10647      ..T.....A. .......... ...A...... ..G..C.... .......... ..........
R6698       ..T.....A. .......... ...A...... .....C.... .......... ..........
R981439     ..T....... .......... ...A..A... .......... .......C.. .........T
R98537      ..T....... .......... ...A...... .......A.. .......C.. ...T..C...
R7801       ..T....... .......... ...A...... .......... .......C.. ...T..C...
R981620     ..T....... .......... ...A...... .......... .......C.. ...T..C...
SAB3        ...T....T. ....A..TT. G..C..A... ..C.....G. .T........ ...T......

R98543      ACAGCCGGGT ACAAAATCTG CAATTACCAC
97-127      .......... .......... ...C......
R982202     .......... .T..G..T.. ......T...
R5118       .....T..T. .......T.. ......T..T
R4083       ..G..T.... ....G..T.. T..C......
R8513       ........A. .......T.. ..........
R9144       .......... ....G..T.. ...C......
R98010      ..G..T.... ....G..T.. T..C......
```

```
R98492      .......... .......T.. T..C......
R98958      .....T.... ....G..T.. T..........
R98702      .......... .......... T..C..T...
97-120      .......... .T.....T.. T..C......
R5914       .......... .......... T..C......
R982077     .......... .......... T.....T...
R98533      .......... .......T.. T..C......
R9721       .......... .......T.. T..C......
R982165     .......... .......T.. T..C......
R10151      ........T. .......T.. T..C......
R5799       .......... .......T.. T..C......
R9252       .......... .......T.. T..C......
R10647      .......... .T.....T.. T..C......
R6698       .......... .......T.. T..C......
R981439     .....T..T. .......T.. ......T..T
R98537      .....A..A. .......A.. T..C..T...
R7801       ..T..A..A. .......A.. ...C..T...
R981620     .....G..A. .......A.. ...C..T...
SAB3        ..T..T..T. ....G..... ...C.....T
```

Figure 24: Genomic distances for sequences in the VP1/2A junction region calculated using Kimura 2 Parameter method of wild poliovirus type 3 isolated from different parts of India (1997-2001).

R98543	0.0000	0.1376	0.1551	0.1551	0.1336	0.1132	0.1028	0.1807
	0.0711	0.0864	0.0706	0.0787	0.0787	0.0636	0.0636	0.0562	0.0787
	0.1028	0.0870	0.0711	0.0711	0.0711	0.1035	0.1387	0.1311	0.1132
	0.3861							
97-127	0.1376	0.0000	0.1463	0.1463	0.1821	0.2025	0.1703	0.1602
	0.1627	0.1992	0.1821	0.1899	0.1366	0.1899	0.1716	0.1627	0.1899
	0.2008	0.2008	0.1627	0.1716	0.1627	0.1836	0.1679	0.1600	0.1587
	0.3366							
R982202	0.1551	0.1463	0.0000	0.1207	0.1914	0.1930	0.1551	0.1992
	0.1716	0.1716	0.2201	0.1807	0.1716	0.1627	0.1627	0.1539	0.1807
	0.2008	0.1914	0.1539	0.1716	0.1716	0.1387	0.1743	0.1486	0.1474
	0.3168							
R5118	0.1551	0.1463	0.1207	0.0000	0.2104	0.1866	0.1914	0.1899
	0.1627	0.1627	0.2008	0.1716	0.1716	0.1716	0.1716	0.1627	0.1899
	0.1792	0.2008	0.1807	0.1627	0.1627	0.1198	0.2259	0.1979	0.1962
	0.3168							
R4083	0.1336	0.1821	0.1914	0.2104	0.0000	0.1652	0.1028	0.1366
	0.0787	0.0787	0.1173	0.1181	0.0864	0.1181	0.1020	0.0942	0.1020
	0.1272	0.1108	0.0942	0.1100	0.1100	0.1116	0.1301	0.1311	0.1387
	0.4007							
R8513	0.1132	0.2025	0.1930	0.1866	0.1652	0.0000	0.1282	0.2501
	0.1124	0.1291	0.1563	0.1207	0.1551	0.1207	0.1042	0.0962	0.1042
	0.1132	0.1132	0.1124	0.1042	0.1124	0.1311	0.1463	0.1387	0.1207
	0.4103							
R9144	0.1028	0.1703	0.1551	0.1914	0.1028	0.1282	0.0000	0.1346
	0.0562	0.0711	0.1190	0.0787	0.0787	0.0942	0.0636	0.0562	0.0636
	0.0870	0.0716	0.0562	0.0787	0.0711	0.1035	0.1474	0.1398	0.1216
	0.3631							
R98010	0.1807	0.1602	0.1992	0.1899	0.1366	0.2501	0.1346	0.0000
	0.1356	0.1356	0.1716	0.1792	0.1441	0.1792	0.1614	0.1527	0.1614
	0.1899	0.1627	0.1527	0.1614	0.1527	0.1914	0.2042	0.2059	0.2140
	0.3723							
R98492	0.0711	0.1627	0.1716	0.1627	0.0787	0.1124	0.0562	0.1356
	0.0000	0.0273	0.0711	0.0343	0.0343	0.0486	0.0203	0.0135	0.0343
	0.0562	0.0417	0.0273	0.0414	0.0273	0.0870	0.0962	0.1216	0.1042
	0.3956							
R98958	0.0864	0.1992	0.1716	0.1627	0.0787	0.1291	0.0711	0.1356
	0.0273	0.0000	0.1020	0.0632	0.0632	0.0632	0.0486	0.0414	0.0632
	0.0864	0.0711	0.0558	0.0706	0.0558	0.0870	0.1207	0.1474	0.1291
	0.3956							
R98702	0.0706	0.1821	0.2201	0.2008	0.1173	0.1563	0.1190	0.1716
	0.0711	0.1020	0.0000	0.0636	0.0787	0.0636	0.0636	0.0711	0.0787
	0.1028	0.0792	0.0864	0.0864	0.0864	0.1198	0.1301	0.1398	0.1216
	0.4628							
97-120	0.0787	0.1899	0.1807	0.1716	0.1181	0.1207	0.0787	0.1792
	0.0343	0.0632	0.0636	0.0000	0.0706	0.0706	0.0273	0.0343	0.0558
	0.0787	0.0636	0.0486	0.0343	0.0486	0.1108	0.1376	0.1474	0.1291
	0.4406							
R5914	0.0787	0.1366	0.1716	0.1716	0.0864	0.1551	0.0787	0.1441
	0.0343	0.0632	0.0787	0.0706	0.0000	0.0706	0.0558	0.0486	0.0706
	0.0942	0.0787	0.0632	0.0782	0.0486	0.1108	0.1124	0.1216	0.1207
	0.3404							
R982077	0.0636	0.1899	0.1627	0.1716	0.1181	0.1207	0.0942	0.1792
	0.0486	0.0632	0.0636	0.0706	0.0706	0.0000	0.0414	0.0343	0.0414
	0.0636	0.0489	0.0486	0.0632	0.0486	0.0948	0.1124	0.1216	0.1042
	0.4103							
R98533	0.0636	0.1716	0.1627	0.1716	0.1020	0.1042	0.0636	0.1614
	0.0203	0.0486	0.0636	0.0273	0.0558	0.0414	0.0000	0.0067	0.0273
	0.0489	0.0345	0.0203	0.0343	0.0203	0.0948	0.1042	0.1132	0.0962
	0.3814							
R9721	0.0562	0.1627	0.1539	0.1627	0.0942	0.0962	0.0562	0.1527
	0.0135	0.0414	0.0711	0.0343	0.0486	0.0343	0.0067	0.0000	0.0203
	0.0417	0.0275	0.0135	0.0273	0.0135	0.0870	0.0962	0.1050	0.0882

0.3814
R982165 0.0787 0.1899 0.1807 0.1899 0.1020 0.1042 0.0636 0.1614
0.0343 0.0632 0.0787 0.0558 0.0706 0.0414 0.0273 0.0203 0.0000
0.0205 0.0067 0.0343 0.0486 0.0343 0.1108 0.1207 0.1301 0.1124
0.3956
R10151 0.1028 0.2008 0.2008 0.1792 0.1272 0.1132 0.0870 0.1899
0.0562 0.0864 0.1028 0.0787 0.0942 0.0636 0.0489 0.0417 0.0205
0.0000 0.0276 0.0562 0.0711 0.0562 0.1181 0.1132 0.1225 0.1050
0.3631
R5799 0.0870 0.2008 0.1914 0.2008 0.1108 0.1132 0.0716 0.1627
0.0417 0.0711 0.0792 0.0636 0.0787 0.0489 0.0345 0.0275 0.0067
0.0276 0.0000 0.0417 0.0562 0.0417 0.1198 0.1301 0.1398 0.1216
0.3768
R9252 0.0711 0.1627 0.1539 0.1807 0.0942 0.1124 0.0562 0.1527
0.0273 0.0558 0.0864 0.0486 0.0632 0.0486 0.0203 0.0135 0.0343
0.0562 0.0417 0.0000 0.0273 0.0135 0.0870 0.0962 0.1050 0.0882
0.3814
R10647 0.0711 0.1716 0.1716 0.1627 0.1100 0.1042 0.0787 0.1614
0.0414 0.0706 0.0864 0.0343 0.0782 0.0632 0.0343 0.0273 0.0486
0.0711 0.0562 0.0273 0.0000 0.0273 0.1190 0.1291 0.1387 0.1207
0.4252
R6698 0.0711 0.1627 0.1716 0.1627 0.1100 0.1124 0.0711 0.1527
0.0273 0.0558 0.0864 0.0486 0.0486 0.0486 0.0203 0.0135 0.0343
0.0562 0.0417 0.0135 0.0273 0.0000 0.1028 0.1124 0.1216 0.1042
0.3814
R981439 0.1035 0.1836 0.1387 0.1198 0.1116 0.1311 0.1035 0.1914
0.0870 0.0870 0.1198 0.1108 0.1108 0.0948 0.0948 0.0870 0.1108
0.1181 0.1198 0.0870 0.1190 0.1028 0.0000 0.0976 0.0901 0.0736
0.3589
R98537 0.1387 0.1679 0.1743 0.2259 0.1301 0.1463 0.1474 0.2042
0.0962 0.1207 0.1301 0.1376 0.1124 0.1124 0.1042 0.0962 0.1207
0.1132 0.1301 0.0962 0.1291 0.1124 0.0976 0.0000 0.0345 0.0273
0.4212
R7801 0.1311 0.1600 0.1486 0.1979 0.1311 0.1387 0.1398 0.2059
0.1216 0.1474 0.1398 0.1474 0.1216 0.1216 0.1132 0.1050 0.1301
0.1225 0.1398 0.1050 0.1387 0.1216 0.0901 0.0345 0.0000 0.0205
0.3580
R981620 0.1132 0.1587 0.1474 0.1962 0.1387 0.1207 0.1216 0.2140
0.1042 0.1291 0.1216 0.1291 0.1207 0.1042 0.0962 0.0882 0.1124
0.1050 0.1216 0.0882 0.1207 0.1042 0.0736 0.0273 0.0205 0.0000
0.3910
SAB3 0.3861 0.3366 0.3168 0.3168 0.4007 0.4103 0.3631 0.3723
0.3956 0.3956 0.4628 0.4406 0.3404 0.4103 0.3814 0.3814 0.3956
0.3631 0.3768 0.3814 0.4252 0.3814 0.3589 0.4212 0.3580 0.3910
0.0000

Genomic relationship of wild type 3 poliovirus from India (1997-2000)

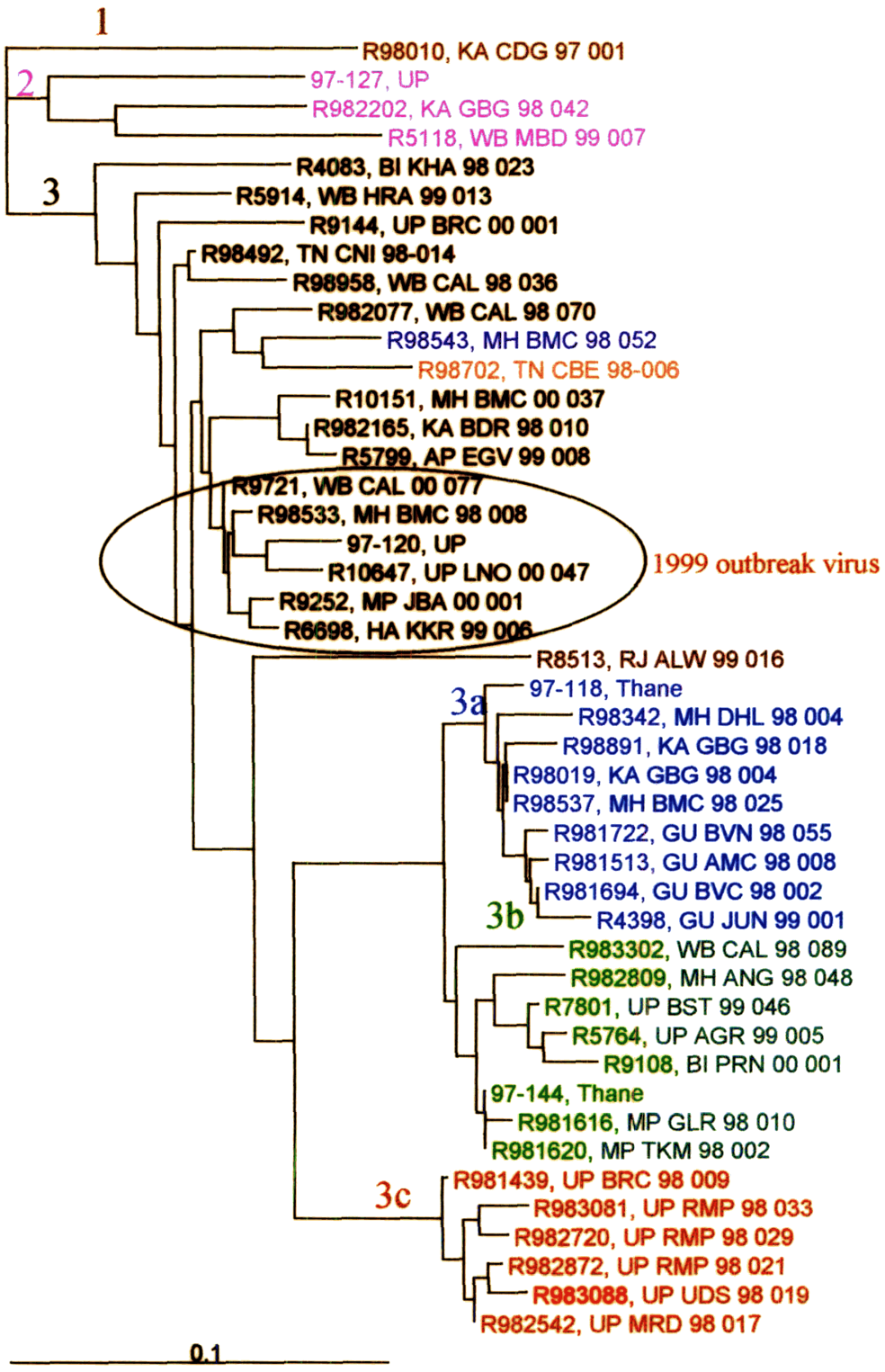

Figure 25: Phylogenetic relationship of wild poliovirus type 3 based on VP1/2A sequence database. The relationship was determined by drawing dendrogram using NJ method of package PHYLIP v3.5c.

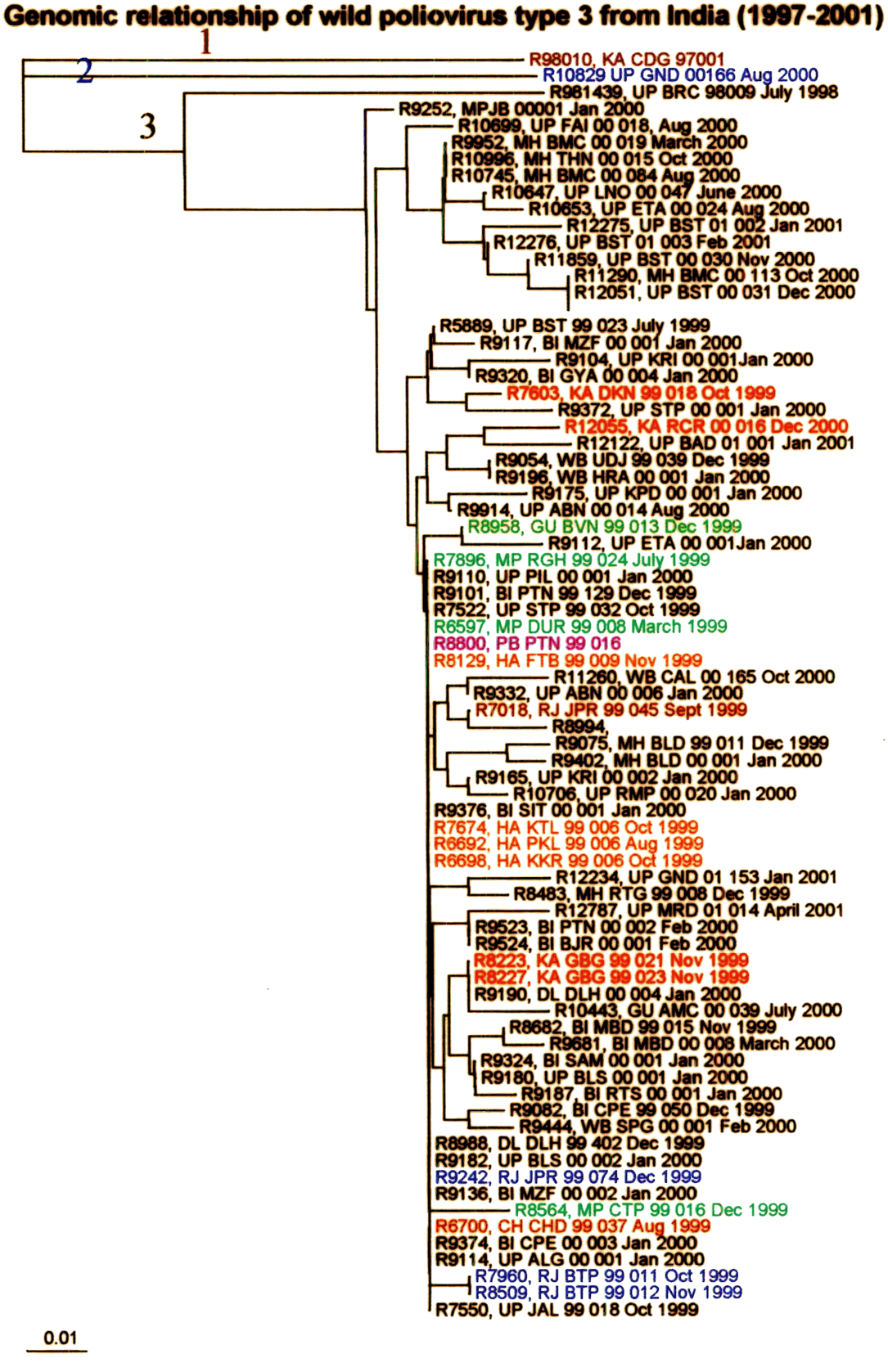

Figure 26: Phylogenetic relationship of wild poliovirus type 3 based on VP1/2A sequence data. The relationship was determined by drawing dendrogram using NJ method of package PHYLIP v3.5c.

Figure 27: Amino acid sequence comparison of wild type 3 poliovirus isolates in VP1/2A junction isolated from different parts of India (1997-2001)

