Enteroviruses: Polio

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1 Introduction

Six decades ago, every child faced the threats of lifelong paralysis or death from poliomyelitis. Poliomyelitis, an infectious disease dating back to antiquity (Fig. 13.1), suddenly appeared in epidemic form in the late nineteenth century in northern Europe and the United States and emerged as one of the great epidemic diseases of the twentieth century [1–3]. The threat of poliomyelitis quickly receded in developed countries following the introduction of the inactivated poliovirus vaccine (IPV) in 1955 [4, 5] and the oral poliovirus vaccine (OPV) in 1961 [6, 7] and had all but disappeared in high-income countries by the early 1970s [8, 9]. In sharp contrast, poliomyelitis remained largely uncontrolled in the developing countries of Latin America, Asia, and Africa and continued to threaten the majority of the world's children with crippling disease [8, 9].

Today, poliomyelitis is on the verge of eradication, and its etiologic agents, the three poliovirus serotypes, are on the brink of extinction from the natural environment (Figs. 13.2 and 13.3) [12]. Circulation of indigenous wild type 2 poliovirus ceased in 1999 [13], and wild type 3 poliovirus may be nearing eradication [12]. Wild type 1 poliovirus circulation is localized to a small and decreasing number of districts in parts of three countries (for updates see http://www.polioeradication.org/) [12].

This brightening picture is the direct result of the initiatives launched in 1985 by the Pan American Health Organization (PAHO; the Regional WHO Office for the Americas) to eradicate poliomyelitis in the Americas by 1990 [14], and subsequently by the World Health Organization (WHO) to eradicate poliomyelitis worldwide by the year 2000 [15]. The Global Polio Eradication Initiative (GPEI), established by a landmark 1988 resolution of the World Health Assembly (the governing body of the WHO) [15], has grown to become the largest public health program in history [16], engaging key segments of both the public and private sectors [17]. The launch of the GPEI was made in light of dramatic progress by PAHO toward achieving its regional eradication goal, attained in 1991 [18]. Like the PAHO initiative, the GPEI achieved early rapid progress, reducing poliomyelitis incidence worldwide by >99 %, from an estimated 350,000 cases in 125 countries in 1988 to a low of 493 cases reported in 10 countries in 2001, raising the long-held hope that a polio-free world would soon be realized [19]. Optimism was fueled by the eradication of wild poliovirus type 2 and reinforced by the cessation of wild poliovirus transmission in many highly challenging settings. However, progress stalled between the years 2000 and 2010 as the global incidence poliomyelitis stabilized at ~500–2,000 cases per year (Fig. 13.2a) [10, 19, 20]. With intensified efforts, the GPEI steadily reduced the number of endemic reservoirs, such that by the end of 2012 the global poliomyelitis case count again fell to a new all-time low of 223, and only three countries (Nigeria, Pakistan, and Afghanistan) had never stopped wild poliovirus transmission (Figs. 13.2 and 13.3) [12, 21]. Despite setbacks, the GPEI achieved many landmark successes: coordinating the vaccination of 2.5 billion children, many of them among the most vulnerable living in the most under-resourced communities in the world, and saving more than ten million people (mostly children <2 years of age) from lifelong paralysis and sparing the lives of more than 250,000 others [22]. The WHO GPEI is now developing a detailed endgame strategic plan to secure forever the many gains achieved by polio eradication (http://www.polioeradication.org/portals/0/document/resources/strategywork/endgamestratplan_20130414_eng.pdf) [22].

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The findings and conclusions in this chapter are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

R.A. Kaslow et al. (eds.), *Viral Infections of Humans*,
DOI 10.1007/978-1-4899-7448-8_13, © Springer Science+Business Media New York 2014

Fig. 13.1 Thirty-three centuries of poliomyelitis. (**a**) Stele from the Eighteenth Dynasty of Egypt (c.1550 to c.1292 BCE) portraying a young man with an atrophied right leg and flaccid foot drop characteristic of the long-term sequelae of paralytic poliomyelitis. The stele is in the Ny Carlsberg Glyptotek, Copenhagen, Denmark; the photograph was downloaded from Wikimedia Commons (http://commons.wikimedia.org/wiki/File:Polio_Egyptian_Stele.jpg). (**b**) Poliomyelitis in Delhi, India, 2002. The last case of poliomyelitis associated with wild poliovirus in India had onset in January 2011. The photograph was downloaded from the WHO Media Centre (http://www.who.int/mediacentre/multimedia/2002/ind_polio211460.jpg)

Polioviruses are members of the *Enterovirus* genus of the family *Picornaviridae* (*pico*, L., small; *rna*, RNA genome) (Chap. 11) [23]. The *Enterovirus* genus, comprising >100 serotypes, is divided into 12 species (enterovirus species A to J and rhinovirus species A to C); poliovirus, along with >20 other serotypes are members of human enterovirus species-C (for updates see: http://www.ictvonline.org/) [24, 25]. Enteroviruses inhabit the intestinal tracts and/or the nasopharyngeal tissues of humans and other mammals. Polioviruses, for which humans are the only natural reservoir host, occasionally invade the central nervous system (CNS) and cause destruction of motor neurons in the spinal cord, resulting in acute flaccid paralysis (AFP). However, poliovirus invasion of the CNS occurs in less than 1 % of infections and represents a dead end for transmission, which occurs by the fecal–oral or respiratory routes [23, 26]. The abrupt appearance of large poliomyelitis outbreaks generated intense interest in the disease and prompted intensive studies of poliovirus epidemiology, pathology, immunology, and virology, leading to the development and worldwide deployment of effective poliovirus vaccines and many groundbreaking contributions to public health, medical science, and basic research [3, 19, 23, 27].

2 Historical Background

The rich history of research on poliomyelitis and polioviruses has been chronicled in numerous excellent books, chapters, and reviews and in an extensive scientific literature

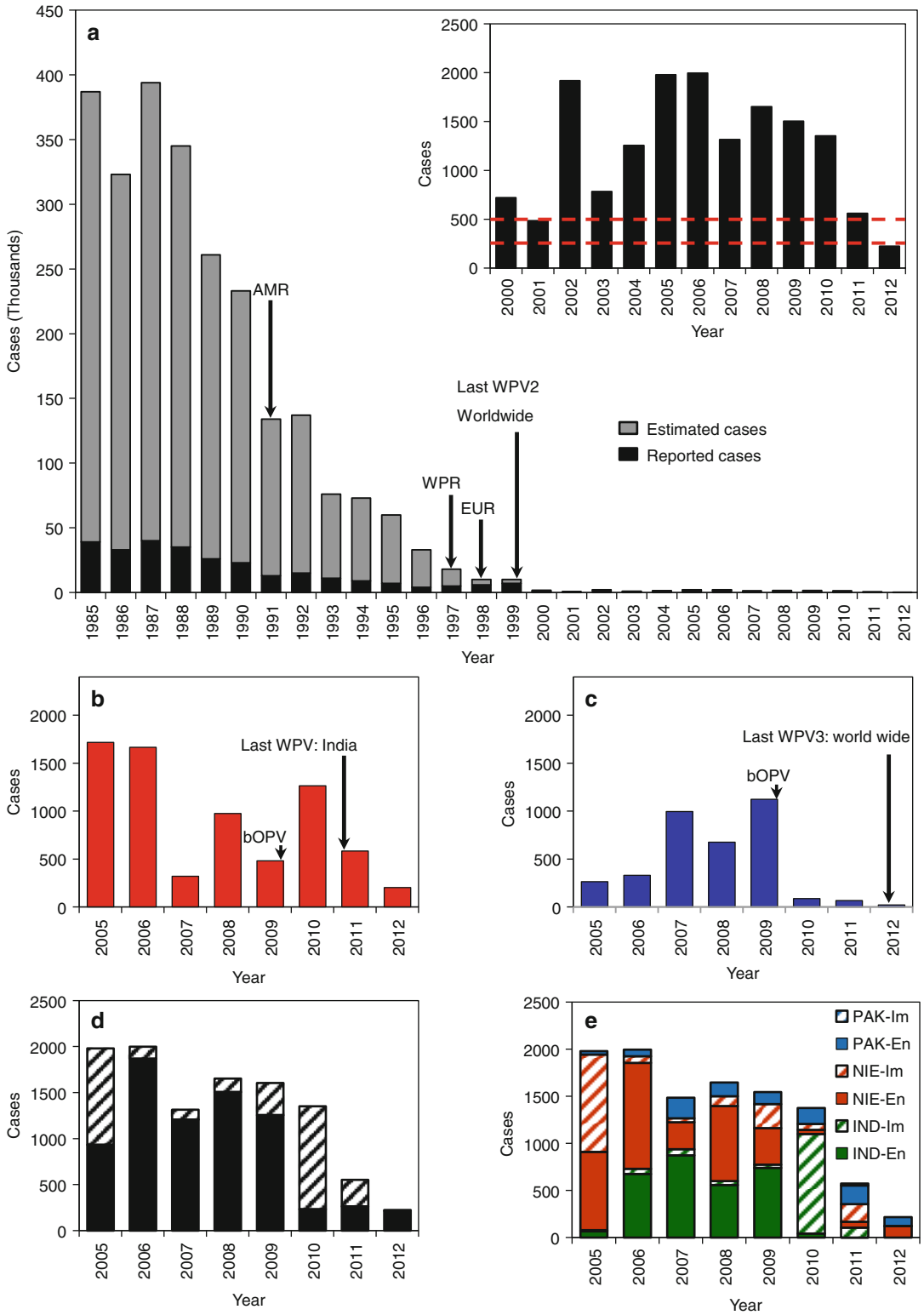

Fig. 13.2 (**a**) Incidence of paralytic poliomyelitis cases associated with wild poliovirus (*WPV*) infections worldwide, 1985–2012 (source http://www.polioeradication.org/). Estimated cases are shown as gray bars; reported, clinically confirmed, and virologically confirmed cases are shown as *black bars*. Starting in 2001, all WPV case counts were based on virologic confirmation by the GPLN. *Arrows* below three-letter codes for WHO regions (*AMR* Americas, *EUR* Europe, *WPR* Western Pacific) indicate year of last detection of indigenous WPV. *Red dashed lines* in inset indicate estimated number (250–500) of cases of VAPP per year worldwide. (**b**) Wild poliovirus type 1 (*WPV1*) poliomyelitis cases, 2007–2012. Introduction of bivalent OPV (bOPV; types 1 + 3) in late 2009 is indicated by the arrow. (**c**) Wild poliovirus type 3 (*WPV3*) poliomyelitis cases, 2007–2012. (**d**) Poliomyelitis cases from endemic (*solid bars*) and imported (*hatched bars*) WPV, 2007–2012. (**e**) Poliomyelitis cases associated with endemic (En) and imported (Im) WPV, 2007–2012 (*PAK* Pakistan [and Afghanistan], *NIE* Nigeria, *IND* India). (**e**) Poliomyelitis cases associated with endemic and imported WPV3, 2007–2011 (Modified from reference Kew [10])

