Vol 3 (2), July 2012; 28-34

The Global Polio Eradication Initiatives: From Past to Present

Shazia Tabassum Hakim[1], Sayyada Ghuffrana Nadeem[2], Shaista Malik[1], Urooj Javed[2], Abdul Basit Khan[3]

[1]Virology and Tissue culture Laboratory, Department Of Microbiology, Jinnah University for Women, Karachi

[2]Medical Mycology Research and Reference Laboratory, Department of Microbiology, Jinnah University for Women, Karachi

[3]PCSIR Labs. Complex, Off University Road, Karachi

INTRODUCTION

The war against infections is one that has been remuneration all through the history of human. In this centuries-long effort the worldwide population has prepared remarkable advancement in stopping infections, therapeutic infections, and enlarging living expectancies. Up till now for all our successes, only once in history 30 years ago, with smallpox made completely eliminate an infection from the earth. Now a days we are on the entrance of eradicating the earth of polio an infection able of crippling and killing many children. The story of polio is both lengthy and hesitant. The arrival of efficient vaccines in the 1950s facilitated polio prevention, and the resultant struggles were victorious wildly, however confronts still continue (Plan, 2011).

Abbreviations: PV (Polio Virus), VAPP (Vaccine Associated Paralytic Poliomyelitis), cVDPV (Circulating Vaccine Derived Polio Virus).

THE POLIO VIRUS

Polio is a well-recognized infection and has its roots bottomless in the history of various cultures and civilization (Global Eradication Initiative, 2010b; Valtanen, et al. 2000). The causative agents of poliomyelitis is Polio viruses, characterize into three serotypes in the genus *Enterovirus* (family *Picornaviridae*). The virions have a single-stranded, messenger-sense RNA and 60 copies of each of the 4 capsid proteins VP1 to VP4 (Mick, et al. 1999). The RNA genome of poliovirus is about 7,500 nucleotides (nt) in length and has the polarization of mRNA, therefore describing it as positive stranded (Belov, et al. 2012) . The positive-sense genome RNA of PV, which is surrounded by a protein capsid shell, be able to be translated into a large polyprotein in a host cell, and after that the large polyprotein is sliced by viral proteases into a dozen of different proteins (Shen, et al.2012). The proteolytic procedure flow produces about 10 mature proteins and numerous intermediate products, several of which carry out their self-determining jobs in the life cycle of virus (Belov, et al. 2012).

*Corresponding author: g-sarwaar@hotmail.com

SYMPTOMS OF POLIO

The majority of illnesses are unapparent (72% or more). A more 5–24% may have an insignificant disease by flu-like symptoms including headache, gastrointestinal disorders, malaise, neck and back stiffness. About 4% build up nonparalytic poliomyelitis, below 1% in total, and 0.5% of children having infection, comprise paralytic infection. Paralysis is may be spinal (79%), bulbar (2%), or mixed spinal bulbar (19%) (Mayer and Neilson, 2010).

PATHOGENECITY

PV briefly lives in the GIT (gastrointestinal tract). The virus go into the mouth and make copies in the pharynx and gastrointestinal cells, after that moves towards the blood stream through local lymphoid tissue, then move towards the CNS (central nervous system) and replicate in the motor neurons of anterior

horn cells of the spinal cord and brain stem, cells of the roof of the cerebellum and the motor cortex, having the reason of their demolition (Mayer and Neilson, 2010). Direct neural increase of PV may also arise in certain circumstances, for example in tonsillectomy with following bulbar paralysis or subsequent injection of an annoying matter into a limb leading to following paralysis of that limb (Robertson, 1993). PV disease yields in various alters to the host cell, and perhaps one of the mainly prominent is the huge buildup of cytosolic double-membrane vesicles and these vesicles are the hallmark of autophagy, a degradative lane of homeostasis and pressure response. Poliovirus specially persuades autophagic signaling, and virus construction associates to the level of autophagic activity in cells (Richards and Jackson, 2012). A site consist of amino acid 89 to 100 of VP1 is a main immunogenic place for type 2 and type 3 polioviruses, as evaluated by monoclonal antibodies produced in mice (Robertson, 1993). Subsequent natural exposure, IgM and IgG emerge in the serum about 7 to 10 days after disease. Adequately elevated levels can block poliovirus entrance into the CNS (central nervous system).Originally, the IgM response is 2- to 8-fold larger than the IgG response (Robertson, 1993). Diagnosis can be done on the basis of clinical, with demonstration by stool testing or serology (Mayer and Neilson, 2010).