```
#R98543   RPPRAVPYYG PGVDYKDGLA PLSEKGLTTY GFGHQNKAVY TAGYKICNYH
#97-127   .......... .......... .......... .......... ..........
#R982202  .......... .......... .......... .......... ..........
#R5118    .......... .......... .......... .......... ..........
#R4083    .......... .......... .......... .......... ..........
#R8513    .......... .......... .......... .......... ..........
#R9144    .......... .......... .......... .......... ..........
#R98010   .......... .......... .......... .......... ..........
#R98492   .......... .......... .......... .......... ..........
#R98958   ....V..... .......... .......... .......... ..........
#R98702   .......... .......... .......... .......... ..........
#97-120   .......... .......... .......... .L........ ..........
#R5914    .......... .......... .......... .......... ..........
#R982077  .......... .......... .......... .......... ..........
#R98533   .......... .......... .......... .......... ..........
#R9721    .......... .......... .......... .......... ..........
#R982165  .......... .......... .......... .......... ..........
#R10151   .......... .......... .......... .......... ..........
#R5799    .......... .......... .......... .......... ..........
#R9252    .......... .......... .......... .......... ..........
#R10647   .......... .......... .......... .......... ..........
#R6698    .......... .......... .......... .......... ..........
#R981439  .......... .......... .......... .......... ..........
#R98537   .......... .......... .......... ..E....... ..........
#R7801    .......... .......... .......... .......... ..........
#R981620  .......... .......... .......... .......... ..........
#SAB3     .......... .....RNN.D .......... .......... ..........
```

Figure 28: Nucleotide sequence alignment of the wild type 3 poliovirus sequenced in the VP1 region (1998-2002) isolated from different parts of India.

```
UPBST99023   GGAGTAGAAG ATTTGATATC CGAAGTGGCG CAAGGAGCCT TAGCACTTTC ACTCCCTAAA
UPBST99046   .......... ....A...A. T......... .......... .......... ...T..C...
UPGND00152   .......... .......... .......... .......... .......C.. ..........
UPGND00166   .......... .C......A. T..G...... ........T. .......... ..........
UPRMP00020   .......... .......... .......... .......... .G........ ......C...
UPABN01014   .......... ....A..... .........A .......... .......... ..........
UPBRL01086   ..G....... .......... .......... .......... .......... ......C...
UPBST01002   ........G. .......G.. .........A ..G....... .......... ..........
UPMRD01107   .......... .......... .......... .......... .G........ ......C...
UPGND01153   .....G.... .......... .......... .......... .......... ..........
DLDLH02108   .......... .......... .......... .......... .......... ......C...
UPBAD02008   .......... .......... .......... .......... .G........ ......C...
UPBRP02012   .....G..G. .......... .......... .......... .......... ......C...
GUAMC00039   .......... .......... T......... .......... .......... ..........
MHBMC00037   .....T.... ........A. T......... .......... ....G..... ..........
WBMBD99007   .......... ..C.A...A. T........A ..G..G.... .G.....C.. ......C...
WBBBM99002   .....G.... ........A. T......... .......... .......... ..........
WBMBD99011   .......... .C..A...A. T......... .......... .......C.. ..........
WBHRA99013   .......... ....A...A. T.G....... ....A..... .G.....C.. ......C...
WBMBD00006   .......... .......... .......... .......... ....T..... ......C...
WBCAL00077   .......... .....T.... .......... .......... .......... ..........
TNCBE98006   .......... ....A..TA. T......... .......... .......C.. ..........
KARCR00016   .......... .......... .......... .......... .......... ..........
APEGV99012   .......... ........A. T......... .......... .......... ..........
SAB3VP1      ..TA.T.... .......T.. T.....T..A ..G..C...C ..A.TT.G.. ......G..G

UPBST99023   CCTCAAAACA GCCTCCCGGA CACCGTAGCA AGTGGTCCTG CTCATTCCAA AGAGGTACCT
UPBST99046   .....T.... .T..T..... T...A..... .......... .......T.. .........A
UPGND00152   .......... .......... .......... .......... .C........ ...A......
UPGND00166   .......... .......... T...A..... .......... .......... ...A.....A
UPRMP00020   .......... .......... .......... .......... .......... ..........
UPABN01014   ......C... .......... .......... .......... .......... ......G...
UPBRL01086   .......... .......... .....C.... .....C.... .......... G.....G...
UPBST01002   .......... .......... ...T...... .......... .......... ..........
UPMRD01107   .......... .......... ......G... .......... .......... ...A......
UPGND01153   .T........ .......... .......... .......... .......... ......G...
DLDLH02108   .......... .......... .......... .......... .......... ..........
UPBAD02008   .......... .......... .....C.... .......... .......... ..........
UPBRP02012   .......... .......... .......... .......... .......... ..........
GUAMC00039   .......... .......... .......... .......... .......... ..........
MHBMC00037   .........G .......... ....A.G... .......... .......... .........A
WBMBD99007   ..C....... A......... ....A.G... .......... .......... ......G..A
WBBBM99002   .......... .T........ ....A.G... .......... .......... .........G
WBMBD99011   .......... .......... ....A..... .....A.... .......... .........A
WBHRA99013   .......... .T........ ....A..... .......... .......... .........A
WBMBD00006   .......... .......... .......... .......... .......... ..........
WBCAL00077   .......... .......... .......... .......... .......... ..........
TNCBE98006   .......... .......... ....A..... .....A.... T......... G........A
KARCR00016   .......... .......... ...T...... .......... .......... ..........
APEGV99012   .......... .......... T..TA.G... .......... .......... .........A
SAB3VP1      .AA..GG.T. ..T.A..T.. T..TAAG..C .....C..G. .G........ G.........

UPBST99023   GCTCTGACCG CGGTTGAAAC GGGTGCCACC AATCCACTTG TGCCATCAGA CACAGTGCAA
UPBST99046   ........T. .......... .......... ........C. .A..C..... ...T......
UPGND00152   .......... .......G.. .......... .......... .......... ...G..A...
UPGND00166   .....A.... ....C..G.. A..C..T... .......... .A..G..... ..........
UPRMP00020   ........T. .......... .........T .......... .......... .........G
UPABN01014   .......... ....C..... .......... .....G.... .......... ..........
UPBRL01086   ........T. .......... ...C...... ........C. .......... ..........
UPBST01002   .......... ....C..... .......... .......... .......... ..........
UPMRD01107   ........T. .......... .........T .......... .......... .........G
UPGND01153   ........T. .......... .......... .......... .......... ..........
DLDLH02108   ........T. .......... .........T .......... .......... .........G
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UPBAD02008   ........T. .......... .........T .......... .......... T........G
UPBRP02012   ........T. .......... .......... ..C....... .......... ..........
GUAMC00039   ...T...... .......G.. A..C...... .......... .......... ..........
MHBMC00037   .......... ....A..... ...C...... ........C. .......... ..........
WBMBD99007   ..C.....T. .......... A..C...... .......... .......... ...T..T...
WBBBM99002   ........T. ....C..... ...C...... .......... .......... ..........
WBMBD99011   ........T. .......... A..C..T... ........C. .......... ..........
WBHRA99013   ........T. .A..C..... .......... .......... .A........ .........G
WBMBD00006   .......... .......... .......... .......... .......... ..........
WBCAL00077   ...T...... .......... .......... .......... .A........ ..........
TNCBE98006   ........T. .......... ...C...... ........C. .......... ..........
KARCR00016   ........T. .......G.. .......... .......... .......... T..T......
APEGV99012   .......... ....A..... ...C...... .....T.... ....T..... ..........
SAB3VP1      ..A..C..T. .A..C..G.. T..A...... .....T..G. CA.....C.. ......T...

UPBST99023   ACACGTCACG TCATACAGCG ACGCAGCAGA ACAGAGTCAA CCATTGAGTC ATTCTTCGCA
UPBST99046   ........T. ..G......A .........G .......... .......... ..........
UPGND00152   .......... .......... .......... .......... .......... ..........
UPGND00166   ........T. ..G....... .........G .......... .......... ..........
UPRMP00020   .......... .......... .......... .......... ....C..... ..........
UPABN01014   .......... .......... .......... .......... .......... ..........
UPBRL01086   .......... .......... .........G ..G....... .T........ .........G
UPBST01002   .....C.... .......... ......T... .......... .A.....A.. ..........
UPMRD01107   .......... .......... .......... .......... ....C..... ..........
UPGND01153   .......... .......... .......... .......... .......... ..........
DLDLH02108   .......... .......... ......T... .....A.... .T..C..... ..........
UPBAD02008   .....C.... .......... .......... .......... ....C..... ..........
UPBRP02012   .......... .......... ......T... .......... .......... G.........
GUAMC00039   .......... .......... .......... .......... .......... ..........
MHBMC00037   ........T. .......... ......T... .......... .......... ......T...
WBMBD99007   ........T. .T..T..A.. ......T... .......... ....C..... ......T...
WBBBM99002   ........T. .......... .......... .......... .......... ......T...
WBMBD99011   ........T. .......... .......... .......... .......... G.........
WBHRA99013   .......... .T........ .......... .......... .......... .........G
WBMBD00006   .......... .......... .......... .......... .......... ..........
WBCAL00077   .......... .T........ .......... .......... .......... ......T...
TNCBE98006   ........T. .......... .......... .......... .......... ..........
KARCR00016   ..G....... .......... G.....T... G......... .......A.. ..........
APEGV99012   ........T. .......... ......T... .......... .......... ......T...
SAB3VP1      ..G..C.... .AG.C..A.. .........G T.......C. .A..A..A.. ..........