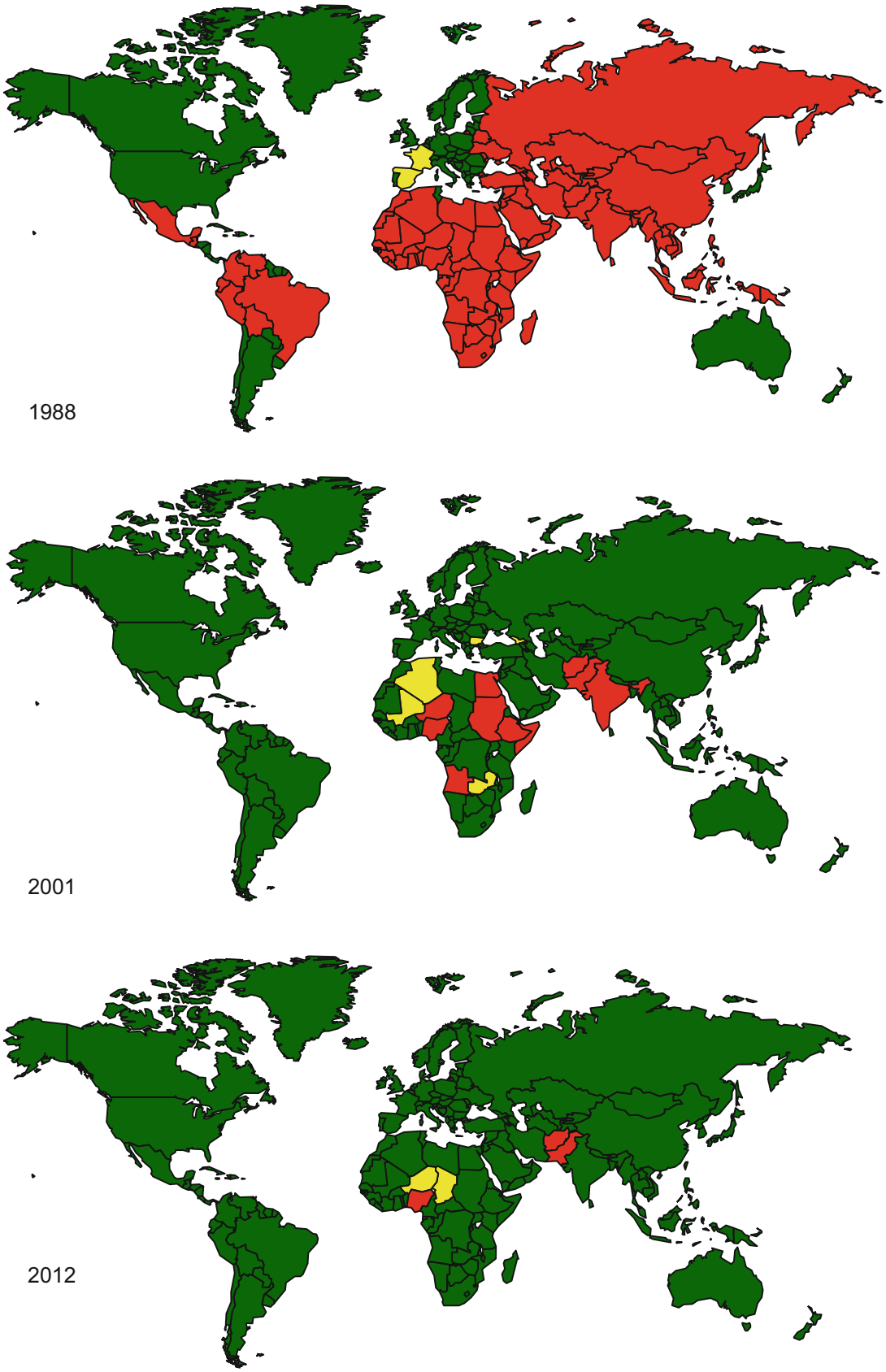

Fig. 13.3 Progress toward global polio eradication, 1988 to 2012. *Red* countries with indigenous wild polioviruses (WPVs), *yellow* countries with one or more case associated with imported WPV, *green* polio-free countries (Modified from reference Kew and Pallansch [11])

dating back over a century [1–3, 7, 23, 28–35]. The disease probably emerged at the dawn of civilization, when population centers grew in size and density sufficient to support continuous endemic circulation. The earliest evidence for endemic poliomyelitis comes from Egypt, recorded on a small funerary stele from the Eighteenth Dynasty (c.1550 to c.1292 BCE), depicting a crippled young man, standing with the aid of a staff, with an atrophied right leg and flaccid foot drop characteristic of the long-term sequelae of paralytic poliomyelitis (Fig. 13.1) [1]. Three millennia later, in 1789, Underwood in England wrote the first clear clinical description of poliomyelitis as a "debility of the lower extremities" [36]. In 1840, the German orthopedist, von Heine, described "Spinale Kinderlähmung" (infantile spinal paralysis) and postulated that the disease could be contagious [37]. In Sweden, Medin conducted the first investigations of the epidemiology of poliomyelitis during the outbreak in Stockholm in 1887 [38]. Sporadic small outbreaks of paralytic disease had been described in the United States since 1841 [33, 39], but the first large outbreak (132 cases) occurred in Rutland, Vermont, in 1894 [40]. In northern Europe, major epidemics (>500 reported cases) erupted in Norway and Sweden (1905), Austria (1908–1909), Germany (1909), and England and Wales (1911) [34, 39]. During this period, Wickman in Sweden firmly established that poliomyelitis was transmitted by person-to-person contact, that the disease spread along the major lines of transportation, and that it gave differing clinical presentations. Wickman hypothesized that all infections, both severe and mild, contributed to spread, and that the incubation time was 3–4 days [41]. In Vienna in 1908, Landsteiner and Popper demonstrated that monkeys became paralyzed after intraperitoneal injection of a filtered homogenate from the spinal cord of a 9-year-old boy (who died within 3 days of onset of paralysis), and that they developed neural lesions similar to those observed in paralyzed humans [42]. Landsteiner and Popper were unable to passage the virus, but several groups, including Flexner and Lewis in 1909, achieved serial passage of poliovirus by nasal inoculation of monkeys [43]. With continued passage in monkeys, they selected a strictly neurotropic type 2 variant, MV, leading them to postulate that poliovirus grew only in neural tissues. However, in 1912, Kling, Wernstedt, and Pettersson isolated poliovirus not only from neural tissues but also from the oropharynx and small intestine, as well as from intestinal contents and throat swabs [44], but the views of Flexner and colleagues prevailed, and the critical findings from human pathology were overlooked for nearly three decades [1, 2].

After 1906, large epidemics appeared in the northeastern and north central states of the United States, culminating in the epidemic of 1916 centered around New York City, far larger at 23,000 reported cases than any previous outbreak [34, 45]. Nationwide poliomyelitis surveillance had begun in the United States in 1910, when all states were requested to forward monthly poliomyelitis case counts to the Surgeon General's Office [46]. The Drinker respirator, commonly known as the "iron lung," was introduced in 1929 as a device to save the lives of patients with respiratory paralysis (many of whom would subsequently recover unassisted respiratory function) [47]. In 1931, reinfection experiments in monkeys by Burnet and Macnamara provided the first evidence of more than one poliovirus serotype [48]. Seven years later, President Franklin D. Roosevelt, who had been paralyzed in both legs by poliomyelitis in 1921, cofounded with Basil O'Connor the National Foundation for Infantile Paralysis and the March of Dimes campaigns, providing a critical source of support for poliovirus vaccine research [1, 3]. Adaptation of the type 2 Lansing strain to cotton rats and mice by Armstrong in 1939 opened the way for much broader and more quantitative virologic and serologic studies on a scale previously unattainable with titrations in monkeys [49]. By the early 1940s, Trask and Paul [50, 51] and Sabin and Ward [52] recognized that poliovirus replicated in the tissues of the intestinal tract as well as the CNS, confirming the earlier observations of Kling. In 1949, Enders, Weller, and Robbins cultivated the type 2 Lansing strain of poliovirus in nonneural cells from human embryonic tissue—including skin, muscle, and intestine—yielding large quantities of virus, thereby accelerating the pace of poliovirus research and opening the way for expanded vaccine development and large-scale vaccine production [53]. That same year, Bodian and colleagues established that there were only three poliovirus serotypes [54, 55]. In 1953, following the peak year for poliomyelitis cases reported in the United States (57,628) [56], Hammon et al. demonstrated that administration of immune gamma globulin was protective against paralytic disease [57], and the following year Horstmann et al. showed that viremia preceded paralysis in humans [58]. With these strong experimental underpinnings, the stage was set in 1954 for Francis to conduct a field trial enrolling 1,800,000 children in the United States [59], demonstrating the safety and efficacy of the IPV developed by Salk and colleagues [4]. The new IPV was promptly licensed and distributed following announcement of the field trial findings in April 1955 [1, 3, 35]. In 1959, large field trials of the OPV of Albert Sabin were conducted in the Soviet Union, Poland, and Czechoslovakia [60], leading to the licensure and distribution of monovalent OPV types 1 and 2 in 1961 and type 3 in 1962 [6].