EPIDEMIOLOGY

Infection with Polio Virus was general globally, with seasonal max outs and epidemics in summer and autumn in temperate regions (CDC, 2010; Mayer and Neilson, 2010). Polio has been filed in history for thousands of years, preliminary with very old Egyptian that ranges from 1580-1359 B.C. This infection was first analyzed as an epidemic in the United States and Europe in the late 1800s. While polio has influenced people as ancient times, it was solitary earliest medically accounted in 1840. Unfortunately, only fifty years later in 1890, epidemics start to occur and polio was a main concern for doctors and researchers. Through that time, polio was habitually exclusively observed in children; that's why it was often submitted to as childhood paralysis (Valtanen, et al. 2000). In 1952, more than 21000 cases of paralytic poliomyelitis were filed in the United States (Alexander, et al. 2004; CDC, 1981).

The worldwide documentation of smallpox eradication in 1980 made significant attention in additional infectious disease eradication attempts. Subsequent the quick development towards break off aboriginal wild poliovirus spread in the Americas in the early 1980s, the worldwide Polio eradication initiative was launched with a declaration of the World Health Assembly (WHA) in 1988 (Global Eradication Initiative, 2010a; Aylward and Tangermann, 2011), and has since developed to turn into one of the biggest internationally corresponding health plans in history (Aylward and Tangermann, 2011) (Fig. 1).

Though, imported wild polioviruses from regions where polio is endemic have reason of epidemics in countries previously reported to be polio free and in which specific subpopulations either show a weak general immunity, such as in Finland in 1984 and 1985 (Hovi, et al.1986), or lack immunity, such as in The Netherlands in 1978 (Bijkerk, 1979) and 1992 (Oostvogel, 1994)), Bulgaria (WHO, 1992), and Romania (Strebel, et al.1994). The reemergence of poliomyelitis has also been reported in countries where political and financial alterations have made it hard to retain immunization plans, for example various areas of the previous Soviet Union in the 1990s (Oblapenko and Sutter, 1997; Patriarca, et al. 1997; Wassilak, et al. 1997; Fiore, et al. 1998).In spite of the extraordinary development of the WHO plans for worldwide eradication of infection, endemic poliovirus spread still continues in a lot of Asian countries also in Pakistan. Patients with acute infection of polio from all over the province of Sindh in Pakistan have been admitted to the Civil Hospital Karachi, and since 1989 they have been followed clinically and observed virologically (Huovilainen, et al. 1995). Pakistan started polio virus eradication work in 1994 and has had significant achievement (CDC, 2002). These actions are performed with the

continuing World Health Organization (WHO) prolonged plans on Immunization, which search for to vaccinate children against poliomyelitis, measles, diphtheria, pertussis, tuberculosis, tetanus, and hepatitis B (Lowther, et al. 2005). Through 2011, following setbacks that yield in cases being exported to new countries, 650 verified cases of poliomyelitis were accounted provisionally from 16 polio-influenced countries: 4 countries where the infection was endemic and 12 countries with restored diffusion (lasting =12 months) or outbreaks (lasting <12 months) after importations (CDC, 2011d; CDC, 2011e; CDC, 2012b)(Fig. 2) . In 2010 worldwide reporting of children with 3 doses of oral trivalent vaccine was approximately 85% but is uneven at the national and sub national levels (CDC, 2011d; Hopkins, 2013).

In January 2012, India commemorated a complete year with no cases of poliomyelitis, except Nigeria, Afghanistan, and Pakistan as countries with endemic infection here eradication was difficult for the reason that of political unsteadiness or frighten about immunization (CDC, 2011c; CDC, 2011a; CDC, 2011b)(fig.3). In 2011 among countries with restored spread Chad and the Democratic Republic of Congo identified the majority of cases (132 and 93, respectively (CDC, 2012b). Recently the polio virus has been discovered in sewage in Egypt which was thought to be imported from Pakistan because genetic analysis revealed that Egyptian virus linked to one that was last seen in Pakistan in September 2012 however Egypt has been polio free since 2004 this is the second time that polio virus from Pakistan has infected any country other than neighboring Afghanistan; the first was china where a virus from Pakistan made an outbreak in 2011(Roberts, 2013). The aim is to break off spread of poliovirus types 1 and 3 by December 2012. The major challenges to poliomyelitis eradication are contributor tiredness; political unsteadiness in areas of Afghanistan, Pakistan, and some other influenced countries; public tiredness with again immunizations against PV alone; and weak regular immunization systems. This plan is expected to expenditure of $9.5 billion for the