UPBST99023   AGAGGGGCGT GCGTTGCCAT AATTGAGGTG GACAATGAGC AACCAACAAC CAGAGCCCAG
UPBST99046   .......... .......... ...A.....A .....C.... .......... ......T..A
UPGND00152   .......... .......... .......... .......... .......... ..........
UPGND00166   ........A. .......... .........A ........A. .......... ..........
UPRMP00020   .......... .......... .........A .......... .......... ..........
UPABN01014   .......... .T........ .......... .......... .......... ..........
UPBRL01086   .......... .......... .........A .....C.... .......... ..........
UPBST01002   .......... .......... .......... .......... .......G.. ..........
UPMRD01107   .......... .......... .........A .......... .......... ..........
UPGND01153   .......... ....C..... .......... .......... .......... ..........
DLDLH02108   .......... .T........ .........A .......... .......... ..........
UPBAD02008   .......... ....C..... .........A .......... .......... ..........
UPBRP02012   .......... ....C..... .......... .......... .......... ......A...
GUAMC00039   .......... .......... .......... .......... .......... ..A.......
MHBMC00037   .......... .......... ......A..A .....C.... .......... ..........
WBMBD99007   .....A.... .......T.. .......... .....C.... ........T. .........A
WBBBM99002   .......... ....C..... .........A .......... .......... ..........
WBMBD99011   .......... .......... .........A .......... .......... T.A......A
WBHRA99013   .......... .......... .........A .......... .......... .........A
WBMBD00006   .......... .......... .......... .......... .......... ..........
WBCAL00077   .......... .......... ......A... .......... .......... ..A.......
TNCBE98006   .......... .......... .........A .......... .......... T........A
KARCR00016   .......... .......... .......... .......... .......... ..........
APEGV99012   .......... .......... ......A..A .......... .......... ..........
SAB3VP1      C.C....... ....C..T.. T......... ........A. .......C.. .C.G..A...

UPBST99023   AAGTTGTTTG CAACTTGGCG CATCACTTAC AAAGACACAG TGCAATTGCG CCGCAAACTG
UPBST99046   .......... .C..C..... ......G... .......... .......... ...T......
UPGND00152   .......... .......... .......... .......... .....C.... ..........
UPGND00166   ...C...... ....C..... ......A... .....T.... .......... ..........
UPRMP00020   .......... .G........ .......... .......... .....C.... ..........
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UPABN01014   ........C. .......... .......... .......... .....C.... ..........
UPBRL01086   .......... .......... .......... .....T.... .....C.... ..........
UPBST01002   ........C. .......... .......... .......... .....C.... .......T..
UPMRD01107   .......... .G........ .......... .......... .....C.... ..........
UPGND01153   .......... .T..G..... .......... .......... .....C.... ..........
DLDLH02108   .......... .G..C..... .......... .......... .A...C.... .........A
UPBAD02008   .......... .G........ .......... .......... .....C.... ..........
UPBRP02012   .......... ....G..... .......... .......... .....C.... ..........
GUAMC00039   .......... .......... .......... .......... .....C.... ..........
MHBMC00037   .......... ....C..... ......G... .......... .......... ..........
WBMBD99007   .......... .C........ ......G... ..G....... .......... ..........
WBBBM99002   .......... ....C..... ......A... ........G. .......... ..........
WBMBD99011   .......... ....C..... ......G... ..G....... .......... ......G...
WBHRA99013   ...C...... ....C..... ......G... .......... .......... ..........
WBMBD00006   .......... .......... .......... .......... .....C.... ..........
WBCAL00077   .......... .......... .......... .......... .....C.... ..........
TNCBE98006   ...C...... ....C..... ......G... .....T.... .......... ..........
KARCR00016   ........C. .......... .......... .......... .....C.... .........T
APEGV99012   .......... ....C..... ...T..G... .......... .......... ...T......
SAB3VP1      ..AC.A.... .C.TG..... ...T..A... .....T.... ....G..... ...T..GT..

UPBST99023   GAGTTTTTCA CCTACTCTCG CTTTGACATG GAGTTTACAT TCGTGGTGAC TGCCAACTTC
UPBST99046   .....C.... .......... T......... .....C.... .T..A..... ...T..T...
UPGND00152   .......... .......... T......... .......... .......... ..........
UPGND00166   .....C.... .......... T..C...... ........G. .T........ ..........
UPRMP00020   .......... .......... T......... .......... .......... ..........
UPABN01014   .......... .......... T......... .....C.... .......... ......T...
UPBRL01086   .......... .......... T......... .......... ........... C.........
UPBST01002   ..A....... .......... T......... .....C.... .......... ..........
UPMRD01107   ..A....... .......... T......... ..A....... .......... ..........
UPGND01153   .......... .......... T......... .......... .......... ..........
DLDLH02108   ..A....... .......... T......... ..A....... .......... ..........
UPBAD02008   ..A....... .......... T......... ..A....... .......... ..........
UPBRP02012   .......... .......... T......... .......... .......... ..........
GUAMC00039   .......... .......... T......... .......... .......... ..........
MHBMC00037   .......... .T........ A......... .......... .T........ ..........
WBMBD99007   .....C..T. .......... .......... .......... .T........ ...T......
WBBBM99002   .....C..T. .......... T......... .......... .T........ .........T
WBMBD99011   ..A..C.... .......... T......... .....C.... .T........ .........T
WBHRA99013   .....C.... .T.....C.. T......... .......... .T.....A.. ..........
WBMBD00006   .......... .......... T......... .......... .......... ..........
WBCAL00077   .......... .......... T......... .......... .......... ...T......
TNCBE98006   .....C.... .......... T......... .......... .T........ .........T
KARCR00016   .......... .......... T......... .......... .......... ..........
APEGV99012   .....C.... .T........ T......... .......... .......... ..........
SAB3VP1      .......... .A........ T......... ..A..C..C. .......A.. C.........

UPBST99023   ACCAATTCAA ATAATGGGCA CGCTCTCAAC CAAGTGTACC AAATAATGTA CATTCCCCCA
UPBST99046   .......... .C........ .......... .......... .G........ ..........
UPGND00152   .......... .C........ .......... ..G....... .......... ..........
UPGND00166   ..T..C.... .......... .......... .......... .G........ ...C.....G
UPRMP00020   .......... .......A.. .......... .......... .......... ..........
UPABN01014   G....C.... .C........ .......... .......... .......... ..........
UPBRL01086   .......... .......... T.....T... .....A.... .......... ..........
UPBST01002   ..T....... .......... .......... .......... .......... ..........
UPMRD01107   .......... .......A.. T......... .......... .......... ..........
UPGND01153   .......... .......... T......... .......... .......... ..........
DLDLH02108   .......... .......A.. T......... .......... .......... ..........
UPBAD02008   .......... .......A.. T......... .......... .......... ..........
UPBRP02012   .....C.... .......... T......... .......... .......... ..........
GUAMC00039   .......... .......... .......... .......... .......... .........C
MHBMC00037   ......A... .......... .......... .......... .......... ..........
WBMBD99007   .....C.... .......... .......... ..G....... .G........ ...C......
WBBBM99002   .......... .......... .......... ..G....... .G........ ..........
WBMBD99011   ..T..C.... .......... .......... ..G....... .G........ ..........
WBHRA99013   .....C.... .......A.. .......... .......... .G..T..... ...C......
WBMBD00006   .......... .C........ .......... .......... .......... ..........
WBCAL00077   .......... .......... .......... .......... .......... ..........
TNCBE98006   ..T..C.... .C........ .......... .......... .G........ ..........
KARCR00016   .......... .......... .........T .......... .......... .G........
APEGV99012   ......A... .......... .......... .......... .......... ..........
SAB3VP1      .....CG.T. .......... T..A...... ..G....... .G........ ...C......
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UPBST99023   GGCGCCCCCA CACCAAAATC GTGGGATGAC TACACATGGC AGACCTCCTC AAACCCATCT
UPBST99046   .....T.... .G..G...G. A......... .....G.... ....T..... ..........
UPGND00152   .......... .......... .......... .....G.... ......A... ..........
UPGND00166   ..T....... ....G..... ......C... .....G.... .......... ..........
UPRMP00020   .......... .......... .......... .....G.... .......... G........C
UPABN01014   .......... .......G.. .......... .....G.... .A........ ..........
UPBRL01086   .......... .G........ .......... .....G.... .......... ..........
UPBST01002   ..T....... .......... .......... .....G.... .......... ..........
UPMRD01107   .......... .......... .......... .....G.... .......... G.........
UPGND01153   .......... .......... .......... .....G.... ......A... ..........
DLDLH02108   .......... .......... .......... .....G.... .......... ..........
UPBAD02008   .......... .G........ .......... .....G.... .......... G.........
UPBRP02012   .......... .......... .......... ..T..G.... .......... ......G...
GUAMC00039   .......... .......... .......... .....G.... .......... ..........
MHBMC00037   ..T....... .......G.. A......... .....G.... ....T..... .........C
WBMBD99007   .......... ....G..... ......C... .....G.... ....T..... T.........
WBBBM99002   .......... .......... A......... .....G.... .A..T..... .........C
WBMBD99011   .....T.... ....G..... A......... ..T..G.... ....A..... .........C
WBHRA99013   ..T..T.... .......GA. A......... .....G.... ....T..... ..........
WBMBD00006   .......... .......... .......... .....G.... .......... ..........
WBCAL00077   .......... .......... .......... .....G.... ......A... ..........
TNCBE98006   ..T..T.... .......... A.....C... .....G.... ....T..T.. .........C
KARCR00016   .......... .......... .......... ....TG...A .C....A..T .........C
APEGV99012   .......... .G.....G.. A......... .....G.... .A..T..... ...T.....C
SAB3VP1      ..G..A.... .......G.. A.....C... .....T.... .A..A..T.. C.....G..C

UPBST99023   ATATTCTACA CCTATGGTGC TGCCCCAGCC CGGATATCAG TGCCATATGT GGGATTAGCA
UPBST99046   .......... .T........ .......... .......... ....G..C.. A.........
UPGND00152   .......... .......... .......... .......... .......... ..........
UPGND00166   .....T.... .......... .......... .......... ....T..... A..CC.....
UPRMP00020   .......... .......... ...T...... .......... .......... ......G...
UPABN01014   .......... .......... .......... .......... ....G..... ...G..G...
UPBRL01086   .......... .......... .......... .......... .......... ..........
UPBST01002   .......... .......... .......... ..A....... .......... ...G..G...
UPMRD01107   .......... .T........ ...T...... .......... .......... ......G...
UPGND01153   .......... .......... .......... .......... .A........ A.........
DLDLH02108   .......... .T........ ...T...... .......... .......... ......G...
UPBAD02008   .......... .T........ ...T...... .......... .......... ......G...
UPBRP02012   .......... .T........ .......... ..A....... .A.....C.. A.........
GUAMC00039   .......... .......... .......... .......... .......... ..........
MHBMC00037   .......... .......... .......... .......... .......... .........T
WBMBD99007   .......... .......... C.....G... .......... .......... A..C......
WBBBM99002   .......... .......... .......... .......... .......... .........C
WBMBD99011   .......... ....C..C.. .......... .......... .......... ..........
WBHRA99013   .......... .......... ...T..G... .......... .......... ......G...
WBMBD00006   .......... .......... .......... ........G. .......... ..........
WBCAL00077   .......... .......... .......... .......... .......... ..........
TNCBE98006   .......... ....C..... .......... .......... .......... ..........
KARCR00016   .......... .......... .......... .......... .A........ ..........
APEGV99012   .......... .......... .......... .......... .......... .........T
SAB3VP1      .....T.... .......G.. ......G..G ..A..C.... .......C.. ...G.....C

UPBST99023   AATGCCTATT CACACTTCTA TGATGGTTTT GCTAAAGTGC CCTTGAAGTC TGATGCAAAC
UPBST99046   ........C. .......... C......... .......... ..C........ C...AT...T
UPGND00152   .......... .......... .......... .......... .......... ..........
UPGND00166   ..C.....C. .......... ......C... .......... ..C....A.. ..........
UPRMP00020   .......... .......... .......... .......... .......... ..........
UPABN01014   .......... .......... ...C...... .......... .......... ..........
UPBRL01086   .......... .......... .......... .......... .......... ..........
UPBST01002   .......... .......... .......... .......... .......... ..........
UPMRD01107   ........C. .......... ......C... .......... ....A..... ...C......
UPGND01153   .......... .......... .......... .......... .......... ..........
DLDLH02108   .......... .......... ......A... .......... ....A...A. ...C......
UPBAD02008   ........C. .......... .......... .......... ....A..... C..C......
UPBRP02012   .......... .......... .......... ..C....... .......... ..........
GUAMC00039   .......... .......... .......... ........A. .T........ ..........
MHBMC00037   .......... .......... C........C ..C....... .......... ......G...
WBMBD99007   .......... .......... ......C... .......... .TC....... ..........
WBBBM99002   ........C. .......... C......... .......... .......... ..........
WBMBD99011   .......... .......... C.....C... ..C....... .......... .........T
WBHRA99013   .......... .......... C.....C... ..C....... ..C....... ..........
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WBMBD00006  .......... .......... .........C .......... .......... ......G...
WBCAL00077  .......... .......... ......C... .......... ....A..... ..........
TNCBE98006  .......... .......... C........C ..C....... ..C....... ..........
KARCR00016  .......... .......... .......... .......... .......... ..........
APEGV99012  .......... .......... C.....A... ..C....... .......... ..........
SAB3VP1     .....T..C. .G.....T.. C..C..C..C ..C..G.... .A......A. A.....C..T