3 Methodology Involved in Epidemiologic Analysis

3.1 Sources of Data

Poliomyelitis has been a notifiable disease in the United States since 1910 [46], when case reports from state and territorial boards of health and health departments were summarized

monthly (1910–1926) and then weekly (1927–1951) in *Public Health Reports* [61]. Starting in 1952 [62], reports were regularly published in the CDC (Communicable Disease Center, later Centers for Disease Control and Prevention) *Morbidity and Mortality Weekly Report* (*MMWR*) [63]. Special annual poliomyelitis surveillance summaries were also published by CDC through 1974 [64]. In addition to the United States, many European countries and Canada established systems early in the last century for reporting cases of poliomyelitis, allowing epidemiologists to monitor rising disease incidence up to mid-century [65, 66] and the sharp decline after the introduction of poliovirus vaccines [8, 9]. In contrast, data on poliomyelitis incidence in developing countries was very incomplete, with only a small fraction of cases reported and with many populous countries not reporting any cases at all [8, 9]. In 1969, the World Health Assembly adopted a resolution that placed poliomyelitis "under international surveillance" [67]. However, systematic and sensitive surveillance for poliomyelitis in developing countries only followed the launch of polio eradication efforts in the Americas [14, 18] in 1985 and the GPEI in 1988 [15] and the establishment of field surveillance for cases of acute flaccid paralysis (AFP) [68] closely integrated with virologic testing of clinical specimens [69]. The quality of the integrated surveillance data improved gradually, usually in step with improvements in OPV coverage, and the findings were published regularly in reports by PAHO [70], WHO [71], and CDC [72]. Current weekly and monthly reports are posted on the WHO website (http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/.aspx), and lists of wild polioviruses by country and year are posted on http://www.polioeradication.org/Dataandmonitoring/Poliothisweek/Wildpolioviruslist.aspx.

Over the past century, the large majority of case counts were based on clinical diagnoses, and it was soon recognized that the most accurate counts were obtained in outbreak settings [73]. Cases not associated with large outbreaks were more likely to be underreported. In the PAHO and WHO eradication initiatives, AFP cases were systematically reported and investigated, and as national and regional laboratory networks developed, all specimens from AFP cases were tested for the presence of poliovirus [69, 74, 75]. Countries and regions shifted from a clinical case definition to a virologic case definition once field surveillance for cases of AFP was tightly integrated with laboratory investigations for poliovirus. By 2001 global poliomyelitis case counts were based on virologic findings.

Data on case/fatality (CFR) ratios are not routinely available as ratios vary with the age distribution of population susceptibility and by setting [9, 23]. CFRs increase with age and are generally on the order of 2–5 % in children <5 years of age and 10–30 % in adults [23, 76, 77]. The epidemics early in the twentieth century were associated with high CFRs (27 % in the 1916 New York epidemic) [45], and more recent outbreaks from importation of wild poliovirus into previously polio-free countries have also been characterized by high CFRs in older age groups: Albania, 1996 (18 % for ages 19–24 years) [78]; Cape Verde Islands, 2000 (57 % for ages >15 years) [79]; Namibia, 2006 (31 %; most paralytic cases were among adults) [80]; the Republic of Congo, 2010–2011 (43 %; most paralytic cases were among adults) [81]; and Xinjiang, China, 2011 (10 %) [82]. During the outbreak year of 2006 in India, highly sensitive surveillance documented a CFR of 7.1 % among children <2 years [77].

3.2 Serologic Surveys

Serologic studies of infectious diseases were initially applied to the diagnosis of individual cases [83]. The first applications of serology to epidemiology were the studies by Aycock and Kramer in 1930, who used the newly developed neutralization test to show that antibodies to poliovirus appeared at younger ages in urban compared with rural populations [84]. Despite the methodological limitations (neutralization tests were performed in monkeys and predated recognition of more than one poliovirus serotype), these early studies heralded a powerful new tool to address fundamental questions about the epidemiology of poliomyelitis. In the pre-vaccine era, serologic surveys played an indispensable role in defining the prevalence of poliovirus infection, the intensity of transmission of each poliovirus serotype, the age profiles of exposure in different settings, the years and the associated serotypes of past outbreaks in isolated populations, the duration of type-specific immunity, the identification of susceptible populations, and key aspects of the pathogenesis of paralytic disease—including estimates of age-specific case/infection ratios [23, 28, 85, 86]. In the post-vaccine era, seroepidemiology has been used to detect immunity gaps in underserved populations [87, 88], to estimate the extent of wild poliovirus circulation in populations [89], to determine the efficacy of different OPV formulations in inducing neutralizing antibodies [90], and to provide evidence for eradication of indigenous wild polioviruses [91]. In the GPEI (and in the earlier PAHO initiative), serology was found not to be useful in the diagnosis of individual cases, because the response to detection of an AFP case was prompt administration of trivalent OPV (tOPV) to the patient and to the community ("mop-ups"; Sect. 10.4), such that many initially seronegative children had seroconverted to all three poliovirus serotypes by the time of the second blood sample [18]. However, seroprevalence studies continue to be important in measuring the immunogenicities of different OPV formulations [92], in detecting otherwise inapparent spread of OPV-derived viruses in unimmunized populations [93, 94], and in providing objective data on population immunity [95, 96],

including in polio-free countries where OPV coverage rates have fallen and the rising risks of outbreaks might not otherwise be recognized [22]. In recent outbreaks, primarily associated with poliovirus type 1 (Sect. 10.6), determination of the prevalence of neutralizing antibodies to poliovirus type 2 is a surrogate for vaccine-induced population immunity when the initial immunization response is deployment of type 1 monovalent OPV (mOPV1).

3.3 Lameness Surveys

Severe underreporting of poliomyelitis cases in many developing countries led to the misperception that the disease was not a source of serious morbidity in the tropics [8, 66]. Lameness surveys conducted in the 1970s and 1980s in Africa, Asia, and the Middle East confirmed the high prevalence of paralytic disease in developing countries and prompted many countries to begin polio vaccination programs [97, 98]. For example, in India in 1981–1982, estimates of the incidence of poliomyelitis from lameness surveys were as high as 200,000 cases per year, more than tenfold higher than officially reported case counts, with 83 % of cases occurring before 3 years of age [99]. Methods to improve the comparability of lameness surveys in different settings, including the use of standardized case definitions, has been reviewed [97].

3.4 Acute Flaccid Paralysis (AFP) Surveillance

AFP is the most serious clinical manifestation of wild poliovirus infection (Sect. 8.1) [100]. Poliomyelitis outbreaks are readily recognized, but low-level circulation in interepidemic periods may be missed in the absence of a sensitive surveillance system. This is especially true for endemic circulation of poliovirus types 2 and 3, which have much lower paralytic case/infection ratios than type 1 (estimated case/infection ratios [assuming an overall case/infection ratio of 1/150]: type 1, ~1/190; type 2, ~1/1,900; type 3, ~1/1,150) [34, 101]. Recent importation of wild poliovirus or emergence of circulating vaccine-derived polioviruses (cVDPVs; Sect. 10.8) may also be missed by the AFP surveillance system unless high sensitivity is maintained. Starting in 1985, PAHO built an AFP surveillance system to support the regional Polio Eradication Initiative [18]. Performance indicators for reporting of AFP cases were established assuming a background rate of nonpolio AFP of at least 1 case per 100,000 population <15 years. In addition, surveillance sites were required to report weekly, including "zero reporting" when no AFP cases were identified during the previous week [75]. AFP surveillance was closely integrated with virologic surveillance whereby stool samples from at least 80 % of patients with AFP were tested for the presence of poliovirus (Sect. 3.6) [69]. The successful PAHO strategy was adopted by the GPEI and implemented in all polio-endemic countries [68, 74, 102]. The benchmark AFP rate was raised to at least 2 cases per 100,000 population <15 years in endemic areas, and the global rate has been >4 since 2007 (http://apps.who.int/immunization_monitoring/en/diseases/poliomyelitis/afpextract.cfm). In the last stages of polio eradication in India, AFP surveillance sensitivity reached the extraordinarily high levels of >25 AFP cases per 100,000 population <15 years in the remaining polio-endemic states of Uttar Pradesh and Bihar (http://www.npspindia.org/bulletin.pdf) [91]. Only a small fraction of the AFP case-patients had wild poliovirus infections, and the integrated AFP and poliovirus surveillance system was approximating a community stool sampling survey of a population of ~300 million. It was critical to integrate AFP surveillance with laboratory-based poliovirus surveillance because AFP has multiple etiologies (including Guillain–Barré syndrome, transverse myelitis, infections by other neurotropic viruses, and traumatic neuritis; Sect. 8.1) [7], and the large majority of wild poliovirus infections are inapparent.

3.5 Environmental Surveillance

Sewage sampling was used as early as the 1940s to monitor the seasonal variation of poliovirus circulation in urban communities [51, 103]. Because most poliovirus infections are inapparent (Sect. 3.4 and 8.1), sewage sampling can greatly increase the overall sensitivity of poliovirus surveillance. For example, during the pre-vaccine era in the United States, poliovirus could be detected in sewage shortly before and after the seasonal appearance of paralytic cases, and the combined clinical data and environmental poliovirus isolation rates permitted estimation of the ratio between inapparent infections and paralytic cases [103]. Sewage sampling is widely implemented in Europe (conducted by 20 countries, including Israel [104–108]) and Japan [109] as a component of enterovirus (and poliovirus) surveillance. During the 1984 poliovirus outbreak in Finland, environmental surveillance demonstrated widespread circulation of the wild type 3 outbreak virus and provided a basis for the estimate of the occurrence of at least 100,000 inapparent infections despite the appearance of only nine paralytic cases [104, 110]. Wild poliovirus type 3 was found to be present in the environment 3 weeks before the appearance of the first paralytic case during the 1992–1993 outbreak in the Netherlands, and circulation of the outbreak virus was found to be localized to communities that refused immunization [106]. Environmental surveillance coupled with sequencing of wild poliovirus isolates was introduced on a limited basis in the PAHO program [111, 112]. Sampling of wastewater in a high-risk community