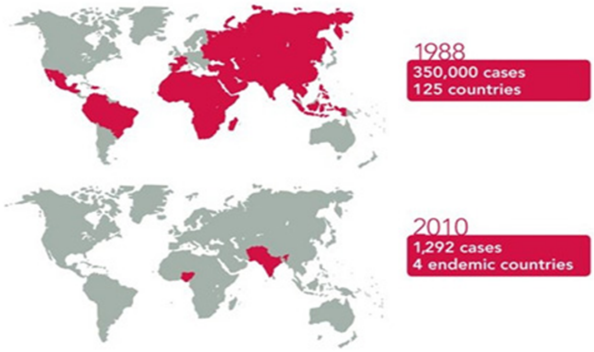

Fig. 1: WHO Polio Initiative
The World Health Organization spearheads an international program to eradicate polio around the world. Polio cripples an estimated 350,000 children that year. (cdc.gov)

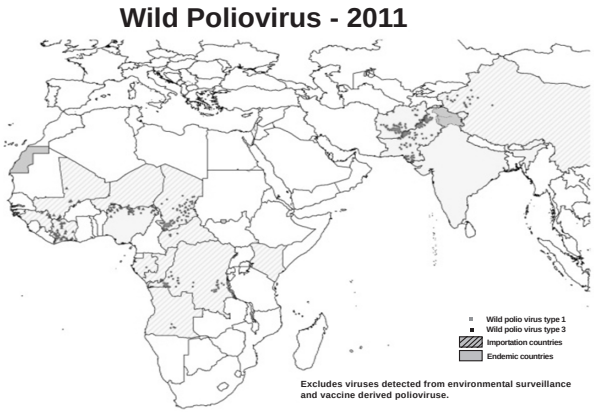

Fig. 2: Reported Cases of Poliomyelitis as of 2011. (polioeradication.org)

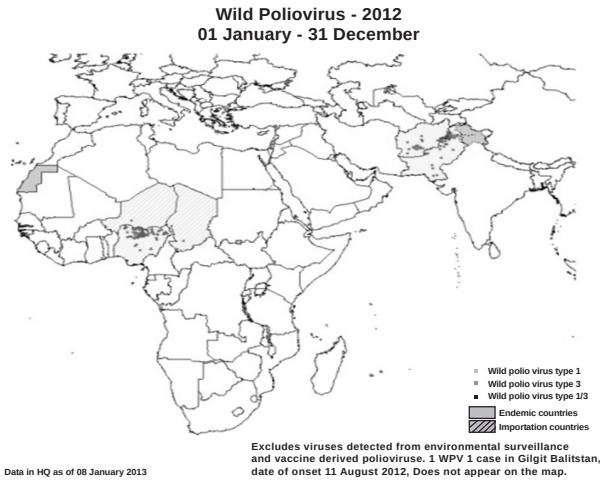

Fig. 3: Reported Cases of Poliomyelitis as of 2012. (polioeradication.org)

ERADICATION BY POLIO VACCINE

There are two polio vaccines which are utilized all over the world to eradicate PV. Inactivated polio vaccine (IPV) was the first vaccine produced in 1955 by Dr Jonas Salk also called the "Salk vaccine". It comprises of inactivated (killed) poliovirus strains of all 3 poliovirus types (Global Polio Eradication Initiative, 2010b) in which viral infection has been inactivated by treatment of formaldehyde. As a result, the utilization of IPV does not have the threats of VAPP or cVDPV outbreaks. Incubation with formaldehyde partly alters the structure of antigen of PV however inactivated vaccines have been observed to defend powerfully against the infection and have been the only vaccine utilized to control and eradicate PV in several countries (Martin, et al. 2013). IPV is administrated by intramuscular injection and requires to be managed by an educated health worker (Global Polio Eradication Initiative, 2010b). This polio vaccine generates antibodies in the blood to all 3 types of PV. In the incident of disease, these antibodies stop the growth of the virus to the central nervous system and defend against paralysis. IPV produces very small levels of immunity in the intestine therefore, when an individual immunized with IPV is infected with wild poliovirus, the virus be able to still reproduce within the intestines and be shed in the faeces. It is risky carry on distribution and is above five times extra costly than oral polio vaccine (Global Polio Eradication Initiative, 2010e).