UPBST99023  GACCAGGTGG GAGACTCCCT CTATAGTGCA ATGACTGTGG ATGACTTCGG TGTTCTTGCT
UPBST99046  ..T....... ....T..T.. ......C... .......... .......... .........C
UPGND00152  ......A... .......... .......... .......... .......... ..........
UPGND00166  .....A.... ....T..T.. ...C.....G .....C.... .......T.. ......A..C
UPRMP00020  .......... .......... .......... .......... .......... ..........
UPABN01014  .......... .......... .......... ........A. .......... .........C
UPBRL01086  ........A. .......... .......... .......... .......... ..........
UPBST01002  .......... .......... .......... .......... .C........ ..........
UPMRD01107  .......... .......... ......C... .......... .......... ..........
UPGND01153  .......... .......... .......... .......... .......... ..........
DLDLH02108  .......... .......... .........T .......... .......... ..........
UPBAD02008  .......... .......... ......C... .......... .......... ..........
UPBRP02012  .......... .......... .......... .......... .......... ..........
GUAMC00039  .......... .......... .......... .......... .......... ..........
MHBMC00037  ..T....... .......T.. ...C...... .....C.... .......... ...C..C..C
WBMBD99007  .......... ....T..T.. ......C... .....C.... .C.....T.. ......A..C
WBBBM99002  .......... .......T.. .......... .......... .C........ ......C..C
WBMBD99011  .......... ....T..T.. .......... .......... .......... .........C
WBHRA99013  .......... ....T..T.. .......... .......... .......... C.........C
WBMBD00006  .......... .......... .......... .......... .......... ..........
WBCAL00077  .......... .......... .......... .......... .......... .........C
TNCBE98006  .....A.... ....T..T.. .......... .......... .......... .........C
KARCR00016  .......... .......... .......... ........A. .......... ..........
APEGV99012  .......... .......T.. .........G .......... .......... .........C
SAB3VP1     ......A.T. .T..T...T. G..C..C..C .....A..T. .......T.. ...AT.G..A

UPBST99023  ATAAGAGTTG TGAACGATCA CAATCCCACA AAAGTTACAT CAAAGGTGCG GATTTACATG
UPBST99046  ........A. ....T..C.. ...C...... .......... .......... A.........
UPGND00152  .......... .......... .......... .......... .......... .G.......
UPGND00166  .....G..A. .T........ ......T... .....C.... .G........ C..C......
UPRMP00020  .......... .......... .......... .......... .......... ..........
UPABN01014  ........A. .......... .......... .....C.... .......A.. ..........
UPBRL01086  .....G.... .......... .......... .......... .......... ..........
UPBST01002  ........A. .......... .......... .......... .......... ..........
UPMRD01107  .......... .......... .......... .......... .......... ..........
UPGND01153  .....G.... .......... .......... .......... .......... ..........
DLDLH02108  .......... .......... .......... .......... .......... ..........
UPBAD02008  .......... .......... .......... .......... .......... ..........
UPBRP02012  .....G.... .......... .......... .......... .......... ..........
GUAMC00039  .......... .......... .......... .......... .......... ..........
MHBMC00037  .......... .......... .........T .......... .......... ..........
WBMBD99007  .....G.... .T.....C.. .......... ..........  .......... A..C......
WBBBM99002  .......... .......... .......... .......... .......... ......T...
WBMBD99011  .......... ....T..C.. .......... ...A...... .......... ..........
WBHRA99013  .......... .......... ...C...... .....A.... .......... A.........
WBMBD00006  .......... .......... .......... .......... .......... .G.......
WBCAL00077  .......... .......... ...C...... .......... .......... ..........
TNCBE98006  .......... ....T..... .......... .......... .......... ..........
KARCR00016  .......... ....T..... .......... .......... .......... ..........
APEGV99012  .......... .......... .......... .......... ....A..... ..........
SAB3VP1     G.TC.T.... .C..T..... ...C.....T .....A..C. .C..A..C.. C.........

UPBST99023  AAACCCAAAC ACGTGAGAGT ATGGTGCCCA CGGCCCCCAC GAGCGGTGCC ATACTACGGA
UPBST99046  ..G.....G. .T........ G......... ........G. ....A..A.. ...T......
UPGND00152  .......... .......... .......... .........A .......A.. ..........
UPGND00166  ..G.....G. .T.....G.. G......... ..A....... ....A..... G.........
UPRMP00020  .......... .......... .......... .......... .......A.. ..........
UPABN01014  .......... .T........ G......... .......... .......... ..........
UPBRL01086  .......... .......... ......T... .......... .......A.. ..........
UPBST01002  .......... .......... .......... .......... .......... ..........
UPMRD01107  .......... .......... .......... .......... .......A.. ..........
UPGND01153  .......... .......... .......... .....T..T. .......A.. ..........
DLDLH02108  .......... .......... T......... .......... .......A.. G........G
UPBAD02008  .......... .......... T......... .......... .......A.. ..........
UPBRP02012  .......... .......... G........G .....T..T. .......A.. .........G
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GUAMC00039  ..........  ..........  ..........  ..........  .......A..  ..........
MHBMC00037  ..G.....G.  .T........  G.........  ........G.  .......A..  ..........
WBMBD99007  ..G.....G.  ..........  G.........  ..A.......  ....A.....  ..........
WBBBM99002  ........G.  ..........  ..........  ..........  ..........  ..........
WBMBD99011  ........G.  .T........  G.........  ..........  ..........  .........T
WBHRA99013  ..G.....G.  .T........  G.........  ..........  .......A..  ..........
WBMBD00006  ..........  ..........  ..........  ........G.  .......A..  ..........
WBCAL00077  ..........  ..........  ..........  ..........  .......A..  ..........
TNCBE98006  ........G.  .T........  G.........  .....T....  ..........  G........T
KARCR00016  ..........  .T........  ..........  ..........  .......A..  ..........
APEGV99012  ........G.  .T........  G.........  ..........  ..........  T.........
SAB3VP1     ..........  ....AC.T..  C........T  A.A..G..G.  .C.....A..  T..T..T...

UPBST99023  CCAGGGGTGG  ACTATAAGGA  TGGTTTAGCG  CCTCTATCAG  AGAAGGGACT  CACAACTTAT
UPBST99046  ..........  ....C.....  ......G...  .........G  ..........  ..........
UPGND00152  ..........  ..........  ..........  ..........  ..........  ..........
UPGND00166  ..G.......  ....C.....  C.........  ..AT......  .......G..  ...C..C...
UPRMP00020  ..........  ..........  ..........  ..........  ..........  ..........
UPABN01014  ..........  .T..C.....  .........A  ..........  ..........  ..........
UPBRL01086  ..........  ..........  ...C......  .....G....  ..........  ..........
UPBST01002  ..........  ....C.....  ...A......  ..........  ..........  ..........
UPMRD01107  ..........  ......G...  ..........  ..........  ..........  ..........
UPGND01153  ..........  ..........  ..........  ..........  ..........  ...G......
DLDLH02108  ..........  ..........  ..........  ...T......  ..........  ..........
UPBAD02008  ..........  ......G...  ..........  ..........  ..........  ..........
UPBRP02012  ..........  ..........  ..........  ..........  ..........  ...G......
GUAMC00039  ..........  .T.....A..  ..........  ..........  ..........  ..........
MHBMC00037  ..........  .T..C.....  ..........  ..........  ..........  ..........
WBMBD99007  ..G.......  ..........  ......G...  ..CT......  .......T..  ...C..C...
WBBBM99002  ..........  ....C.....  ...A.....A  ..........  ....A.....  ..........
WBMBD99011  ..........  ....C.....  ..........  ........G.  ..........  ..........
WBHRA99013  ..........  ..........  ......G..A  ........G.  ....A.....  ..........
WBMBD00006  ..........  ..........  ..........  ..........  ..........  ..........
WBCAL00077  ..........  ....C.....  ..........  ..........  ..........  ..........
TNCBE98006  ..........  .T..C.....  ..........  ........G.  ..........  ..........
KARCR00016  ..........  .T........  ..........  ..........  ..........  ..........
APEGV99012  ..........  .T..C.....  ..........  ..........  ..........  ..........
SAB3VP1     ..........  ......G.A.  CAAC..G.AC  ..CT....T.  ....A..TT.  G..C..A...
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Figure 29: Genomic distances calculated using Kimura 2 Parameter method of wild poliovirus type 3 sequenced in the VP1 region isolated from different parts of India (1998-2002)