in Cartagena, Colombia, revealed close sequence relationships between sewage isolates, stool survey isolates, and paralytic case isolates obtained from the community over the same period, but also detected circulation of lineages not found by AFP surveillance [111]. Sewage sampling in Israel and adjoining Palestinian territories detected outbreaks of wild poliovirus infections in Gaza, Ashdod, and the West Bank in 1990 (type 3), 1991 (type 1), 1994–1995 (type 1), and 1996 (type 1) [107]. The outbreaks were described as "silent" because no poliovirus-associated paralytic cases had been detected by the AFP surveillance systems. All of the wild poliovirus sewage isolates were found to be related to viruses circulating in Egypt. The continued detection in Gaza of wild polioviruses of Egyptian origin at times when none were reported by the AFP surveillance system in the source reservoir communities prompted the implementation of environmental surveillance in Egypt in September 2000, which by 2004 sampled 33 sites in 18 governorates [113–115]. This approach, combined with strengthened AFP surveillance, improved the overall sensitivity of the poliovirus surveillance system, and wild polioviruses disappeared from the environment soon after their disappearance in specimens collected from patients with AFP [113, 115]. Implementation of sewage sampling in the open canals in the large slum communities of Mumbai, India [116], coupled with sequencing (section "Nucleotide sequencing of poliovirus isolates"), confirmed the disappearance of the local wild polioviruses and the repeated importation of wild polioviruses from known reservoirs in the northern Indian states of Uttar Pradesh and Bihar [91]. Because no suitable sampling sites were available in the highest-risk rural reservoir communities, additional sites were established in Delhi and Patna, Bihar, which receive migrants from the endemic rural areas [91]. As in Egypt, the findings from the environmental and AFP surveillance systems were in agreement: the last environmental wild poliovirus isolate (a type 1) was found in Mumbai sewage in November 2010, and the last wild poliovirus isolate (a type 1) from an AFP case-patient was in West Bengal in January 2011 (http://www.npspindia.org/bulletin.pdf) [91]. The GPEI and the Global Polio Laboratory Network (Sect. 3.6.1) have extended environmental surveillance to six cities in Pakistan and three cities in Nigeria and are planning further expansions in countries at high risk of reestablishment of poliovirus circulation either by importation or by the emergence of cVDPVs (see below and Sect. 10.8) [22].

VDPVs closely resembling those excreted by individuals with primary immunodeficiencies (Sect. 10.8.2) have been detected in sewage in Israel [117], Estonia [118], Slovakia [119], and Finland [120]. Despite efforts in each country to identify the source of the excreted virus, no poliomyelitis cases or chronically infected individuals have so far been identified.

Environmental surveillance has also helped inform the endgame strategy for the GPEI (Sect. 11.2). A key question is the persistence of vaccine-related viruses in the population and environment following cessation of OPV use. In Cuba, where OPV is delivered only in mass campaigns in the form of two rounds of National Immunization Days (NIDs), vaccine-related viruses were detected in stool surveys for up to 8 weeks and in the environment for up to 15 weeks after the second NID round [121]. New Zealand shifted from OPV to IPV in February 2002, and vaccine-related viruses were regularly detected in sewage samples until May 2002. Sporadic vaccine-related isolates detected subsequently showed very limited sequence divergence from the parental OPV strains, indicating that they were recent imports from OPV-using countries rather than persistence of vaccine-related viruses in the community [122].

An important difference between environmental sampling and AFP surveillance is that environmental sampling is most sensitive in locations with developed sewage systems or open sewage canals in large slums and therefore is usually established in more urbanized settings. Sewage sampling sites in urban centers are selected to include communities of migrant populations from rural areas. Environmental surveillance is necessarily localized, targeted, and intermittent, in contrast to a well-functioning AFP surveillance system that monitors the entire population on a continuous basis. Consequently, poliovirus isolates obtained by the two surveillance approaches may yield different kinds of public health information. For example, a wild poliovirus isolate from an AFP case is directly linked to a specific patient from a specific locale and usually signals many other inapparent infections in the community. In contrast, the high sensitivity of environmental sampling can result in multiple poliovirus isolations from a single infected person but does not yield further information about the specific source of infection. However, some information about the extent of poliovirus circulation can be obtained from the extent of genetic diversity of polioviruses obtained at a sampling site [111, 115].

3.6 Laboratory Methods

3.6.1 The Global Polio Laboratory Network (GPLN)

The GPLN was established by the WHO to support the GPEI [123]. Currently the GPLN consists of 145 laboratories, initially organized in three tiers: (1) National and Subnational Laboratories ($n=122$), (2) Regional Reference Laboratories ($n=16$), and (3) Global Specialized Reference Laboratories ($n=7$) (Fig. 13.4). As the GPLN developed, activities once assigned to Regional Reference Laboratories are frequently performed by many National and Subnational Laboratories, and Regional Reference Laboratories currently perform

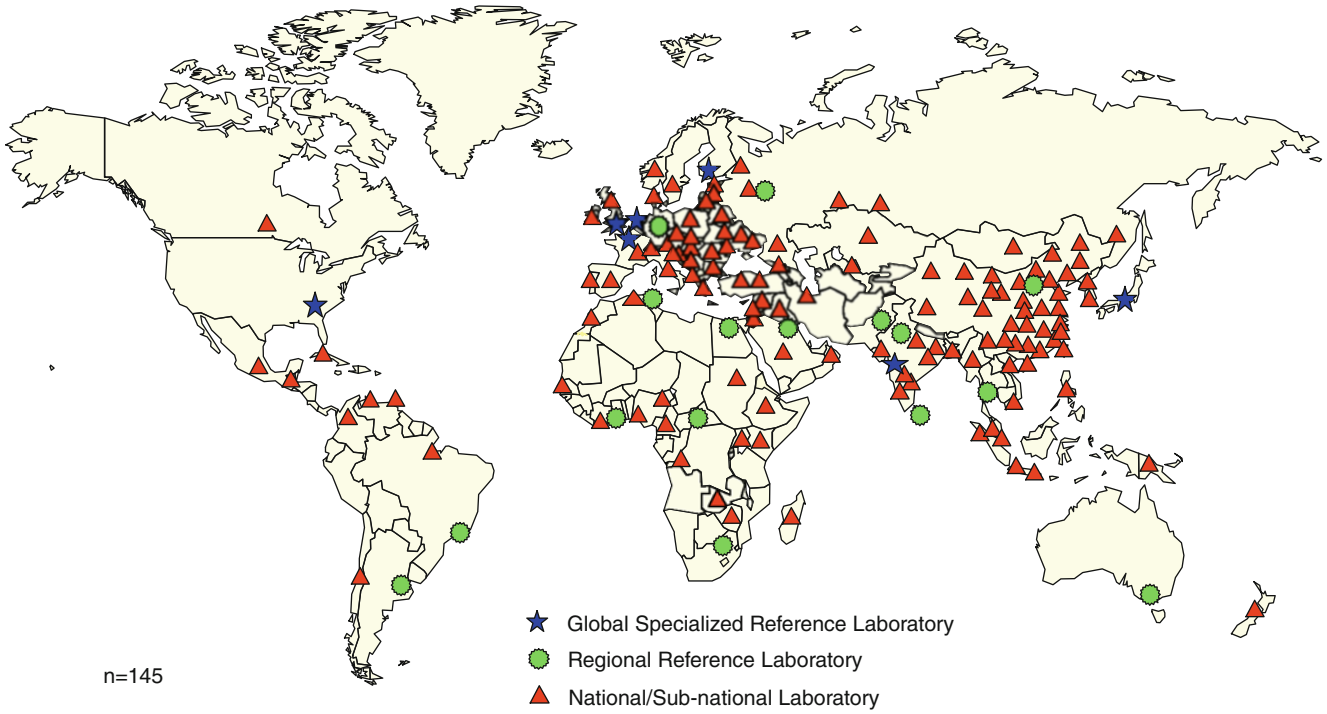

Fig. 13.4 Distribution of laboratories of the Global Polio Laboratory Network (GPLN). *Triangles* National and Subnational Laboratories, *circles* Regional Reference Laboratories, *stars* Global Specialized Reference Laboratories

many functions (such as genomic sequencing) originally assigned to Global Specialized Reference Laboratories. These trends have strengthened the GPLN, permitted continuous technical innovation, and moved many of the diagnostic activities closer to the endemic areas of highest priority. The GPLN was patterned after the PAHO Regional Laboratory Network established in parallel with development of AFP surveillance [69]. Close integration of AFP and poliovirus surveillance was facilitated by the use of standardized case ("EPId") numbers accessible to surveillance officers, virologists, and program managers [100, 124].

Methods for poliovirus isolation, identification, and serology have been described in detail previously [23, 125, 126]. Laboratory manuals were developed by the GPLN to standardize methods for detecting and characterizing polioviruses in clinical specimens and environmental samples [124], and a GPLN Quarterly Update was published by the WHO to keep GPLN virologists and others abreast of new developments and innovations [127]. Because the overriding emphasis of the GPLN is to monitor poliovirus circulation, less attention has been given to the routine typing and characterization of nonpolio enterovirus isolates, and readers are referred to Chap. 11 for details on those methods. The WHO Polio Laboratory Manual [124] is regularly updated as new methods are developed and tested for suitability for use by GPLN laboratories. New methods, designed to increase sensitivity, specificity, and work efficiency, are developed in concert with the rising technical capabilities of the GPLN and in anticipation of (or in response to) increasingly focused surveillance questions posed by the GPEI [22]. All GPLN laboratories participate in a formal accreditation process which includes review of performance in standardized proficiency tests as well as routine diagnostic work as confirmed by GPLN reference laboratories [128]. Well-characterized cells, reference OPV virus stocks, serologic reagents, and molecular reagents are distributed by GPLN reference laboratories to ensure a high degree of standardization, and internal quality control procedures are regularly implemented by all GPLN laboratories to ensure high routine performance [128]. GPLN laboratories participate in annual regional and global meetings to review performance, discuss effective implementation of new methods, develop approaches to improve coordination, and plan research and other future activities (http://www.polioeradication.org/Dataandmonitoring/Surveillance/GlobalPolioLaboratoryNetwork.aspx) [128]. The GPLN is interdependent, applying common approaches to problem solving, parallel testing as needed, training, and other kinds of technical support. The GPLN is guided by expert WHO virologists who serve as global and regional laboratory coordinators. The GPLN, with its close integration with program, has served as the model for newer regional and global networks supporting laboratory-based surveillance for other viral and bacterial vaccine-preventable diseases [129–131]. Many of the methods and reagents used by the GPLN have also been shared with state laboratories in the United States [132].