The oral polio vaccine (OPV) was developed in 1961 by Albert Sabin. It is also called "trivalent oral polio vaccine" or "Sabin vaccine". This polio vaccine consists of a mixture of live, attenuated (weakened) poliovirus strains of all three poliovirus types. OPV produces antibodies in the blood to all three types of poliovirus. In the event of infection, these antibodies protect against paralysis by preventing the spread of wild poliovirus to the nervous system. It also produces a local, mucosal immune response in the mucous membrane of the intestines therefore these mucosal antibodies bound the replication of the wild poliovirus inside the intestine. This intestinal immune response to OPV is thought to be the main reason why mass campaigns with OPV can rapidly stop person-to-person transmission of wild poliovirus (Global Polio Eradication Initiative, 2010c). On the other hand, a serious consequence of the use of this live-virus vaccine, vaccine-associated paralytic poliomyelitis (VAPP), was recognized as early as 1962 (Luther, 1962; Henderson, 1964). From 1961 through 1989, an average of 9 cases of VAPP (range, 1-25 cases) were confirmed each year (Schonberger, et al.1976; Nkowane, et al.1987; Strebel, et al.1992; Alexander, et al. 2004) (fig.4). In immunocompetent individuals, the risk of VAPP is very low evaluated with that of immunodeficient patients (Yang, et al. 2005). Susceptible individuals usually excrete polioviruses for two to six weeks and occasionally for up to 137 days after they have been immunized with oral poliovirus vaccine (Sutter, et al.2003). The shedded viruses commonly show increased neurovirulence and are frequently transmitted to close contacts. Although immunodeficiencies are listed as a contraindication for receiving OPV, patients with these clinical conditions may sporadically receive the poliovirus live vaccine before their immunodeficiency is diagnosed and/or may be infected with OPV strains excreted by a vaccinee or that is present in the community (Galal, et al.2012).

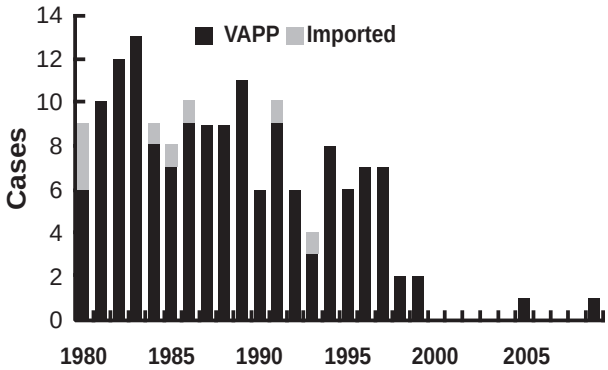

Fig. 4 Poliomyelitis - United States, 1980-2009

The duration and extent of spread are dependent on the magnitude of the immunity gap and the intensity of other risk factors favoring poliovirus circulation. This long-discussed hypothetical concern (Fine and Carneiro, 1999) has been realized by the recent occurrence of outbreaks of paralytic polio associated with circulating VDPVs (cVDPVs). Though several important themes are common to all of the outbreaks, each outbreak has taught its own important lesson about the parameters for the safe

administration of OPV in a world free of circulating wild polioviruses (Kew, et al. 2004). Developed or rich countries are replacing OPV with IPV to eliminate VAPP. Currently, 22 countries are using IPV exclusively and eight more have a sequential schedule of IPV and OPV (D. Wood, personal communication 2003). This situation has begun to evolve as rich–poor disparity. Global public health leaders are divided on the acceptability of VAPP in developing countries. Some recognize the double standard, as developing countries will be exposed to a risk that the industrialized nations will avoid (Nathanson and Fine, 2002).