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UPBST99023  0.0000  0.0946  0.0181  0.1026  0.0192  0.0367  0.0343  0.0309
  0.0319  0.0216  0.0392  0.0367  0.0356  0.0169  0.0589  0.1003  0.0488
  0.0686  0.0762  0.0135  0.0204  0.0661  0.0333  0.0540  0.3109
UPBST99046  0.0946  0.0000  0.1041  0.1219  0.0999  0.1052  0.1079  0.1175
  0.1065  0.1028  0.1165  0.1053  0.1083  0.1040  0.0936  0.1165  0.0919
  0.0829  0.0853  0.0947  0.0975  0.0842  0.1150  0.0935  0.3428
UPGND00152  0.0181  0.1041  0.0000  0.1137  0.0285  0.0464  0.0439  0.0428
  0.0391  0.0286  0.0489  0.0463  0.0452  0.0239  0.0728  0.1114  0.0612
  0.0789  0.0866  0.0181  0.0273  0.0763  0.0368  0.0691  0.3163
UPGND00166  0.1026  0.1219  0.1137  0.0000  0.1175  0.1161  0.1243  0.1190
  0.1243  0.1153  0.1291  0.1342  0.1262  0.1054  0.1111  0.1080  0.1052
  0.1067  0.1147  0.1135  0.1110  0.0987  0.1321  0.1072  0.3243
UPRMP00020  0.0192  0.0999  0.0285  0.1175  0.0000  0.0475  0.0378  0.0415
  0.0146  0.0286  0.0262  0.0192  0.0415  0.0273  0.0688  0.1070  0.0574
  0.0774  0.0699  0.0215  0.0308  0.0749  0.0392  0.0651  0.3194
UPABN01014  0.0367  0.1052  0.0464  0.1161  0.0475  0.0000  0.0633  0.0427
  0.0609  0.0501  0.0649  0.0646  0.0623  0.0451  0.0791  0.1237  0.0712
  0.0866  0.0879  0.0415  0.0464  0.0814  0.0538  0.0691  0.3227
UPBRL01086  0.0343  0.1079  0.0439  0.1243  0.0378  0.0633  0.0000  0.0572
  0.0486  0.0380  0.0536  0.0486  0.0524  0.0402  0.0802  0.1220  0.0723
  0.0903  0.0968  0.0367  0.0463  0.0825  0.0574  0.0791  0.3130
UPBST01002  0.0309  0.1175  0.0428  0.1190  0.0415  0.0427  0.0572  0.0000
  0.0524  0.0465  0.0563  0.0549  0.0538  0.0416  0.0805  0.1225  0.0673
  0.0880  0.0984  0.0380  0.0428  0.0867  0.0490  0.0742  0.3073
UPMRD01107  0.0319  0.1065  0.0391  0.1243  0.0146  0.0609  0.0486  0.0524
  0.0000  0.0392  0.0239  0.0112  0.0499  0.0402  0.0828  0.1138  0.0685
  0.0890  0.0813  0.0343  0.0391  0.0916  0.0549  0.0778  0.3173
UPGND01153  0.0216  0.1028  0.0286  0.1153  0.0286  0.0501  0.0380  0.0465
  0.0392  0.0000  0.0454  0.0416  0.0227  0.0298  0.0729  0.1100  0.0589
  0.0842  0.0894  0.0251  0.0309  0.0791  0.0393  0.0717  0.3088
DLDLH02108  0.0392  0.1165  0.0489  0.1291  0.0262  0.0649  0.0536  0.0563
  0.0239  0.0454  0.0000  0.0274  0.0501  0.0476  0.0871  0.1268  0.0790
  0.0933  0.0894  0.0416  0.0477  0.0920  0.0589  0.0780  0.3311
UPBAD02008  0.0367  0.1053  0.0463  0.1342  0.0192  0.0646  0.0486  0.0549
  0.0112  0.0416  0.0274  0.0000  0.0512  0.0451  0.0894  0.1235  0.0736
  0.0956  0.0878  0.0391  0.0463  0.0956  0.0574  0.0830  0.3188
UPBRP02012  0.0356  0.1083  0.0452  0.1262  0.0415  0.0623  0.0524  0.0538
  0.0499  0.0227  0.0501  0.0512  0.0000  0.0440  0.0805  0.1197  0.0738
  0.0854  0.0945  0.0368  0.0476  0.0854  0.0539  0.0793  0.3062
GUAMC00039  0.0169  0.1040  0.0239  0.1054  0.0273  0.0451  0.0402  0.0416
  0.0402  0.0298  0.0476  0.0451  0.0440  0.0000  0.0639  0.1072  0.0574
  0.0725  0.0852  0.0215  0.0239  0.0725  0.0369  0.0602  0.3178
MHBMC00037  0.0589  0.0936  0.0728  0.1111  0.0688  0.0791  0.0802  0.0805
  0.0828  0.0729  0.0871  0.0894  0.0805  0.0639  0.0000  0.1129  0.0538
  0.0739  0.0790  0.0589  0.0652  0.0626  0.0794  0.0381  0.3167
WBMBD99007  0.1003  0.1165  0.1114  0.1080  0.1070  0.1237  0.1220  0.1225
  0.1138  0.1100  0.1268  0.1235  0.1197  0.1072  0.1129  0.0000  0.1016
  0.1043  0.1057  0.1112  0.1086  0.1111  0.1294  0.1157  0.3213
WBBBM99002  0.0488  0.0919  0.0612  0.1052  0.0574  0.0712  0.0723  0.0673
  0.0685  0.0589  0.0790  0.0736  0.0738  0.0574  0.0538  0.1016  0.0000
  0.0623  0.0736  0.0587  0.0587  0.0611  0.0740  0.0489  0.3094
WBMBD99011  0.0686  0.0829  0.0789  0.1067  0.0774  0.0866  0.0903  0.0880
  0.0890  0.0842  0.0933  0.0956  0.0854  0.0725  0.0739  0.1043  0.0623
  0.0000  0.0749  0.0788  0.0763  0.0379  0.0908  0.0689  0.3314
WBHRA99013  0.0762  0.0853  0.0866  0.1147  0.0699  0.0879  0.0968  0.0984
  0.0813  0.0894  0.0894  0.0878  0.0945  0.0852  0.0790  0.1057  0.0736
  0.0749  0.0000  0.0813  0.0789  0.0661  0.1013  0.0791  0.3142
WBMBD00006  0.0135  0.0947  0.0181  0.1135  0.0215  0.0415  0.0367  0.0380
  0.0343  0.0251  0.0416  0.0391  0.0368  0.0215  0.0589  0.1112  0.0587
  0.0788  0.0813  0.0000  0.0250  0.0712  0.0381  0.0640  0.3083
WBCAL00077  0.0204  0.0975  0.0273  0.1110  0.0308  0.0464  0.0463  0.0428
  0.0391  0.0309  0.0477  0.0463  0.0476  0.0239  0.0652  0.1086  0.0587
  0.0763  0.0789  0.0250  0.0000  0.0789  0.0428  0.0603  0.3227
```

TNCBE98006 0.0661 0.0842 0.0763 0.0987 0.0749 0.0814 0.0825 0.0867
0.0916 0.0791 0.0920 0.0956 0.0854 0.0725 0.0626 0.1111 0.0611
0.0379 0.0661 0.0712 0.0789 0.0000 0.0856 0.0626 0.3184
KARCR00016 0.0333 0.1150 0.0368 0.1321 0.0392 0.0538 0.0574 0.0490
0.0549 0.0393 0.0589 0.0574 0.0539 0.0369 0.0794 0.1294 0.0740
0.0908 0.1013 0.0381 0.0428 0.0856 0.0000 0.0718 0.3289
APEGV99012 0.0540 0.0935 0.0691 0.1072 0.0651 0.0691 0.0791 0.0742
0.0778 0.0717 0.0780 0.0830 0.0793 0.0602 0.0381 0.1157 0.0489
0.0689 0.0791 0.0640 0.0603 0.0626 0.0718 0.0000 0.3088
SAB3VP1 0.3109 0.3428 0.3163 0.3243 0.3194 0.3227 0.3130 0.3073
0.3173 0.3088 0.3311 0.3188 0.3062 0.3178 0.3167 0.3213 0.3094
0.3314 0.3142 0.3083 0.3227 0.3184 0.3289 0.3088 0.0000

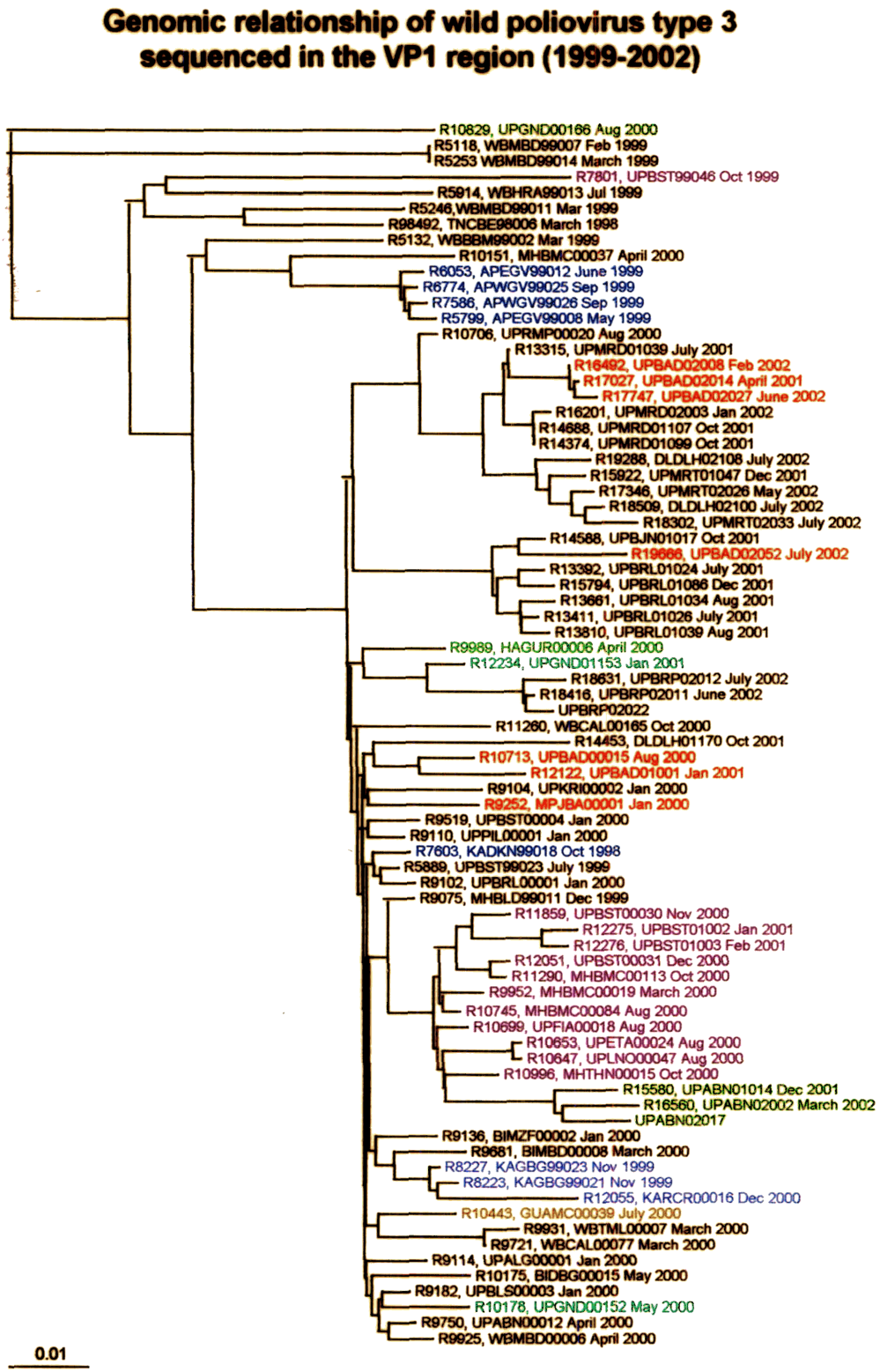

Figure 30: Phylogenetic relationship of wild poliovirus type 3 based on complete VP1 sequence (900bp) of viral RNA. The relationship in a form of dendrogram was constructed using NJ method of package PHYLIP v3.5c.

Figure 31: Amino acid sequence comparison of wild type 3 poliovirus isolates in VP1 region isolated from different parts of India (1998-2002)

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#UPBST99023 GVEDLISEVA QGALALSLPK PQNSLPDTVA SGPAHSKEVP ALTAVETGAT NPLVPSDTVQ
#UPBST99046 ......T... .......... .H......I. .......... .......... ..........
#UPGND00152 .......... .......... .......... .......... .......... ..........
#UPGND00166 ......T... .......... ........I. .......... .......... ..........
#UPRMP00020 .......... .......... .......... .......... .......... ..........
#UPABN01014 .......... .......... ..H....... .......... .......... ..........
#UPBRL01086 .......... .......... ........A. .......... .......... ..........
#UPBST01002 .....M.... .......... .......... .......... .......... ..........
#UPMRD01107 .......... .......... .......... .......... .......... ..........
#UPGND01153 .......... .......... L......... .......... .......... ..........
#DLDLH02108 .......... .......... .......... .......... .......... ..........
#UPBAD02008 .......... .......... ........A. .......... .......... ..........
#UPBRP02012 .......... .......... .......... .......... .......... ..........
#GUAMC00039 .......... .......... .......... .......... .......... ..........
#MHBMC00037 ......T... .......... ...G....M. .......... .......... ..........
#WBMBD99007 ......T... .......... ...N....M. .......... .......... ..........
#WBBBM99002 ......T... .......... ........M. .......... .......... ..........
#WBMBD99011 ......T... .......... ........I. .......... .......... ..........
#WBHRA99013 ......TG.. .E........ ........I. .......... .......... ..........
#WBMBD00006 .......... .......... .......... .......... .......... ..........
#WBCAL00077 .....L.... .......... .......... .......... .......... ..........
#TNCBE98006 ......T... .......... ........I. ...V...... .......... ..........
#KARCR00016 .......... .......... .......... .......... .......... ..........
#APEGV99012 ......T... .......... ........M. .......... .......... ..........
#SAB3VP1    .I........ ....T..... Q.D.....K. .......... .......... ...A......

#UPBST99023 TRHVIQRRSR TESTIESFFA RGACVAIIEV DNEQPTTRAQ KLFATWRITY KDTVQLRRKL
#UPBST99046 ....V.Q... .......... .......... .......... .......... ..........
#UPGND00152 .......... .......... .......... .......... .......... ..........
#UPGND00166 ....V..... .......... .......... .......... .......... ..........
#UPRMP00020 .......... .......... .......... .......... .......... ..........
#UPABN01014 .......... .......... .......... .......... .......... ..........
#UPBRL01086 .......... .......... .......... .......... .......... ..........
#UPBST01002 .......... .......... .......... .......... .......... ..........
#UPMRD01107 .......... .......... .......... .......... .......... ..........
#UPGND01153 .......... .......... .......... .......... .......... ..........
#DLDLH02108 .......... .......... .......... .......... .......... ..........
#UPBAD02008 .......... .......... .......... .......... .......... ..........
#UPBRP02012 .......... .......... .......... .......... .......... ..........
#GUAMC00039 .......... .......... .......... .......K.. .......... ..........
#MHBMC00037 .......... .......... .......... .......... .......... ..........
#WBMBD99007 .......... .......... .......... ......S... .......... ..........
#WBBBM99002 .......... .......... .......... .......... .......... ..........
#WBMBD99011 .......... .......... .......... .......K.. .......... ..........
#WBHRA99013 .......... .......... .......... .......... .......... ..........
#WBMBD00006 .......... .......... .......... .......... .......... ..........
#WBCAL00077 .......... .......... .......... .......K.. .......... ..........
#TNCBE98006 .......... .......... .......... .......... .......... ..........
#KARCR00016 .......... A......... .......... .......... .......... ..........
#APEGV99012 .......... .......... .......... .......... .......... ..........
#SAB3VP1    ....V..... S......... .......... .......... ....M..... ..........

#UPBST99023 EFFTYSRFDM EFTFVVTANF TNSNNGHALN QVYQIMYIPP GAPTPKSWDD YTWQTSSNPS
#UPBST99046 .......... .......... .......... .......... ......A... ..........
#UPGND00152 .......... .......... .......... .......... .......... .....Y....
#UPGND00166 .......... .......... .......... .......... .......... ..........
#UPRMP00020 .......... .......... .......... .......... .......... ..........
#UPABN01014 .......... .......... A......... .......... .......... ..........
#UPBRL01086 .......... .......... .......... .......... .......... ..........
#UPBST01002 .......... .......... .......... .......... .......... ..........
#UPMRD01107 .......... .......... .......... .......... .......... ..........
#UPGND01153 .......... .......... .......... .......... .......... .....Y....
#DLDLH02108 .......... .......... .......... .......... .......... ..........
#UPBAD02008 .......... .......... .......... .......... .......... ..........
#UPBRP02012 .......... .......... .......... .......... .......... ..........
#GUAMC00039 .......... .......... .......... .......... .......... ..........
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#MHBMC00037 ..........  ..........  ..T.......  ..........  ..........  ..........
#WBMBD99007 ..........  ..........  ..........  ..........  ..........  ..........
#WBBBM99002 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD99011 ..........  ..........  ..........  ..........  ..........  ..........
#WBHRA99013 ..........  ..........  ..........  ..........  ......T...  ..........
#WBMBD00006 ..........  ..........  ..........  ..........  ..........  ..........
#WBCAL00077 ..........  ..........  ..........  ..........  ..........  .....Y....
#TNCBE98006 ..........  ..........  ..........  ..........  ..........  ..........
#KARCR00016 ..........  ..........  ..........  .......V..  ..........  .M.N.YL...
#APEGV99012 ..........  ..........  ..T.......  ..........  ..........  ..........
#SAB3VP1    ..........  ..........  ..A.......  ..........  ..........  ..........

#UPBST99023 IFYTYGAAPA  RISVPYVGLA  NAYSHFYDGF  AKVPLKSDAN  DQVGDSLYSA  MTVDDFGVLA
#UPBST99046 ..........  ..........  ..........  ........I.  ..........  ..........
#UPGND00152 ..........  ..........  ..........  ..........  ..M.......  ..........
#UPGND00166 ..........  ..........  ..........  ..........  ..........  ..........
#UPRMP00020 ..........  ..........  ..........  ..........  ..........  ..........
#UPABN01014 ..........  ..........  ..........  ..........  ..........  ..........
#UPBRL01086 ..........  ..........  ..........  ..........  ..........  ..........
#UPBST01002 ..........  ..........  ..........  ..........  ..........  ..........
#UPMRD01107 ..........  ..........  ..........  ..........  ..........  ..........
#UPGND01153 ..........  ..........  ..........  ..........  ..........  ..........
#DLDLH02108 ..........  ..........  ..........  ......T...  ..........  ..........
#UPBAD02008 ..........  ..........  ..........  ..........  ..........  ..........
#UPBRP02012 ..........  ..........  ..........  ..........  ..........  ..........
#GUAMC00039 ..........  ..........  ..........  ..........  ..........  ..........
#MHBMC00037 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD99007 ..........  ..........  ..........  ..........  ..........  ..........
#WBBBM99002 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD99011 ..........  ..........  ..........  ..........  ..........  ..........
#WBHRA99013 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD00006 ..........  ..........  ..........  ..........  ..........  ..........
#WBCAL00077 ..........  ..........  ..........  ..........  ..........  ..........
#TNCBE98006 ..........  ..........  ..........  ..........  ..........  ..........
#KARCR00016 ..........  ..........  ..........  ..........  ..........  ..........
#APEGV99012 ..........  ..........  ..........  ..........  ..........  ..........
#SAB3VP1    ..........  ..........  ..........  ......T...  ..I.......  ..........

#UPBST99023 IRVVNDHNPT  KVTSKVRIYM  KPKHVRVWCP  RPPRAVPYYG  PGVDYKDGLA  PLSEKGLTTY
#UPBST99046 ..........  ..........  ..........  ..........  ..........  ..........
#UPGND00152 ..........  .......V..  ..........  ..........  ..........  ..........
#UPGND00166 ..........  ..........  ..........  ..........  ..........  ..........
#UPRMP00020 ..........  ..........  ..........  ..........  ..........  ..........
#UPABN01014 ..........  ..........  ..........  ..........  ..........  ..........
#UPBRL01086 ..........  ..........  ..........  ..........  ..........  ..........
#UPBST01002 ..........  ..........  ..........  ..........  ..........  ..........
#UPMRD01107 ..........  ..........  ..........  ..........  .....R....  ..........
#UPGND01153 ..........  ..........  ..........  ..........  ..........  ..........
#DLDLH02108 ..........  ..........  ..........  ..........  ..........  ..........
#UPBAD02008 ..........  ..........  ..........  ..........  .....R....  ..........
#UPBRP02012 ..........  ..........  ..........  ..........  ..........  ..........
#GUAMC00039 ..........  ..........  ..........  ..........  ..........  ..........
#MHBMC00037 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD99007 ..........  ..........  ..........  ..........  ..........  ..........
#WBBBM99002 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD99011 ..........  .I........  ..........  ..........  ..........  ..........
#WBHRA99013 ..........  ..........  ..........  ..........  ..........  ..........
#WBMBD00006 ..........  .......V..  ..........  ..........  ..........  ..........
#WBCAL00077 ..........  ..........  ..........  ..........  ..........  ..........
#TNCBE98006 ..........  ..........  ..........  ..........  ..........  ..........
#KARCR00016 ..........  ..........  ..........  ..........  ..........  ..........
#APEGV99012 ..........  ..........  ..........  ..........  ..........  ..........
#SAB3VP1    V.........  ..........  ..........  ..........  .....RNN.D  ..........
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CHAPTER 6

DISCUSSION

Discussion