3.6.2 Virus Isolation and Identification

Categories of Poliovirus Isolates

The primary purpose of infectious disease surveillance is to identify agents that present potential public health risks. Poliovirus isolates of each serotype are grouped into three categories, correlating with the risk of transmission and spread, and based on the extent of divergence of the VP1 nucleotide region compared to the corresponding OPV strain: (1) wild polioviruses (no genetic evidence of derivation from any vaccine strain and demonstrated capability of continuous person-to-person transmission); (2) vaccine-derived polioviruses (VDPVs) (vaccine-related polioviruses that are >1 % divergent [types 1 and 3] or >0.6 % divergent [type 2] from the corresponding OPV strain and potentially capable of causing paralytic disease and establishing person-to-person transmission) (Sect. 10.8); and (3) "OPV-like" polioviruses (vaccine-related polioviruses that are ≤1 % divergent [types 1 and 3] or ≤0.6 % divergent [type 2] from the corresponding OPV strain) that are ubiquitous wherever OPV is used [133]. VDPVs are further categorized as (1) circulating VDPVs (cVDPVs), when there is evidence of person-to-person transmission in the community; (2) immunodeficiency-associated VDPVs (iVDPVs), which are isolated from persons with primary immunodeficiencies who have prolonged VDPV infections; and (3) ambiguous VDPVs (aVDPVs), which are either clinical isolates from persons with no known immunodeficiency or sewage isolates whose ultimate source is unknown [133].

Clinical Specimens

The specimens of choice for AFP and poliovirus surveillance are stool samples collected as soon after onset of paralysis as possible. The GPEI has defined "adequate" stool specimens as "two stool specimens of sufficient quantity (~8 g) for laboratory analysis, collected at least 24 h apart, within 14 days after the onset of paralysis, and arriving in the laboratory in good condition and with proper documentation" (http://www.polioeradication.org/Dataandmonitoring/Surveillance.aspx). Two samples are collected because poliovirus shedding is often intermittent [134]. The GPEI has established clear performance guidelines and training procedures for proper specimen transport to the laboratory via a "reverse cold chain" similar to the forward cold chain used for deployment of OPV [100, 124]. Such importance has been assigned to specimen collection from AFP cases that runners with specimens in insulated backpacks were allowed safe passage through combat lines in war zones where use of motorized transport was very hazardous.

Poliovirus may be isolated at lower frequencies from rectal swabs [135] and throat swabs (if taken within the first few days of infection) and only rarely from cerebrospinal fluid (CSF); however, none of these specimens are recommended by the GPEI and the GPLN [100, 124].

Virus Isolation in Cell Culture

The most critical and basic procedure is virus isolation in cell culture. Polioviruses can be grown in a wide range of human cells (RD, HeLa, HEp-2, WI-38, MRC-5, HEK293) and simian cells (from rhesus macaques and African green monkeys; primary monkey kidney cells, Vero, LLC-MK2, BGM) (http://www.atcc.org/) [23], but two cell lines are routinely used in combination by the GPLN for virus isolation [124]: (1) RD cells (a continuous line from human rhabdomyosarcoma [136]) which are highly sensitive to poliovirus infection and yield virus at high titers [23] and (2) L20B cells (a derivative of the mouse L cell line engineered to express the human poliovirus receptor, CD155) which are highly selective for growth of poliovirus [124, 137]. Viruses that grow in L20B are usually polioviruses (although some Coxsackie A viruses can grow in L20B cells [124, 138]) and are further characterized by molecular identification methods.

Molecular Characterization and Intratypic Differentiation of Isolates

The original methods for identification of polioviruses and other enteroviruses were based on antigenic properties. Virus isolates were typed by testing for growth in the presence of pools of antisera containing different combinations of high-titer neutralizing antibodies [23]. Typing of individual poliovirus (or enterovirus) isolates was then confirmed by use of type-specific antisera. Heterotypic poliovirus mixtures were resolved by growth in the presence of different pairs of type-specific neutralizing antisera.

Intratypic differentiation (ITD; distinguishing wild polioviruses from vaccine-related isolates) was originally based on antigenic or phenotypic properties [139]. Before the development of molecular methods, the most reliable of these were the antigenic methods, and isolates were described as "vaccine-like," "non-vaccine-like," or "intermediate." Most assignments based on antigenic methods were confirmed by the more precise molecular methods. It is remarkable that the antigenic methods worked so well in view of the fact that the Sabin OPV strains undergo frequent antigenic evolution toward "non-vaccine-like" antigenicity during replication in the human intestine [139–141], and that the wild polioviruses themselves are antigenically diverse [139, 142]. Although the Sabin type 1 OPV strain (Sabin 1) has multiple non-consensus antigenic changes in its neutralizing antigenic sites [139, 143, 144], Sabin 2 and Sabin 3 are usually less antigenically divergent from the corresponding wild polioviruses [139]. ITD based on antigenic properties was improved by use of highly specific cross-absorbed monotypic sera which contained antibodies that reacted specifically with "vaccine-like" or "non-vaccine-like" antigens [143]. ITD using cross-absorbed antisera was adapted to an ELISA format [145] and widely used by the GPLN, especially in recent years for the characterization of VDPVs

Table 13.1 Poliovirus identification by real-time RT-PCR

RT-PCR primer–probe set	Poliovirus/enterovirus isolate category[a]								
	NPEV	WPV1[b]	WPV3[b]	S1	S2	S3	WPV1/WPV3	VDPV1	VDPV2
panEV	+	+	+	+	+	+	+	+	+
panPV	–	+	+	+	+	+	+	+	+
Sero1	–	+	–	+	–	–	+	+	–
Sero2	–	–	–	–	+	–	–	–	+
Sero3	–	–	+	–	–	+	+	–	–
Sab1	–	–	–	+	–	–	–	+	–
Sab2	–	–	–	–	+	–	–	–	–
Sab3	–	–	–	–	–	+	–	–	–
VDPV1	–	–	–	–	–	–	–	+	–
VDPV2	–	–	–	–	–	–	–	–	+
VDPV3	–	–	–	–	–	–	–	–	–

Based on data from references [151, 153, 157–159]. Reagents for identification of VDPV3 isolates have also been prepared. All suspected wild poliovirus and VDPV isolates are routinely further characterized by VP1 sequencing. Special sequencing primer sets have been developed to resolve both heterotypic and homotypic mixtures. The combinations shown, along with others (especially different combinations of Sabin vaccine-related isolates), have been observed in clinical isolates

[a]*Abbreviations*: *NPEV* nonpolio enterovirus, *WPV1* wild poliovirus type 1, *WPV3* wild poliovirus type 3, *S1* Sabin type 1 vaccine-related, *S2* Sabin type 2 vaccine-related, *S3* Sabin type 3 vaccine-related, *VDPV1* vaccine-derived poliovirus type 1, *VDPV2* vaccine-derived poliovirus type 2

[b]As previously described for other wild poliovirus genotypes [152], wild genotype-specific real-time RT-PCR primers and probe sets have been prepared for the Nigeria wild type 1, Nigeria wild type 3, Pakistan–Afghanistan wild type 1, and Pakistan–Afghanistan wild type 3 genotypes (D. Kilpatrick, manuscript in preparation)

[133]. Other ITD methods based on antigenic properties used panels of neutralizing monoclonal antibodies [140, 142, 145, 146]. However, none of the antigenic ITD methods could overcome the basic biological limitations arising from the antigenic evolution of the OPV strains [140, 141, 145], and antigenic methods have been replaced by methods based on the nucleotide sequence properties of poliovirus isolates.

The earliest molecular method for routine ITD was oligonucleotide fingerprinting [147, 148]. This approach had the high reliability required for poliovirus surveillance in support of eradication, but it was also laborious, expensive, and difficult to scale up and required the use of radioisotopes. Therefore, oligonucleotide fingerprinting was not appropriate for developing country laboratories. Consequently, oligonucleotide fingerprinting was replaced by nucleic acid probe hybridization [149, 150], and the transfer of this ITD method to the PAHO Polio Laboratory Network commenced in the late 1980s. The reverse transcriptase-polymerase chain reaction (RT-PCR) offered specificities and sensitivities unattainable with probe hybridization, and it became the method of choice within the GPLN [151, 152], although full deployment awaited adaptation to a real-time format, which greatly reduced the risks of contamination by PCR products [153]. RT-PCR coupled to restriction fragment length polymorphism analysis was also widely used, because this routine test provided insights into the origins of wild poliovirus isolates [154]. An elegant approach based on microarrays was also developed [155, 156], but it was less readily transferrable to developing country laboratories at the front lines of global poliovirus surveillance.

Currently the GPLN uses real-time RT-PCR for ITD [124]. A series of primer pairs and specific fluorescent probes have been developed that identify isolates hierarchically: (1) as enteroviruses (panEV), (2) as polioviruses (panPV), (3) by poliovirus serotype (Sero1, Sero2, Sero3), and (4) whether vaccine-related (Sab1, Sab2, Sab3) (Table 13.1) [153, 157, 158]. The sets of real-time RT-PCR reagents are deployed as kits for routine use by the GPLN [160] and can be supplemented with additional real-time RT-PCR reagents that further identify wild polioviruses by genotype (Sects. 3.6.3 and 10.7) and can facilitate screening for genetically divergent VDPVs (VDPV1, VDPV2, VDPV3) [133, 161]. The rapid evolution and high genetic diversity within and across poliovirus serotypes presented special challenges to development of the panPV and serotype-specific primer and probe sets (Table 13.1), as it was necessary to use degenerate and inosine-containing oligonucleotides to base pair with the appropriate specificities at positions of codon degeneracy [157, 158]. Although the nondegenerate Sabin vaccine strain-specific RT-PCR reagents can be used in a multiplex format, the complexity of the degenerate reagents limits the number of reactions that can be combined in multiplex.