CONCLUSION

Polio is one of the diseases that can be eradicated. Eradication is more than just bringing the number of cases to zero. Eradicating polio means that polioviruses will be wiped off the face of the earth and that vaccination will no longer be necessary (WHO, 1999). In present we are facing polio endemic in developing and poor countries which include Pakistan, Afghanistan, Chad, Ethiopia, DR Congo and Nigeria because of large populations, high birth rates, overcrowding, lack of education, poor sanitary condition, poorly functioning immunization systems and various controversial rumors about vaccine. It is vital to finish polio eradication in Pakistan, for the health of the nation, and for the whole global community. The highest levels of Government have committed to finishing this job as a national responsibility (Global Polio Eradication Initiative, 2010f). So this is the time to educate the people about vaccine. All we need is to immunize every infant with the oral vaccine to eradicate this endemic and make the globe free of this infection. If the eradication initiative stops prematurely, the disease will return with a vengeance.

REFERENCES

Alexander LN, Seward JF, Santibanez TA, Pallansch MA, Kew OM, Prevots DR, Strebel PM, et al. 2004. Vaccine policy changes and epidemiology of poliomyelitis in the United States. JAMA, 292(14): 1696–701. doi:10.1001/jama.292.14.1696.

Aylward B and Tangermann R. 2011. The global polio eradication initiative: lessons learned and prospects for success. Vaccine, 29 Suppl 4, D80–85. doi:10.1016/j.vaccine.2011.10.005.

Belov GA, Nair V, Hansen BT, Hoyt FH, Fischer ER, Ehrenfeld E. 2012. Complex Dynamic Development of Poliovirus Membranous Replication Complexes. J Virol, 86(1): 302–312.

Bijkerk H. 1979. Poliomyelitis in the Netherlands. Dev. Biol. Stand., 43:195– 206.

CDC. 1981. Annual Summary: Reported Morbidity and Mortality in the United States. Atlanta, Ga: Centers for Disease Control.

CDC. 2002. Progress toward poliomyelitis eradication—Pakistan and Afghanistan, January 2000–April 2002. MMWR, 51(24):523–4.

CDC. 2010. Travelers' health: yellow book. In: CDC Health Information for International Travel 2010. Atlanta: U.S. Department of Health and Human Services, Public Health Service, 2009.

CDC. 2011(a). Progress toward poliomyelitis elimination — Nigeria, January 2010–June 2011. MMWR, 60: 1053-7.

CDC. 2011(b). Progress toward poliomyelitis eradication — Afghanistan and Pakistan, January 2010–September 2011. MMWR, 60:1523-7.

CDC. 2011(c). Progress toward poliomyelitis eradication — India, January 2010 – September 2011. MMWR, 60:1482-6.

CDC. 2011(d). Progress towards interrupting wild poliovirus transmission worldwide: Janu- ary 2010–March 2011. Wkly. Epidemiol. Rec., 86:199-204.

CDC. 2011(e). Tracking progress toward global polio eradication — worldwide, 2009–2010. MMWR, 60:

441-5.

CDC. 2012(a). Poliomyelitis: Epidemiology and Prevention of Vaccine-Preventable Diseases (Pink Book). Available from:

CDC. 2012(b). Progress towards global interruption of wild poliovirus transmission, January 2011–March 2012. Wkly. Epidemiol. Rec., 87:195-200.

Fine PEM and Carneiro IAM.1999. Transmissibility and persistence of oral polio vaccine viruses: implications for the global poliomyelitis eradication initiative. Amer J Epidemiol, 150:1001-1021.

Fiore L, Genovese D, Diamanti E, Catone S, Ridolfi B, Avoort HG, et al. 1998. Antigenic and Molecular Characterization of Wild Type 1 Poliovirus Causing Outbreaks of Poliomyelitis in Albania and Neighboring Countries in 1996. J Clin Microbiol, 36 (7): 1912-1918.

Galal NM, Bassiouny L, Nasr E and Abdelmeguid N. 2012. Isolation of poliovirus shedding following vaccination in children with antibody deficiency disorders. J Infect Dev Ctries, 6(12):881-885.

Global Polio Eradication Initiative. 2010 (a). Global eradication of poliomyelitis by the year 2000 : World Health Assembly Resolution 41.28. Available from:

Global Polio Eradication Initiative. 2010 (b). Inactivated polio vaccine (IPV). Available from:. Global Polio Eradication Initiative. 2010 (c). Polio Eradication. Available from:

Global Polio Eradication Initiative. 2010 (d). Financial resource requirements 2012–2013. Available from: .