Global eradication of smallpox was certified in the year 1980 after a three years period of intensive search for a smallpox case. The last case of smallpox was reported from Aligarh, India (WHO, 1980). Smallpox eradication was possible due to concerted efforts by all the countries working together under the umbrella of WHO. For the second time in the history of mankind all the member states joined hand to eradicate poliomyelitis, a disease that afflicted mankind since at least 3500 years ago (WHA, 1988). The goal of global polio eradication was set because two highly efficacious anti-polio vaccines (IPV and OPV) have been available, ability to diagnose the disease and the scientific data that indicated humans as the only natural host of the virus. However certain difficulties in the eradication process were also recognized, for example a) existence of more than one serotypes and b) subclinical infections wherein one out of 100 to 400 infections result in a paralytic case but all the infected individuals are able to transmit the virus.

In 1974, the WHO established Expanded Program on Immunization (EPI) with the aim to provide potent vaccines against six childhood vaccine preventable diseases to all the children in the world. Though the EPI was formally launched in 1974, many developing countries could not provide the vaccines to sufficiently large number of children due to economic reasons. Vaccination coverage was low and in more than 150 countries polio was highly endemic.

The strategies for polio eradication were defined and refined through the research work of Sabin, Chumakov, Cruz, Domock and Jacob John (Domok, 1984; Cruz, 1984; John, 1984). WHO recommended the use of OPV in polio eradication program. The strategies of polio eradication are a) high routine OPV coverage (exceeding 90%), b) AFP surveillance, c) supplemental immunization in the form of NIDs (Pulse Polio Immunization in India), d) mopping up in high-risk areas e)

preparedness against importation. The strategies were applied in the South American region from 1985 onwards, ultimately resulting in eradication of the disease in the year 1991 (WHO, 1995). At the beginning of the PEI approximately 350,000 cases of paralytic poliomyelitis occurred worldwide. By the end of the year 2000 only two regions, AMRO and WPRO were awarded the certificates of polio eradication, and the European region was preparing for certification (WHO, 2000). In the year 2000, twenty-five countries continued wild poliovirus transmission. It is heartening to note that at least wild poliovirus type 2 transmission was globally eradicated by the end of the year 1999 (WHO, 2001).

One of the key elements of polio eradication is the ability of the laboratories to isolate and characterize polioviruses from AFP cases. It has been well understood by the scientific communities that no epidemiological studies can be considered as complete until molecular characterization of the infectious agent is achieved. In molecular epidemiologic studies various tools such as PCR, RT-PCR, RFLP and genomic sequencing can be used to characterize the infectious agent.

Seroconversion rates after OPV immunization are poliovirus type 2> type 1> type 3. Although least seroconversion rates were detected for poliovirus type 3, wild polioviruses are eliminated from circulation in order of poliovirus type 2, type 3 and type 1. This may be due to the fact that poliovirus type 1 has more transmissibility and is more neurovirulent (Nathanson, 1979). A change in the proportion of poliovirus type 3 in OPV was established to improve seroconversion rates (Patriarca et al, 1988). Since 1997 the OPV used in polio eradication program since 1997 contains $10^{6.0}$, $10^{5.0}$, $10^{5.8}$ CCID50 per dose of poliovirus type 1, type 2 and type 3 respectively, a change from the proportion of 10:1:3 to 10:1:6 for the three serotypes.

The genome of poliovirus is about 7.5kb (7500 nt) long RNA molecule and all the three serologically distinct groups differ in as much as 30% of their genomic sequences and similar differences are also detected

in the wild and vaccine strains. The genome codes for four structural proteins VP1, VP2, VP3 and VP4. The VP1 protein contains the major antigenic site for all three poliovirus serotypes.

Polioviruses can be characterized on the bases of antigenic differences. However, due to the limited number of antigenic sites (epitopes) on virus particle, methods based on the antigen-antibody reaction have limited application in defining the relatedness or differences between the wild or vaccine virus of the different origin (Emini et al, 1982; Ferguson et al, 1984; Minor et al, 1984). The RNA fingerprinting of poliovirus genome was the first elegant application of molecular technique in defining the origin and relationship of poliovirus isolates (De Watcher and Fiers, 1971; Lee et al, 1979; Nottay et al, 1981). The RNA fingerprinting method was the most sensitive method for identification of vaccine OPV derived poliovirus isolated from cases of vaccine associated paralytic poliomyelitis (VAPP). Soon resolution power of the method was recognized in defining the relationship between wild poliovirus isolates. Epidemiologic linkage between wild poliovirus type 1 isolated from The Netherlands, Canada, New York was unequivocally proved through RNA fingerprinting. Based on these results Nottay et al, 1981 calculated 1-2% nucleotide substitution rate per year over the entire genome of poliovirus (Nottay et al, 1981).

By comparing the 150nt sequences of VP1/2A junction region of poliovirus type 1 isolates from different parts of the world Ricco-Hesse et al (1987) inferred circulation of genomically distinct poliovirus type 1 strains. A genotype of poliovirus was defined as a group of viruses sharing more than 85% sequence similarity, the similarity of 95% or more constitute a lineage and the cases are said to be epidemiologically linked if the sequence similarity was found to be more than 98% (Ricco-Hesse et al, 1987). The explicit goal of the PEI is to break all the wild poliovirus transmission chains. The only method available to determine the transmission pathways of poliovirus is the genomic sequencing.

Substantial progress has been made in India towards polio eradication. The number of wild poliovirus cases declined from 1934 in 1988 to 268 in 2001 (WHO, 2001). Wild poliovirus type 2 has not been isolated since October 1999; therefore it appears that wild poliovirus type 2 has been truly eradicated.

Genomic characterization of wild poliovirus from India was not being performed until recently. The data available was from isolates that were analyzed in countries abroad especially in Center for disease control and prevention (CDC), USA and Pasture institute France, that were made available either on request or through individual laboratory collaborations. Most of these isolates were of a period before the initiation of eradication efforts.

In order to have an overview of genomic characterization of poliovirus type 2, it was decided to study isolates from historical collection. Epidemiologic information was available from the database maintained at ERC. VP1/2A sequences of a total of 14-poliovirus type 2 isolates from Mumbai city, obtained during the period 1971 to 1986 reveals presence (circulation) of one genotype. Using the definition of Ricco-Hesse et al at least 4 lineages of poliovirus type 2 (>5% sequence divergence) were observed. The data also shows high levels of sequence similarity within specific lineage indicating the power of sequence analysis to identify epidemiologically linked cases. The results showed continue transmission of parental strains over many years. It was also possible to document increasing diversity over a period of time.

The 30 isolates of poliovirus type 2 isolated between the years 1991 and 1995 grouped in two genotypes. Both the genotypes co-circulated until 1994. Presence of the two genotypes and multiple transmission chains of wild poliovirus type 2 shows sufficient evidence of low routine OPV coverage especially in the slums. No wild poliovirus type 2 was isolated from Mumbai after 1995. High population immunity achieved through PPI of 1995 and 1996 terminated wild poliovirus type 2

transmissions in Mumbai. More than 120 million children were immunized with OPV in the country on December 1995 and January 1996 (Andrus et al, 1997a). Wild poliovirus type 2 was eliminated from Maharashtra in 1995-1996, indicating high quality mass vaccination is a powerful methodology for polio eradication.

However yearly PPI campaigns were not sufficiently powerful to stop poliovirus transmission in Utter Pradesh and Bihar until year 2000. Out of a total of 1934 wild poliovirus confirmed cases in the year 1998, wild poliovirus type 2 was isolated from 78 AFP cases and out of 1042 wild types in 1999, wild poliovirus type 2 was isolated from 11 AFP cases. Complete VP1 sequence analysis provides high resolution of virus isolated during a short span of time and permits the detection of virus transmission pathways (Mulders et al, 1995). As depicted in Figure 16 all the 37 isolates represent a single genotype of wild poliovirus type 2 and since the genomic distance was found to be less than 5% infer a single lineage. The isolates grouped into 5 clusters, indicating at least 5 major transmission chains. Intense circulation of wild poliovirus type 2 in certain districts (Meerut, Muzaffernager, Moradabad, Breilley) was inferred from detection of multiple transmission chains of the virus within districts. Genetic data corroborated with epidemiologic finds that the continued circulation of poliovirus type 2 indicate serious deficiencies in polio vaccine coverage. Immunization surveys detected >30% of the children had received the routine doses of OPV in Utter Pradesh and Bihar.

The single isolate from Gujarat (1998) showed a genetic distance of 1.4% with isolates from Moradabad and Muzaffernager, Utter Pradesh. The virus therefore has strong epidemiologic link with the highly endemic focus of wild poliovirus type 2 transmission in Utter Pradesh. The 1.4% genetic indicates surveillance gap most probably within Utter Pradesh. This lacuna of information highlights the need to do extensive sequencing analysis. A direct import of wild poliovirus type 2 in West Bengal was

proved on the bases of sequencing analysis. The source of the virus can be predicted to be Sultanpur, Utter Pradesh.

Very small number of wild poliovirus type 2 has been sequenced globally because many other countries with high routine vaccination program through EPI had controlled wild poliovirus type 2 infections in mid eighties. The poliovirus type 2 characterized in this thesis represent globally last wild poliovirus transmission chains.

Although all the three-poliovirus types are capable of causing paralytic disease, epidemiological data shows that wild poliovirus type 1 has been responsible for endemic cases as well as epidemic cases of poliomyelitis (Kew et al, 1995). Poliovirus type 2 was usually isolated from sporadic cases. No large-scale outbreak of wild poliovirus type 2 has been recorded in the history of poliomyelitis. By proportion of the isolates during endamicity of poliomyelitis, poliovirus type 3 qualifies to be the weaker serotype and the transmission remains low. However it is difficult to control poliovirus type 3 infections due to low seroconversion rates (Risi et al, 1984). India was the only country harboring the transmission of wild poliovirus type 3 in the year 2000 was (WHO, 2001).

Four genotypes of wild poliovirus type 3 have been documented in America (Kew et al, 1993) and one in China (Yang et al, 1995). In other polio endemic countries like Pakistan, Afghanistan, Central Asia and South Africa more than one genotypes of wild poliovirus type 3 were found (Kew et al, 1995; Mulders et al, 1995).

A total of 30 isolates of wild poliovirus type 3 from the historical collection were sequenced in the VP1/2A junction region to know the genetic diversity of poliovirus type 3 in Mumbai over a period of 20 years. Such information was thought to be useful in knowing the dynamics of poliovirus circulation, replacement of the lineages etc. Three genotypes of wild poliovirus type 3 were observed between 1972 and 1989. It is noticeable from Figure 20 that the three genotypes were found in three different time period over the 17 years period. Genotype A was found in

the oldest samples available for study i.e. 1972 to 1979. Within the genotype A separate lineages could be documented for example at least two lineages each in the year 1972-1973 and 1974-1975.

The limitations are that we have sequenced a small number of isolates out of a very large collection, but the data are sufficient to indicate multiple chains of virus transmission. Although all the possible lineages could not be documented because of small number of isolates sequenced, it is sufficient to multiple lineages. It is very interesting to find the transition from one genetic lineage to another in Mumbai. The dendrogram shows the appearance of genotype B starting from the year 1979. Genotype A an B co-circulated in the city during1979. A clear displacement of genotype A by B could be observed. Intense transmission of genotype B continued from 1979 until 1984. A close genetic similarity between an isolate from Utter Pradesh V5402 in 1986, and Mumbai V7210 (P ward, 1987), indicates exchange of virus between Utter Pradesh and Mumbai even in the eighties. The arrival of genotype C in the year 1985 (co-circulated with genotype B) indicates another epidemiologic event in the history of poliovirus type 3 in Mumbai.

More than 95% of all the wild poliovirus type 3 strains isolated from paralytic cases detected in Mumbai between 1992 and 2000 were subjected to sequence analysis. Figure 21 shows a composite dendrogram presenting genetic relationship using VP1/2A sequences of virus isolates from years 1972 to 2000. Viruses belonging to genotype A and C were not detected even when more than 95% of all isolates were sequenced. Thus the respective genotypes were completely eliminated from circulation. A single isolate of the year 1997 (97-127) from Utter Pradesh (a case of paralytic poliomyelitis of Utter Pradesh origin, reported from Mumbai) of genotype C. It is therefore possible that this genotype was present in Utter Pradesh over a long period of time and was probably exported from Utter Pradesh to Mumbai.

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Genotype B was detected in Mumbai in 1979 and continued transmission until 1996. Over the 16 years period the four lineages of wild poliovirus type 3 were identified during the period 1992 to 1996. The important features of the isolates (1992-1995) are the reduction in the genetic distance (strain diversity) within each lineage. This is an evidence of good AFP surveillance through which almost all the cases were detected and virologically investigated. Epidemiologic link between the cases was therefore easy to determine by sequence analysis. The last case of wild poliovirus type 3 due to locally circulating virus was identified to be strain number V10177 from N ward in January 1996.

Wild poliovirus type 3 was isolated from one each case in the years 1998, 1999 and 2000 from Mumbai. Sequence analysis proved that these isolates did not belong to the previously detected local lineages and therefore provide clear evidence of importation of the virus from other endemic areas. Thane district is geographically adjacent to Mumbai. Poliovirus type 3 isolated from the cases from Thane in 1997 to 2000 shows close sequence similarity with isolates from Utter Pradesh. Three separate instances of virus importation into Thane were also identified. Evidence also shows that virus established indigenous transmission for a short duration of time.