Nucleotide Sequencing of Poliovirus Isolates

ITD screens for wild polioviruses and VDPVs and screens out OPV-like polioviruses that are unlikely to be of current epidemiologic importance. Since 2001, all wild poliovirus and VDPV isolates are sequenced by GPLN laboratories following standardized procedures and using standardized sequencing primer sets. The ~900-nucleotide interval

(representing ~12 % of the total genome) encoding the major capsid protein, VP1, is routinely sequenced. VP1 sequences are used for routine comparisons because they encode several serotype-specific antigenic sites [162] and evolve primarily by successive fixation of nucleotide substitutions rather than by recombination [163, 164]. Wider genomic intervals, up to the complete genome, may be sequenced to obtain higher epidemiologic resolution or to address specific virologic questions [165–168]. Serotype- and genotype-specific sequencing primers have been developed to specifically amplify components of heterotypic and homotypic poliovirus mixtures, bypassing selective cultivation in the presence of neutralizing antibody or incubation at supraoptimal temperatures [169]. Sequence relationships among poliovirus isolates are summarized in phylogenetic trees and genotypic maps that are distributed monthly by GPLN laboratories to Ministries of Health, WHO country and regional offices, WHO-Geneva, and other GPLN laboratories.

3.6.3 Molecular Epidemiology of Polioviruses

The application of genomic sequencing of poliovirus isolates has added a new dimension and resolving power to the understanding of the epidemiology of poliomyelitis [170]. Because poliovirus genomes evolve rapidly (typically just over 1 % nucleotide substitutions per site per year at all sites, equivalent to one to two nucleotide substitutions per week) [164, 165, 168, 171–174], links between poliomyelitis cases can be determined with precision, and the sources and timing of importations from the remaining poliovirus reservoirs can be established [11, 163, 164, 168, 175–178]. Sequence analyses offer an additional tool to monitor the progress of the GPEI and has shown that poliovirus genotypes (viruses within a genotype differ by <15 % in their nucleotide sequences) and genetic clusters within genotypes (viruses within a cluster differ by <5 % in their nucleotide sequences) disappear sequentially through intensive immunization efforts (Sect. 10.7) [11]. Experience in the Americas has found that in settings of sensitive surveillance, a genotype that is not detected for more than a year has probably become extinct [11, 170]. Molecular epidemiology has established the existence of numerous poliovirus genotypes endemic to different regions of the world (Sect. 10.5.3) [11, 170], demonstrated that poliovirus type 2 is usually the first serotype to be eliminated [13], that poliovirus type 3 appears to circulate more locally than type 1 [10], and that poliovirus type 1 appears to be most commonly associated with importations from neighboring countries and with intercontinental or global spread of the virus (Sect. 10.6) [10, 11, 163, 175, 176, 179–181]. In some settings, different genotypes of poliovirus type 1 have been found to have co-circulated in a geographically limited area [163, 179, 182, 183].

Molecular epidemiologic methods are routinely used to help identify reservoir communities with low population immunity and where demographic and environmental conditions favor poliovirus circulation. During the peak months of poliovirus circulation, virus spreads from the reservoir communities to adjacent non-reservoir indicator communities (where the density of nonimmune susceptible children can support some poliovirus circulation during the peak transmission season). This has led to a refinement in the concept of virus importation, which in previous usage referred to virus transmission across national boundaries. Although many importations over long distances have been documented [10, 180], reservoir communities and their associated indicator communities frequently overlap international borders [10, 184], underscoring the importance of regional synchronization of NIDs and Subnational Immunization Days (SNIDs). Equally important are the patterns of importation from reservoir communities to indicator communities within a country [168, 184, 185]. High vaccine coverage in the reservoir communities, especially in mass campaigns conducted during the low transmission season, prevents the subsequent spread to indicator communities.

Sequence analysis led to the recognition of highly divergent iVDPVs [165, 186] and cVDPVs [166, 187, 188] (Sect. 10.8), and it has been used to resolve at high-resolution chains of cVDPV transmission [132, 188–190] and separate iVDPV lineages in individual immunodeficient patients with prolonged infections [165, 173, 186, 191, 192].

Molecular epidemiologic methods have also opened a new avenue for detecting gaps in polio surveillance. In areas with good surveillance, poliovirus isolates representing frequent sampling of a single chain of transmission are typically closely related (usually >99.5 % VP1 sequence identity among the closest relatives). These closely related viruses are routinely visualized as sequences connected by short branches on phylogenetic trees [193]. Long-branch connections between isolate sequences indicate missing information. If the virus is imported, the missing information may be recovered from the sequence relationships to viruses from the source reservoir [11]. However, in many other circumstances, no closely related viruses can be found, and the recent virologic history of the isolate lineage is indeterminate. For example, gaps in AFP surveillance in southern Egypt were inferred from the sequence data, because indigenous type 3 isolates in 1999 appeared as “orphan lineages” at the tips of long branches on phylogenetic trees, and the closest relatives were isolated nearly 3 years earlier [194], observations that highlighted the importance of environmental surveillance to improve sensitivity. Orphan lineages have been repeatedly found in areas with insensitive surveillance. The GPLN regularly monitors for the appearance of poliovirus orphan lineages as a means to assess surveillance sensitivity.

A serious challenge to the integrity of poliovirus surveillance data is the occurrence of poliovirus contamination of

cultures. High workloads in many GPLN laboratories potentially increase the risk of contamination. Fortunately, sequence analysis can distinguish contaminants from true clinical isolates. Contaminants are easily recognized when they are standard wild reference strains, such as Mahoney, MEF-1, or Saukett (OPV-like contaminants are usually of little current programmatic importance), but are more difficult to recognize when they are the wild polioviruses indigenous to a country or community. However, when wild polioviruses isolated at different times and locations have identical VP1 sequences, contamination is suspected, because such sequence identities are inconsistent with the rapid rate of evolution of the poliovirus genomes. Contamination can be definitively confirmed (or ruled out) by complete genomic sequencing. At the advanced stages of polio eradication, laboratory contamination could have severe programmatic consequences if unrecognized, prompting the diversion of resources into unnecessary immunization campaigns mobilizing large populations and costing many millions of dollars.

3.6.4 Tests for Antibody

Because precise and detailed epidemiologic information is routinely obtained from characterization of poliovirus isolates, virologic methods are the mainstay for global poliovirus surveillance [195]. However, antibody tests, especially those measuring population immunity or vaccine efficacy, have assumed greater prominence in recent years [92, 95, 196–200]. The "gold standard" is the neutralization test, as the presence of neutralizing antibody is regarded as the key indicator of protective immunity to poliovirus [7]. Current automation methods permit tests for neutralizing antibody to be performed at scales previously unattainable.

4 Biological Characteristics of Poliovirus

4.1 General Properties

Polioviruses, as members of species-C of the *Enterovirus* genus, share most properties with other members of that species and genus (Chap. 11) [201, 202]. Polioviruses are small (~30 nm in diameter [203]), non-enveloped viruses with capsids of icosahedral symmetry enclosing a single-stranded, positive-sense RNA genome. The genome is ~7,500 nucleotides long, has a small (22-amino acid) basic protein, VPg, covalently linked to the 5′-end, and is polyadenylated at the 3′-end (Fig. 13.5). The single open reading frame (ORF) is flanked by a long (~740 nucleotides) 5′-untranslated region (5′-UTR) and a short (~70 nucleotides) 3′-UTR. Complete genomic sequences have been determined for numerous representatives of each of the three serotypes, including those of the three Sabin OPV strains [204]. Only the sequences encoding the capsid proteins are unique to polioviruses, as the flanking sequences are frequently exchanged by recombination with the closely related species-C enteroviruses during circulation (Sect. 4.6.2) [24, 166, 188, 205, 206]. The poliovirion consists of 60 copies each of 4 capsid proteins (VP1–4) that form a highly structured capsid shell [203]. The three major proteins (VP1, VP2, VP3) share a similar basic architecture and were probably derived from a common ancestral protein [203]. The smallest protein, VP4, internalized in the native virion, is formed by the cleavage of the precursor VP0 (VP4 + VP2) during final maturation of the virion. The external surface of the poliovirion is decorated by peptide loops extending from VP1, VP2, and VP3, which form the neutralizing antigenic sites (Fig. 13.5) [162, 207]. Polioviruses attach to and enter cells via the specific poliovirus receptor (PVR) on the cytoplasmic membrane; the PVR was later identified as CD155, a glycoprotein of the immunoglobulin superfamily [208–210]. The key distinguishing properties of poliovirus capsids are their antigenic surfaces and their abilities to specifically bind to CD155, as the sequences and structures of the internal capsid domains are largely conserved among species-C enteroviruses.

4.2 Physical Properties

Poliovirus capsids contain no essential lipids, and infectivity is insensitive to inactivation by detergents and lipid solvents such as ether, chloroform, or alcohol [23]. The viruses are stable at pH 3–5 for 1–3 h and can therefore pass through the stomach without inactivation. Exposure to 0.3 % formaldehyde, pH <1, pH >9, or free residual chlorine at 0.3–0.5 ppm causes rapid inactivation. Infectivity is stable indefinitely at –20 °C or lower, and stable for weeks at 4 °C, but is rapidly inactivated at temperatures above 50 °C [211]. Molar concentrations of $MgCl_2$ significantly increase the thermal stability of poliovirions [212], both at elevated and ambient temperatures, and $MgCl_2$ is added to many OPV preparations to preserve potency [7, 213]. High intensity ultraviolet light or desiccation inactivate infectivity by causing an irreversible conformational transition from D-antigenicity to C-antigenicity (Sect. 4.3) [211, 214]. The three-dimensional crystal structures of representatives of all three serotypes have been determined [203, 215, 216]. Poliovirions have a buoyant density of 1.34 g/ml and a sedimentation coefficient of 160S, properties that can be exploited to obtain highly purified virus preparations [217].