Global Polio Eradication Initiative. 2010 (f). Augmented National Emergency Action Plan for Polio Eradication 2012, Available from:.

Global Polio Eradication Initiative. 2010 (e). Oral Polio Vaccine (OPV). Available from: .

Henderson DA, Witte JJ, Morris L, Langmuir AD.1964. Paralytic disease associated with oral polio vaccines. JAMA, 190:41-48.

Hopkins, D. R. 2013. Disease Eradication. New Engl J Medi, 368(1): 54–63. doi:10.1056/NEJMra1200391.

Hovi T, Cantell K, Huovilainen A, Kinnunen E, Kuronen T, Lapin- leimu K, et al. 1986. Outbreak of paralytic poliomyelitis in Finland: widespread circulation of antigenically altered poliovirus type 3 in a vaccinated population. Lancet i: 1427–1432.

Huovilainen A, Mulders M, Agboatwalla M, Tuija P, Stenvik M and Tapani H.1995. Genetic divergence of poliovirus strains isolated in the Karachi region of Pakistan, J Gen Virol, 76: 3075-3088.

Kew OM, Wright PF, Agol VI, Delpeyroux F, Shimizu H, Nathanson N & Pallansch MA. 2004. Circulating vaccine-derived polioviruses: current state of knowledge/ Bull World Health Organ, 82(1): 16 – 23.

Lowther SA, Mir T, Bile MK, Abdul Hafiz R & Mounts AW. 2005. Characteristics of districts in Pakistan with persistent transmission of wild poliovirus, 2000-2001. East Mediterr Health J, 10(4-5): 582–590.

Luther T.1962. The Association of Cases of Poliomyelitis with the Use of Type III Oral Polio Vaccines. Washington, DC: US Dept of Health, Education, and Welfare: 1-8.

Martin J, Crossland G,Wood DJ and Minor PD. 2003. Characterization Of Formaldehyde-Inactivated Poliovirus Preparations Made From Live-Attenuated Strains. J Gen Virol, 84: 1781–1788.

Mayer CA and Neilson AA. 2010. Poliomyelitis Prevention in travelers. Aus Fam Physician, 39 (3): 122 – 125.

Mulders M, Reimerink J, Stenvik M, Alaeddinoglu I, Van der Avoort HG, Hovil T, et al. 1999. A Sabin vaccine-derived field isolate of poliovirus type 1displaying aberrant phenotypic and genetic

features,including a deletion in antigenic site 1. J Gen Virol, 80: 907–916.

Nathanson N and Fine P. 2002.Poliomyelitis eradication – a dangerous endgame. Science, 296:269-70.

Nkowane BM, Wassilak SG, Orenstein WA, et al. 1987. Vaccine-associated paralytic poliomyelitis. JAMA, 257:1335-1340.

Oblapenko, G., and R. W. Sutter. 1997. Status of poliomyelitis eradication in Europe and in Central Asian Republics of former Soviet Union. J Infect Dis, 175(Suppl.1):S576–S581.

Oostvogel PM., Van Wyngarden JK, Van der Avoort HG, Mulders M, Spaedonck M, Rumke HC, et al. 1994. Poliomyelitis outbreak in unvaccinated community in The Netherlands, 1992–93. Lancet, 344:665–670.

Patriarca PA, Sutter RW, and Oostvogel PM. 1997. Outbreaks of paralytic poliomyelitis, 1976–1995. J Infect Dis, 175(Suppl.1):S165–S172.

Plan, S. 2011. Polio eradication. 1–12.

Richards AL and Jackson WT. 2012. Intracellular Vesicle Acidification Promotes Maturation of Infectious Poliovirus Particles. PLoS Pathog, 8(11): e1003046. doi:10.1371/journal.ppat.1003046

Roberts L. 2013. Polio virus spread from Pakistan to Egypt. Science AAAS. Available from:

Robertson, S. 1993. The Immunological Basis for Immunization Series.ÊModule 6: Poliomyelitis.ÊWorld Health Organization. Geneva, Switzerland.

Schonberger LB, McGowan JE, Gregg MB.1976. Vaccine-associated poliomyelitis in the United States, 1961-1972. Am J Epidemiol, 104:202-211.