Polio eradication program was intensified in 1995-96 with the decision to conduct countrywide PPI days. All wild poliovirus transmission chains indigenous to Mumbai were eliminated immediately after the two PPI rounds in the years 1995-1996. Only one case of wild type 3 infection in 1996 and none in year 1997 were reported from Mumbai. All the isolates after that period could be linked to importation from Utter Pradesh. In sharp contrast to the success in Mumbai, wild poliovirus transmission continued in Thane district. It required two additional PPI and intensified mopping up activity to eradicate wild type 3 focus from Thane.

A total of 135 strains were sequenced in the VP1/2A region. For the purpose of legibility the genomic relation is presented in two separate

dendrograms (Figures 25 and 26). Some of the strains were repeated in both the dendrograms to clarify the relations of groups of isolates in the two dendrograms. Large sequence diversity was observed among the isolates of the years 1998 and 1999. Three genotypes were identified using the VP1/2A data set. Multiple lineage transmission was observed in Karnataka, Tamil Nadu, West Bengal, Utter Pradesh, and Bihar. Noteworthy is the finding that genotype 3 was predominant in India. Regional circulation of specific lineages was observed as highlighted by lineage 3a and 3c and wider circulation of 3b.

In the year 1999 more than 600 cases of wild poliovirus type 3 etiologies were reported from Utter Pradesh. Figure 26 clearly shows that the outbreak strain spreads from Utter Pradesh to almost all parts of India. The minimal sequence diversity of the outbreak virus highlights rapid spread. Sequence analysis also provided the information to the program on establishment of the outbreak strain in certain areas. The continued circulation of the outbreak lineage in certain areas permitted evolution and divergence to form newer lineages.

All virus isolated from low polio zone from 1999 were subjected to complete VP1 sequence analysis to know more about the transmission of the wild poliovirus type 3. Within Utter Pradesh a large number of isolates of the 2000 onwards were sequenced. The complete VP1 sequence analysis was used to define the transmission pathways at much higher resolution within a given epidemic (Kew et al, 1993).

Discordance was found in the genotypic classification of the isolates on the basis of VP1 and VP1/2A sequence data. The isolates classified into 3 genotypes on the basis of VP1/2A sequence data could be grouped into single genotype after analysis of VP1 sequences. The VP1 sequence information is at least 6 times larger than VP1/2A sequence information. It has much better resolution of sequence relationship determination. In recent years recombination in non-capsid protein regions of poliovirus has been noted within polioviruses and in enteroviruses (King, 1988;

Cammack et al, 1989). We therefore feel that the diversity within the 2A region resulted in large genomic drifts.

Figure 27 provides the evidence of continued transmission of the year 1999 outbreak strain (1999) in Utter Pradesh. Poliovirus evolves at a rate of 1-2% per year over the entire genome. Evolution of the outbreak strain through continuous transmission in Utter Pradesh over a period of 2 years led to the development of individually identifiable lineages. By the end of the year 2001 the outbreak strain had evolved into 4 lineages specific to Badaun, Moradabad, Bareilly, and Basti districts. The high quality AFP surveillance supported with molecular epidemiology highlighted undetected (silent) transmission of virus in or around Gonda district. The VP1 sequence data of the year 2002 from Bahrampur district was closely related to virus earlier isolated from Gonda in January 2001. The virus of the lineage was not detected for more than 16 months. Similar silent circulation with the wild poliovirus type 3 is of great concern in Egypt. One case of wild poliovirus type 3 etiology was detected from Egypt in the year 2000; however at least 16 wild poliovirus type 3 isolates has been isolated from the environmental samples. The 64 wild poliovirus type 1 cases detected from the environment sampling during 2000 and 2001 in Egypt are epidemilogically linked to the paralytic cases, shown by the partial genome sequence analysis (WHO, 2002a).

Molecular epidemiology has elucidated the process of eradication of polioviruses. Wild poliovirus type 2 was eradicated very rapidly when immunization coverage reached moderately high levels and only two rounds of PPI were sufficient to stop poliovirus transmission. Just prior to elimination of poliovirus type 2 from Mumbai multiple lineages were circulating in the population. All the lineages were eliminated in one good quality immunization activity. The same probably happened in Utter Pradesh as well for poliovirus type 2. Gradual reduction in the strain diversity therefore not necessary for all the serotypes as was suggested earlier (Kew et al, 1995). In contrast to wild poliovirus type 2 wild poliovirus

type 3 showed a gradual decrease in strain diversity before its elimination from Mumbai and other states. In Utter Pradesh, however, the large outbreak in the year 1999 replaced the earlier lineages, therefore observation in the reduction in diversity in Utter Pradesh was due to spread of a single outbreak strain. In last two years, the vaccination program in Utter Pradesh has received a set back. Increased genomic diversity of wild poliovirus type 3 in last two indicates poor quality of vaccination campaign in Utter Pradesh.

The continued circulation of wild polioviruses in India is of great concern for global polio eradication. Almost 80% of the worldwide poliomyelitis cases were reported from India in the year 2002. Therefore success of polio eradication program heavily depends on India. It is hoped that India can complete the most challenging final stages of polio eradication with strong political support, high quality program, as well as continue support from other polio partners

The major focus of eradication has been, and continues to be, completing the task of stopping wild poliovirus transmission. Given the rapid progress made towards stopping wild poliovirus transmission, planning for the post eradication era has begun earnest to minimize the risk of reintroduction of virus into the population from laboratory stock or long term carriers and to prevent vaccine derive polioviruses (cVDPVs) from circulating and causing outbreaks. The outbreaks due the circulating VDPVs in Hispania and Philippines in recent times are the best examples for us to prepare strategies for future for the choice of vaccine to be used (CDC, 2001, 2001b).

After the eradication, the only source of wild polioviruses will be laboratories. Given the small, but not the non-existence risk of transmission of poliovirus from the laboratory is of great concern. The bigger challenge will be to identify all the laboratories with potentially infectious clinical, epidemiological, research, or environmental specimens collected for other purpose, in a geographical area and at a time of wild

poliovirus endamicity. Efforts need to be made to minimize the risk that the poliovirus will escape from the laboratory after transmission of wild poliovirus is interrupted (Wood et al, 2000).

India has yet to achieve the major task of stopping wild poliovirus transmission. Considering the vastness of the country and the large number of institutions involved in bio medical research the laboratory containment of wild poliovirus is going to be equally difficult. Circulating vaccine derive polioviruses (cVDPVs) have been isolated from countries with low vaccination coverage after eradication of wild poliovirus was achieved. The OPV coverage and peoples participation in the program has shown wide variation in the country. Laboratory surveillance for cVDPVs will need to be carried out for many years.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Summary and conclusions

The goal of global polio eradication program goal has been to achieve zero cases of paralytic poliomyelitis due to wild poliovirus infection. Concerted effort by WHO and health ministries of various, supported by the donor agencies has led to significant progress in reducing the disease burden. However eradication of wild poliovirus by the end of year 2000 has not realized on time. At the end of the year 2000, ten countries continued to transmit wild poliovirus.

In India substantial progress has been made towards polio eradication. The number of paralytic cases declined from about 2000 in the year 1998 to 265 cases in the year 2001. Majority of the remaining cases were reported from Utter Pradesh and Bihar. States in the southern parts of India, however, have achieved zero polio. The success towards polio eradication has been credited to establishment of an efficient surveillance system, improvement of OPV coverage, supplemental immunization activities and WHO accredited polio laboratory network that carryout virological diagnosis of AFP cases.

Epidemiology of any disease remains incomplete without molecular characterization of the etiologic agent. Meager information was available on the genomic characterization of poliovirus isolates from India. In support of the polio eradication program in India, molecular characterization of wild poliovirus isolates was desired to, understand pathways of virus transmission, identify the reservoir areas sustaining wild poliovirus transmission, to provide the evidence of poliovirus elimination, to suggests control activities.

The primary aim of this thesis was to generate information about wild poliovirus transmission to refine the polio eradication tactics. In the present study we have tried to study wild poliovirus type 2 and 3 isolated from Mumbai covering a period from 1971 to year 2000. Significant importance is given to the isolates from the year 1991 onwards by studying more than 90%

of all the isolates. The data accrued permitted to draw inference on the genotypes, lineages and genetic diversity of the strains before the initiation of eradication program and since its implementations until their elimination.

Molecular sequencing of poliovirus type 2 and 3 strains from the entire country for the period 1998 to 2000 forms a significant portion of the thesis. Characterization of these strains was done to evaluate the progress of activities of polio eradication initiative in India and also to guide the efforts to special regions.

The sequence studies of the VP1/2A junction region showed circulation of one genotype of wild poliovirus type 2 between the years 1971 and 1986. The genotype diverged sufficiently to establish into separate lineages, which spread to different wards of Mumbai. Between the years 1991 and 1992 two genotypes with multiple lineages were detected in the city. Sequence analysis show that poliovirus type 2 isolated from case from Buldhana district of Maharashtra had close epidemiological link with the isolates from K ward in Mumbai. All wild type 2 poliovirus transmission chains were eliminated from Mumbai by the end of the year 1995.

The sequence data obtained from the entire VP1 region of wild poliovirus type 2 from Utter Pradesh and Bihar inferred circulation of a single genetic lineage in 1998 and 1999. Multiple transmission chains in certain districts helped to identify areas with very low immunization coverage. Only two cases of wild poliovirus type 2 etiologies were reported from areas other than Utter Pradesh and Bihar, one in Gujarat in 1998 and the other in West Bengal in 1999. Complete VP1 sequence comparison revealed close epidemiological links of these isolates with viruses from Utter Pradesh. The data informed the program that there was no indigenous transmission of wild poliovirus type 2 in Gujarat and West Bengal.

The VP1/2A junction region sequence data show presence of three genotypes of wild poliovirus type 3 in Mumbai between 1972 and 2000. One

of the genotype circulated from 1972 to 1979 and then replaced with the other genotype. The poliovirus circulated forming independent lineages. As the polio eradication efforts accelerated, the strain diversity reduced considerably to the extent that only one indigenous lineage of wild type 3 survived during years 1993 to 1996. All the genotypes and lineages indigenous to Mumbai stopped circulating in the year 1997. Sequence data identify that the three cases detected between the years 1998 and 2000 belonged to separate lineages imported from Utter Pradesh. In Bhiwandi, Thane district however, poliovirus type 3 transmission established after virus importation from Utter Pradesh. The information given to the health ministry was useful in planning the mopping up activities in that area. The Bhiwandi focus was eliminated by the end of the year 2000.

Wild poliovirus type 3 sequenced from all over India represents one genotype. As the polio eradication progressed, the elimination of the lineages was documented. In the year 1999 there was an outbreak of wild type 3 etiology in the state of Utter Pradesh. The sequence data shows that the outbreak virus spread to the neighboring states, up to Maharashtra, Gujarat, Karnataka from where these are quickly eliminated through supplemental immunization. In Utter Pradesh however, the continuous circulation the epidemic virus ultimately developed into four lineages.

Sequence information was useful in identification of geographical areas requiring special attention to improve immunization activities such as mopping up, sub-national immunization, and or the quality of pulse polio immunization.

CHAPTER 8

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SYNOPSIS

A SYNOPSIS OF THE THESIS TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

1.	Title of the thesis	:	Molecular Characterization of wild Poliovirus isolates
2.	Name of the candidate	:	Mr. Zaeem Asif Siddiqui
3.	Name of the guide	:	Dr. S. V. Gadre, M.Sc., Ph.D.
4.	Place of Research	:	Haffkine Institute, Parel, Mumbai – 400 012.
5.	Date of registration	:	1st July, 1997
6.	Registration Number	:	30

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Introduction

Paralytic poliomyelitis has afflicted humans since early antiquity. An Egyptian stele from 18th dynasty (1580-1350BC) depicts a crippled young man with a withered and shortened leg with his foot held in a typical equinus position characteristics of flaccid paralysis (Paul, 1971). In 1908, Landsteiner and Levaditi successfully transmitted the disease to monkeys by inoculation of sterile filtrate of spinal cord tissue collected from fatal cases of paralytic poliomyelitis thereby proving viral etiology of the disease. Burnet and Macnamara (1931) recognized more than one antigenic type among poliovirus isolates; finally the three immunological types were identified (Bodian et al 1949).

In 1949 Enders, Weller, and Robbins cultivated poliovirus in cultures of non-neuronal tissues. This discovery paved the way for development of vaccines against poliomyelitis (Salk, 1954; Sabin, 1973). The trivalent oral poliovirus vaccine (Sabin) was introduced in 1963 (Hortsmann, 1960). The widespread use of IPV and OPV rapidly controlled poliomyelitis in United States and other European countries. In 1988, World Health Assembly declared the goal to eradicate poliomyelitis by the year 2000 (WHA, 1988).

Poliovirus enters the body via mouth (Sabin & Ward, 1941). Virus may replicate in pharyngeal lymph nodes, tonsils and in the intestine (Peyer's patches). Infected individuals excrete poliovirus for 2 to 4 weeks. Virus invades regional lymph nodes and reaches the blood stream. Virus can then invade the central nervous system and replicate in the anterior horn cells of the spinal chord. Destruction of the invaded neurons cells results in acute flaccid paralysis (AFP) (Bodian and Horstmann, 1965). Depending upon the anatomical localization of the lesions and the muscular groups paralyzed, poliomyelitis is classified into spinal, bulbar and bulbo-spinal.