4.3 Antigenic Properties

There are three poliovirus serotypes [54, 55]. Three (or four) neutralizing antigenic sites have been identified by patterns

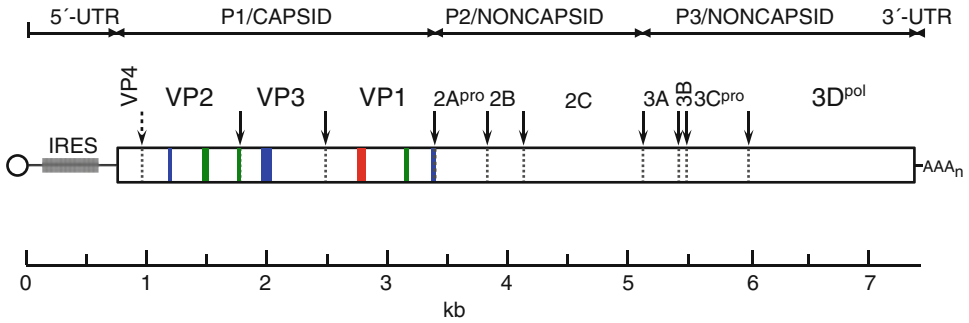

Fig. 13.5 Schematic of the poliovirus genome. The single open reading frame (ORF) is indicated by a rectangle, flanked by the 5′- and 3′-untranslated regions (*UTRs*); the small protein VPg (encoded by the 3B sequence interval) is covalently attached to the 5′-UTR and is represented by a circle at the 5′ end. The internal ribosome entry site (*IRES*; nucleotide positions 130–600) in the 5′-UTR is shown as a shaded rectangle. A single polyprotein is translated from the ORF, which is cotranslationally processed by virus-encoded proteases $2A^{pro}$ (catalyzes cleavage between VP1 and $2A^{pro}$; the cleavage site is indicated by a *dashed arrow*) and $3C^{pro}$ (catalyzes all other cleavages except the VP4/VP2 maturation cleavage; the cleavage sites are indicated by the *solid arrows*). Mature cleavage products are bounded by *dashed lines*. Protein $3D^{pol}$ is an RNA-dependent RNA polymerase (RdRP). *Colored bars* symbolize virion surface loops forming neutralizing antigenic sites 1 (*red*), 2 (*green*), and 3 (*blue*) (Redrawn from reference Kew et al. [94])

of reactivity with neutralizing murine monoclonal antibodies (Fig. 13.5) [162], and the assignments have been confirmed by high-resolution x-ray crystallography [216, 218, 219]. Neutralizing antigenic site 1 is continuous and formed by a loop in VP1; sites 2 and 3 are discontinuous and formed from loops contributed by different capsid proteins. The major type-specific differences in the capsid polypeptides primarily reside on the most surface-accessible peptide loops, which represent less than 4 % of the total capsid protein [204]. Although the neutralizing antigenic sites vary within each serotype [139, 142, 164, 165, 174, 220–223], the range of variability is constrained, possibly because of steric requirements for interaction with CD155 [224, 225], such that all polioviruses within a serotype can be neutralized by type-specific antisera, and poliovirus vaccines (both IPV and OPV) can induce protective immunity to all known antigenic variants. Poliovirus antigenic evolution differs importantly from that of influenza virus in that there is no cumulative antigenic divergence from ancestral viruses during person-to-person transmission, and genetically unrelated viruses may have similar antigenic properties and shared epitopes.

Limited cross-neutralization has been observed for all three poliovirus serotypes [226], and a shared epitope between types 1 and 2 has been identified by mapping escape mutants to cross-reactive neutralizing monoclonal antibody [227]. Recently, chimeric chimpanzee–human monoclonal antibodies have been produced showing patterns of strong cross-neutralization [228]. Epitope mapping with these primate monoclonal antibodies have identified shared determinants not previously recognized by studies using murine monoclonal antibodies [228], suggesting that the poliovirus antigenic surface may be more complex than previously thought.

Within each serotype there are two basic antigenic conformations: D-antigen ("dense"; sometimes also called N or "native" antigen) and C-antigen ("coreless"; corresponding to H or "heated" antigen) [211, 229]. The D-antigen corresponds to that of the intact native virion, and IPV potency is measured in D-antigen units [5]. The C-antigen contains no RNA and is not cross-reactive with the D-antigen [211]. Transitions between the D and C conformations are rapid in empty capsids, but D-antigen is stabilized by RNA packaging [214].

Poliovirus antigenic properties have been reviewed by Minor [162].

4.4 Host Range In Vivo and In Vitro

Humans are the only reservoir host for poliovirus [230]. Chimpanzees, gorillas, and orangutans have been infected while in captivity [231, 232], and chimpanzees can be experimentally infected by the oral route [233]. Poliomyelitis cases appeared in a natural chimpanzee colony following an outbreak in an upstream African village [234]. Old World monkeys are susceptible to experimental poliovirus infection upon intraspinal or intracerebral injection, and macaques (cynomolgus, rhesus, and bonnet) can be infected by the oral route, but high virus titers are required for infection [235–238]. New World monkeys are not susceptible to poliovirus infection by any route of administration because of substitutions in the variable domain of their CD155 orthologs [239, 240]. Paralytic attack rates in humans differ by poliovirus serotype in the order type 1>type3>type 2 (Sect. 3.4) [8, 9, 34, 241]. Susceptibility to oral infection is in the order of humans>chimpanzees>macaques, whereas neural susceptibility is in the order macaques>chimpanzees>humans [242–244].

Poliovirus variants of all three serotypes have been selected for growth in mice [245–247] and a type 2 variant has also been selected for growth in chick embryos [248]. Polioviruses normally cannot directly infect cultured mouse or chick cells, but can replicate efficiently when viral RNA is introduced by transfection, an observation that led to the concept of a specific viral receptor [249]. The CD155 PVR is a transmembrane glycoprotein with three extracellular immunoglobulin-like domains, encoded by a gene mapped to human chromosome 19 [250]. The normal function of CD155 is as a receptor for establishment of intercellular junctions between epithelial cells, a function that is "misused" by poliovirus to gain entry into human cells [251].

4.5 Poliovirus Replication Cycle

An overview of the poliovirus replication cycle is shown in Fig. 13.6. Virus attaches to cells through specific interactions between the amino-terminal variable domain 1 of CD155 and a "canyon" that surrounds the fivefold axis of the virion [225, 253–257]. After endocytosis, viral RNA is uncoated and released into the cytoplasm [257], VPg is cleaved from 5′-end of the RNA, and the RNA is translated. Translation is under the control of the internal ribosome entry site (*IRES*; Fig. 13.5), an element (nucleotides ~130–600) within the 5′-UTR that has a highly conserved stem-loop structure [258, 259]. The translation product is a single polypeptide, the

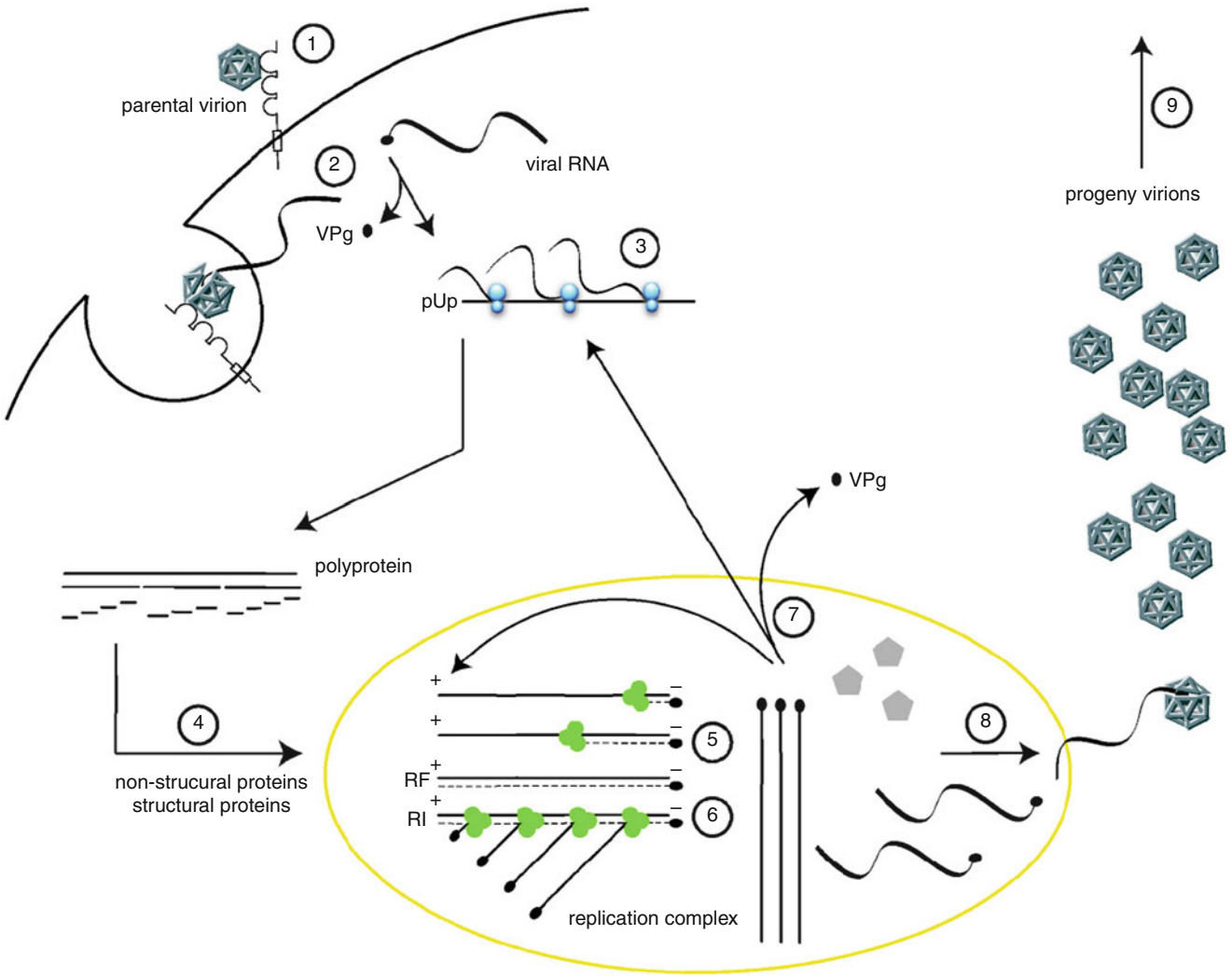

Fig. 13.6 Overview of the poliovirus replication cycle: *1* attachment of polio virion to poliovirus receptor (PVR; CD155) on cytoplasmic membrane, *2* endocytosis and uncoating of RNA, release into cytoplasm, and cleavage of VPg from 5′-end of RNA, *3* translation of viral proteins from viral RNA serving as mRNA, *4* proteolytic processing viral proteins, *5* replication of negative (–) strands of viral RNA by poliovirus RNA-dependent RNA polymerase (RdRP), *6* replication of positive (+) strands of viral RNA by RdRP in replication intermediates (RI), *7* cleavage of VPg from some + RNA strands for programming as mRNA, *8* encapsidation of other + RNA strands into virions, and *9* release from cytoplasm of infected cell. In cell culture, the entire infectious cycle is complete within ~6 h with release of up to 10,000 infectious virions per cell (Reproduced from reference De Jesus [252] with permission from BioMed Central)