Shen H, Sun H, Li G. 2012. What Is the Role of Motif D in the Nucleotide Incorporation Catalyzed by the RNA-dependent RNA Polymerase from Poliovirus? PLoS Comput Biol, 8(12): e1002851. doi:10.1371/journal.pcbi.1002851

Strebel PM, Aubert-Combiescu A, Ion-Nedelcu N, Biberi-Moroeanu S, Combiescu M, Sutter RW,et al. 1994. Paralytic poliomyelitis in Romania, 1984–1992: evi- dence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection. Am J Epidemiol, 140: 1111–1124.

Strebel PM, Sutter RW, Cochi SL, et al.1992. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus associated disease. Clin Infect Dis, 14:568-579.

Sutter RW, Kew OM, Cochi SL. 2003. Poliovirus Vaccine. In: Plotkin SA and Orenstein WA, editors.Vaccines.4th ed. Philadelphia: W.B. Saunders Company. 651-705.

Valtanen S, Roivainen M, Piirainen L, Stenvik M, and Hovi T. 2000. Poliovirus-specific intestinal antibody responses coincide with decline of poliovirus excretion.ÊJ Infect Dis,Ê182**:** 1-5.

Wassilak S, Oblapenko G, Dittmann S. 1997. Progress in Europe towards the goal of poliomyelitis eradication. Eurosurveillance, 2:39–41.

World Health Organization (WHO). 1992. Expanded program on immunization. Poliomyelitis outbreak, Bulgaria. Weekly Epidemiol. Rec. 67:336–337.

World Health Organization (WHO). 1999. A Practical Guide with Polio Eradication as a Case Study. Available from:.

Yang C, Chen H, Jorba J , Sun HC, Yang SJ, Lee HC, et al. 2005. Intratypic recombination among lineages of type 1 vaccine derived poliovirus emerging during chronic infection of an immunodeficient patient. J Virol 79: 12623-12634.

INSTRUCTION TO AUTHORS

"RADS" publishes articles within the whole field of physical science. Generally these articles will be in or related to need of the country. Manuscripts or data will be considered that have not been previously published or submitted elsewhere for publication. However, re-analysis of previously published data can be accepted.

1. All articles submitted for publication will be reviewed by referees. Submit three copies of the article / manuscripts along with a floppy diskette (IBM Computer) containing the manuscript in duplicate. While submitting diskette it should only the latest version of the manuscript.

Name the file clearly, label the diskette with the format of the file and the file name. Provide information of the hardware and software used.

2. Manuscript should be type double space throughout, and print on one side of the A-4 size paper with clear margins on the both sides. Tables as well as illustrations should be typed and drawn on separate paper. Only black & white photo graphs can be submitted.

3. RADS will accept
   a) Guests Editorials on important subjects of current interest.
   b) Original papers / articles.
   c) Review articles.
   d) Short Communications and
   e) Letters to the Editor.

4. The manuscript except short communication & Guest Editorials should contain the following.

a. **Title page:**

The complete title of the manuscript, the name of all the authors with their highest qualifications, the department or institution to which they are attached, address for correspondence with telephone numbers, email & fax number.

b. **Abstract:**

All original articles must accompany a structured abstract up to 150 words. It should state objective of the study, design, setting, main outcome measures, results & conclusions.

c. **Key Words:**

Below the abstract give few words not more than five.

d. **Text:**

It should include Introduction, Materials & Methods, and Results & Discussion.

e. **Tables:**

Should be separate from the text, numbered and with explanatory caption.

f. **Acknowledgement:**

If desired, it should be included after the discussion and before references.

g. **References:**

References within the text should be cited by the author's name followed by the year e.g. (Riaz, 1990). When two authors, e.g. (Riaz and Ali, 2003). In case of three or more than three authors the reference should be cited as e.g. (Kamran, et al. 1995). All references should be arranged alphabetically. The reference should provide the following information: Author's name with initials, year of the publication, full title of the cited article, name of the journal in which the article appeared (in abbreviated form), journal's volume,number and finally first and the last page number such as Carling DE & Brown MF. 1980. Relative effect of vesicular arbuscular mycorrhizal fungi on growth and yields of soybeans. Soil Sci. Soc. Amer. J., 44: 528-532.

5. The manuscript should be accompanied by a covering letter signed by corresponding authors.