Diagnosis of paralytic poliomyelitis depends on clinical findings and virological testing. Minor illness is the most frequent form of the disease characterized by transient illness followed by fever, malaise, drowsiness, headache, nausea, vomiting, constipation, or sore throat in various combinations. An AFP case is most likely to be due to poliovirus infection if it shows fever at onset of paralysis, onset is sudden and paralysis reaches maximum within 48 to 72 hours. Also there is no sensory loss and paralysis is descending. Because anterior horn cells of the central nervous system cannot be regenerated or replaced, the paralysis is permanent. Laboratory confirmation of poliovirus etiology is critical for polio eradication program because other enteroviruses are also known to cause acute flaccid paralysis. In the laboratory poliovirus can be isolated from the stool specimen using susceptible cultured cells followed by serological typing of poliovirus as type 1, 2 or 3 using specific antisera. Poliovirus isolates are differentiated as vaccine or wild type. ELISA, probe-hybridization, PCR, nucleotide sequencing or RFLP assays can differentiate wild virus isolates from vaccine strains. Attenuated poliovirus vaccine stains (Sabin OPV strains) have been fully characterized (complete genome nucleotide sequences, antigenic nature, and attenuation sites). All poliovirus isolates of non-vaccine (Sabin) origin are regarded as wild poliovirus.

Poliovirus is a member of genus Enterovirus of virus family Picornaviridae. Poliovirus is, 27-30nm in diameter and shows icosahedral symmetry. Poliovirus like other Enteroviruses is stable at acid pH (3.0 to 5.0) for 1 to 3 hrs. They are inactivated when heated at 55^0C for 30 min. but $MgCl_2$ retards heat inactivation (Malnick, 1963). The genome of poliovirus is a single molecule of single stranded messenger sense RNA approximately 7500 nucleotides long. A little more then one third coding capacity is used for making the four capsid proteins VP1 to VP4; the rest being used for coding the non-structural proteins. Capsid protein VP1 is the most exposed protein and bears major antigenic site. The nucleotide sequences of the entire genome of each the three types have been

determined (Toyoda, et al 1984). Three-dimensional structure of poliovirus has been elucidated at 2.9^{0}A resolution by X-ray crystallography (Hogle, et al 1985). Thus poliovirus becomes the most studied human virus.

Application of molecular tools, such as RNA fingerprinting, PCR-RFLP and genomic sequencing of poliovirus has added a new dimension and resolution power to our understanding of epidemiology of poliomyelitis (Kew et al 1995). Poliovirus genome incorporates mutations frequently and evolves rapidly because of the lack of proof reading mechanism during RNA replication. Epidemiological link between poliomyelitis cases could be determined by comparing genomes of poliovirus isolates (Kew and Nottay, 1984, Ricco-Hesse et al, 1987, Balanant et al 1991). RNA fingerprinting compared RNase T1 resistant oligonucleotide fragments complete genomes of poliovirus isolates. Though viruses could be differentiated, the experimental procedures were time consuming and laborious (Kew, et al 1995). PCR-RFLP methods differentiate wild and vaccine virus isolates accurately but resolution of different wild virus isolates is not sufficient. Comparison of sequence of only 150 nucleotides at the VP1/2A junction region of wild poliovirus isolates from different parts of the world could distinguish between genetic types of viruses circulating in these regions (Ricco-Hesse et al 1987). Indigenous strains isolated in different geographical regions usually exhibited a significant genetic diversity, on the other hand, a high degree of similarity was observed among the strains isolated from any given epidemic. A genotype in this context has been defined as a group of viruses showing >85% sequence similarity. The viruses showing >95% sequence similarity are considered to have diverted from an ancestral type (lineage) and where wild poliovirus isolates from poliomyelitis cases show >98% sequence identity they are supposed to have diverged only recently and indicate probable epidemiological linkage (transmission chain). Molecular characterization of poliovirus isolates therefore permits the investigator to define whether infections in a community are due to one or many genotypes/ lineages and also provides

information on transmission of virus. The sequence comparison could be used for defining importation of poliovirus from endemic regions to polio free regions when a sequence database of indigenously circulating wild poliovirus strains was available (Mulders et al 1995a, 1995b, Chezzi et al 1997, Fiore et al 1998).

Extraordinary progress towards polio eradication has been made since 1988. The Americas and the Western Pacific Region of the WHO were certified polio free in the year 1994 and 2000, respectively (Robbins and de Quadros 1997, Wkly, Epidemiol Rec 2000). More than 175 countries are now polio free. In 1999, poliomyelitis was still endemic in India, Pakistan, Afghanistan, sub-Saharan African countries and parts of Eastern Mediterranean countries (WHO/Polio/00.05,2000).

Ministry of Heath is responsible for polio eradication from India. A National Polio Surveillance Project was established to oversee the eradication activities, maintain centralized database of poliomyelitis and provide advice. A network of nine laboratories, which is a part of WHO Global Lab-Network, is responsible for virological investigations. In India, transmission of poliovirus has dramatically reduced following application of polio eradication strategies. These include a) High routine immunization courage b) Supplement immunization provided in the form of National Immunization Days c) Acute flaccid paralysis (AFP) surveillance system d) Mopping up (Wright et al, 1991).

The incidence of poliomyelitis has declined from reported figure of 28,380 cases in 1987 to less than 10,000 in 1990 and 5,669 in 1991 (Sokhey, 1992). As of the year 2000, 265 confirmed cases due to wild type poliovirus infections were detected in India. Mumbai reported more than 1000 cases of paralytic poliomyelitis in children annually until 1987. At the end of the year 2000, only 3 wild poliovirus-confirmed cases were detected in spite of good surveillance (Annual Report, 2000).

Introduction to the problem:

It is now well accepted that poliomyelitis can be eradicated. As OPV immunization reaches increasingly larger proportion of infants wild poliovirus transmission is eliminated in order of poliovirus type 2, type 3 and type1. Literature also shows that although wild poliovirus type 1 has caused major outbreaks due to poliovirus type 1, poliovirus type 3 required special attention. Elimination wild poliovirus transmission from South America was difficult, requiring modifications in OPV formulation (Ref). Finland and The Netherlands experienced outbreaks of poliovirus type 3 even though population was very well immunized (Hovi et al, 1986 Ootvogel, 1994). In Egypt a small number of polio 3 cases still continue to appear and very recently a severe outbreak of poliovirus type 3 etiology spread through Angola (Valente et al, 2000).

Very little information was available about the genomic characterization of poliovirus and the number of genotypes circulating in India. Seeing the difficulties in eradicating this serotype mentioned above it was decided to investigate poliovirus type 3 strains from India at the molecular level. While the study was in progress, wild poliovirus type 2 transmission was completely stopped in India, in the year 1999. Thus the Program (PEI) achieved eradication of one poliovirus serotype out of the three types. Thus there came up an opportunity to be able to study the globally last transmission chains of poliovirus type 2.

Aim and Objectives of the study

The main aim of the present investigation was to study the epidemiology of wild poliovirus by molecular methods especially using genomic RNA nucleotide sequence analysis.

The following objectives were defined

1. To determine genetic diversity of wild poliovirus type 3 in India.
2. To study transmission of wild poliovirus type 3 in different parts of India.
3. To characterize isolates from Mumbai to know when certain lineages were eliminated as polio eradication progressed.
4. To sequence and characterize last lineages of wild poliovirus type 2 in India.

Material and methods

Viruses

The Enterovirus Research Center (ERC) is the Regional Reference Laboratory in India. The National Polio Laboratories in India submit poliovirus isolates to ERC for further characterization. Poliovirus isolates from all states in India were thus available.

Wild poliovirus type 3 isolates from Mumbai were made available from the virus bank maintained at ERC. Representative isolates from 1971 to 1989 were studied. All wild poliovirus type 3 strains isolated from paralytic cases reported from Mumbai after 1993 were characterized.

Wild poliovirus type 3 isolates (1998- 2001) from all states in the country onwards were studied to determine genotypes and their transmission in India.

Wild poliovirus type 2 of the years 1998 and 1999 were also studied.

Intratypic differentiation tests

ELISA: Strain specific cross-absorbed polyclonal antibodies were used to determine vaccine vs. wild origin of poliovirus isolates on the basis of antigenic make-up.

Probe-hybridization: DIG- labeled RNA probes were used to differentiate vaccine vs. wild origin of poliovirus isolates on the basis of the nucleotide sequences in VP1 protein region.

RNA Sequencing

Polymerase chain reaction: Tissue culture harvest containing the virus was used as the starting material for amplifying poliovirus RNA. The RNA is extracted using Guanidium iso-thiocyanate, phenol-chloroform extraction (Tri-Pure, Roche Mole Biochem). Reverse transcription (AMV-RT) was used to convert poliovirus RNA to cDNA for further use in PCR. RT- PCR protocol developed by Yang et al (1992) was used. Primers were initially obtained from Centers for Disease Control and Prevention (CDC), Atlanta. Primers designed or modified at the ERC were used whenever required (Nadkarni, 2000).

For the amplification of VP1/2A		
Primers used	Position	Product size
Q8 (reverse)	5' 3476-3496 3'	Q8/ S14F: 296bp
S14F (forward)	5' 3203-3223 3'	
P3/PCR49 (forward)	5' 3245-3252 3'	Q8/ PCR4: 270bp
For the amplification of entire VP1 region		
Q8 (reverse)	5' 3476-3496 3'	Q8/S2B 653bp
S2B (forward)	5' 2852-2871 3'	
Y7 (forward)	5' 2399-2421 3'	Y7/S1 608bp
S1 (reverse)	5' 2987-3006 3'	

After RT-PCR the product was checked by electrophoresis in 1% agarose gel. Amplification product was purified gel extraction kit (Qiagen, Germany).

Cycle sequencing: Primer extension, dideoxy derivative chain termination was used with modifications for cycle sequencing for fully automated DNA sequencer (Sanger et al, 1977 Ausubel et al 1998). The ABI PRISM dye terminator cycle sequencer ready reaction kit protocol (Perkin-Elmer Cat No 4303149) was used for setting up of the sequencing reactions. The sequences were determined by using an automated DNA sequencer model 310 ABI PRISM.

Sequence analysis: Sequences were edited using DNASIS, aligned in CLUSTAL. DNADIST and Neighbor Joining programs of the PHYLIP 3.5c were used to calculate pair-wise genetic distance and drawing the dendrograms, respectively.

Results

A total of 65 wild poliovirus type 3 (wild) isolates from cases of paralytic poliomyelitis reported from Mumbai during the years 1992 to 2000 were studied. These isolates were from poliomyelitis cases among Mumbai residents as well as those cases brought to the city for treatment. The goal was to sequence most of the isolates so that all lineages could be detected and represented in the database for genetic analysis. This goal was achieved.

VP1/2A junction region was amplified in RT-PCR reactions to obtain a cDNA segment of 274 nucleotides long. Both strands of the cDNA were sequenced for obtaining unambiguous data. Finally 150nt fragment representing 90 bases of VP1 protein and 60 bases of 2A protease was used for determining genetic relationship of the isolates.

Two genotypes of wild poliovirus type 3 were identified to have circulated in Mumbai in the eight years period (1992- 2000). Elimination of previously circulating lineages could be documented. It was also found the all the indigenously transmitting lineages from Mumbai stopped circulating (because of polio eradication activities) in the year 1997. However, wild poliovirus entered Mumbai environments due to population influx and through infected children. These results also highlight the problems of polio eradication in metropolitan cities.

93 isolates from different parts of India were also sequenced for genetic analysis. Independently circulating lineages were detected in the various states. In the year 1999, wild poliovirus was isolated from more than 600 cases of poliomyelitis from Uttar Pradesh. Our analysis showed that the UP outbreak strain spread to far and wide states like Punjab, Rajasthan, Maharashtra, Karnataka as well as West Bengal.

The sequence window was widened to entire VP1 protein gene (900 nucleotides) for more sensitive differentiation between isolates obtained in short time frame. With this high resolution it was possible to detect poliovirus type 3 transmission chains in various districts of Uttar Pradesh. Five independent transmission chains of wild poliovirus type 3 were detected in Utter Pradesh in 2000-01. Similarly a growing focus of wild poliovirus type 3 in Thane district in Maharashtra was progressively documented for the purpose of intensification of polio control measures.

The classic example of the power of molecular epidemiology in identifying links between poliomyelitis cases was that of determining the origin of the case reported in Mumbai to Basti district in UP even though the same lineage was known to be circulating in Thane district.

In addition, a total of 34 isolates from Mumbai (1971-89) representing isolates of high endemic period were also characterized. Details will be discussed in the thesis. The genetic characterization of last lineages of wild poliovirus type 2 isolates in the world (Uttar Pradesh, 1998-99) will form a chapter of interest in the thesis. Sequence analysis

will be presented in the thesis as sequence alignments, distance matrices, and dendrograms depicting the genetic relationship of poliovirus isolates.

This work was carried out in support of WHO polio eradication initiative in India.

Summary and conclusions:

1. Two genotypes of poliovirus type 3 were identified in Mumbai during 1992-96.
2. Sequence analysis of majority of isolates from Mumbai revealed elimination of indigenously circulating wild P3 lineages and importation of new lineages from the endemic region in the country. All indigenous P3 lineages were eliminated in 1996.
3. Importation of viruses from Uttar-Pradesh to Maharashtra and its continued transmission was proved by sequence analysis.
4. Spread of Uttar-Pradesh outbreak (1999) virus to other states has been documented through sequence analysis.
5. VP1 sequence analysis allowed high-resolution molecular epidemiology thereby determining individual transmission chain of the viruses (2000-2001). The information was provided to PEI to undertake control measures.
6. A complete database of wild poliovirus type 3 has been created for future use.

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