polyprotein, which is cleaved by virus-encoded proteinases, $2A^{pro}$ and (primarily) $3C^{pro}$, into mature viral proteins [260]. Host protein synthesis is rapidly inhibited by the cleavage by $2A^{pro}$ of the translation initiation factor eIF4G, required for initiation of translation of capped host messenger RNA but not for the internal initiation of translation from the poliovirus IRES [259, 261]. One cleavage product is $3D^{pol}$, an RNA-dependent RNA polymerase (RdRP), that catalyzes the synthesis of negative-polarity (–) RNA strands from the genomic and mRNA-polarity (+) strands forming a duplex called the replicative form (RF) [262, 263]. Multiple copies of positive RNA strands are produced from negative-strand templates in replicative intermediates (RI) arrayed in intracellular membrane complexes [262, 263]. VPg is cleaved from some newly synthesized positive RNA strands for programming as mRNA and further translation [260]. Other positive strands are encapsidated during the maturation step in which the VP0 precursor to VP4 and VP2 is cleaved followed by release of infectious virions from the infected cell. The entire replication cycle takes place within the cytoplasm, and poliovirus can replicate in anucleate cells. Infected cells show cytopathic effects within 6 h and can release up to 10,000 infectious virus particles upon cell lysis and death. This rapid rate of cellular destruction accounts for the rapid progression of paralysis when poliovirus infects motor neurons [264].

Poliovirus (and picornavirus) replication has recently been reviewed in depth [27, 202, 259].

4.6 Poliovirus Genetics

4.6.1 Rapid Evolution of Poliovirus Genomes

Poliovirus is one of the most rapidly evolving viruses known [147, 164, 171, 172, 265]. Most of the nucleotide substitutions generate synonymous codons [164], and the basic biological properties of wild polioviruses remain unchanged, although the Sabin OPV strains can undergo important phenotypic changes (Sects. 9.1.4 and 10.8). Estimates of the rates of total nucleotide substitution into poliovirus capsid regions average ~10^{-2} substitutions per site per year [164–166, 171–173, 188, 191]. The rates appear to be similar across the three poliovirus serotypes and for both circulating polioviruses and polioviruses associated with chronic infections, and constitute a robust poliovirus molecular clock. Underlying the rapid pace of poliovirus genomic evolution are the high rates of base misincorporation (in the range of 10^{-5} to 10^{-3} per base per replication) by the poliovirus RdRP [266–271]. These high mutation rates are attributable to the absence of $3' \rightarrow 5'$ exonuclease proofreading mechanisms for the viral RNA polymerases [267], although other mechanisms may also be involved [272]. This exceptionally rapid rate of genomic evolution has facilitated high-resolution molecular epidemiologic studies (Sects. 3.6.3 and 10.7), even as deeper evolutionary relationships among poliovirus genotypes are obscured by saturation of variable nucleotide sites [164].

Poliovirus populations in cell culture and in humans [173] are a spectrum of mutational variants termed "quasispecies" [273, 274]. On average, each genome in a virus population contains one nucleotide substitution difference from the consensus "master sequence" of the quasispecies population. Two important consequences are that the virus populations in the live, attenuated OPV contain preexisting variants of higher potential neurovirulence [275], and that antigenic variants can be rapidly selected in cell culture [218, 276] and in humans [141, 220–223].

4.6.2 Recombination

Recombination occurs continuously during poliovirus infection of cultured cells [271, 277, 278] and individuals [173, 279]. Wild polioviruses undergo frequent recombination with the closely related human species-C enteroviruses during circulation [24, 167, 205, 206]. Indeed, the 5′-UTR and P2/P3 noncapsid sequences (Fig. 13.5) of wild polioviruses are drawn from a potentially large and constantly exchanging genetic pool that includes the locally circulating human species-C enteroviruses [167, 206, 280]. Crossovers usually map outside the capsid region when the exchange partners are different poliovirus or enterovirus serotypes, but crossovers may occur within the capsid region when the partners are of the same poliovirus serotype [173, 271, 277]. The biological role of recombination in poliovirus is unclear. Recombination may facilitate maintenance of replicative fitness by countering the accumulation of deleterious mutations [281]. However, natural selection apparently maintains wild poliovirus near its fitness optimum, and multiple recombinational variants can co-circulate locally [168]. Children fed tOPV regularly excrete vaccine/vaccine recombinants [279] and most circulating VDPVs (Sect. 10.8) are vaccine/non-vaccine recombinants [133, 166–168].

The genetics of poliovirus and other RNA viruses has been comprehensively reviewed [268, 273, 274, 277, 282, 283].

5 Descriptive Epidemiology

The epidemiology of poliomyelitis remained obscure until inapparent infections and mild cases were recognized [40, 41]. Unlike smallpox, where every infection of a susceptible person is associated with overt and characteristic clinical signs [284], the first poliomyelitis outbreaks erupted with no evident source [1, 34]. Five phases in the natural history of poliomyelitis can be recognized: (1) the endemic phase, (2) the epidemic phase, (3) the vaccine era, (4) the eradication era, and (5) the post-eradication era [22, 23]. Most developed countries eradicated their indigenous wild polioviruses four

to five decades ago [8, 9, 33, 34]. Similar progress has now been achieved by all but three developing countries (Fig. 13.3), parts of which remain in the endemic and epidemic phases because the vaccine era has not yet been fully implemented [12].

5.1 Endemic, Epidemic, Vaccine Era, and Eradication Phases

During the endemic phase, virtually all children were exposed to wild polioviruses at an early age. Large outbreaks were rare because large cohorts of nonimmune susceptible children rarely accumulated. Frequent exposure to wild polioviruses maintained population immunity and had the potentially important additional beneficial effect of boosting the immunity of women of childbearing age. Outbreaks were more likely to occur in smaller, more isolated populations than in large populations that could support continuous poliovirus circulation. The endemic phase was inevitably followed by an epidemic phase [1, 8, 9, 23, 28, 29, 33, 34, 66]. In the United States and Europe, outbreaks of increasing size and severity occurred for six decades until the mid-1950s and were halted only by the introduction of IPV [8, 9, 23, 29, 34]. Unfortunately, the vaccine era arrived unequally in the world, starting first with the most developed countries of Europe, North America, Australia, and New Zealand and progressing to Japan, the Soviet Union and Eastern Europe, and the countries of temperate South America [8, 9]. As the vaccine phase progressed in more developed countries, periodic epidemics appeared in less developed countries [8, 9]. Incomplete vaccine coverage in some developing countries had the perverse effect of reducing, but not eliminating, poliovirus circulation, potentially increasing the risk of explosive epidemics following the buildup of nonimmune susceptible persons in the population. The eradication phase has been permanent in most countries, but continued wild poliovirus circulation in a few areas carries ongoing global risks, and some countries have allowed immunity gaps to widen after eradication of indigenous wild polioviruses and suffered outbreaks from imported wild polioviruses [10, 12] (Sect. 10.6) or from the emergence and spread of cVDPVs (Sect. 10.8) [133]. The global post-eradication phase (Sect. 11.2) is far more complex than originally envisioned and is a key element of the current WHO strategic plan [22].

5.2 Geographic Distribution

Before the vaccine era, all three poliovirus serotypes had virtually a worldwide distribution. Virus circulated continuously in populous tropical areas, and the intensity of wild poliovirus circulation ("force of infection") was high, especially in areas with high population densities [180]. For example, up to the 1990s in Mumbai, India, all three serotypes of wild poliovirus could be found in the community, and children in high-risk urban slums were occasionally found to be concurrently infected with all three wild poliovirus serotypes [285, 286]. In recent years, some children in low-coverage endemic communities were coinfected with wild poliovirus types 1 and 3.

As with other enteroviruses, poliovirus circulation had a distinct seasonality in temperate zones. Paralytic cases peaked during summer and early autumn and could disappear altogether in winter. Sewage sampling could detect the presence of virus before and after the appearance of cases, but generally not throughout the year in smaller communities in cooler climates [103]. In both temperate zones and tropical areas, different serotypes predominated in different years. Poliovirus circulation would stop completely in small, rural populations, which would then be subject to outbreaks once poliovirus was reintroduced into the community [28].

Very isolated communities had no poliovirus infections for years. For example, age-stratified seroprevalence data have shown that Eskimo communities in Canada and the United States experienced sharp outbreaks covering a broad age distribution preceded and followed by many years with no serologic evidence of poliovirus circulation [85, 287]. A similar pattern of infrequent outbreaks also occurred in isolated tropical communities. Following introduction of poliovirus from the mainland, a large outbreak in the Andaman and Nicobar Islands in the Bay of Bengal in 1947–1948 affected a broad age distribution, with an overall paralytic attack rate of 10 % and a CFR of 14 % [288].

In the vaccine era, poliovirus type 1 had the widest geographic distribution, and poliovirus type 2 the most restricted [180]. It is difficult to separate out the effects of immunization, even at low rates of coverage, from the intrinsic biological properties of wild polioviruses. For example, type 1 is most frequently associated with large outbreaks and appears to be able to spread over wider geographic areas than type 3 [180] and (especially) type 2. Wild poliovirus type 2 was the first to be eradicated globally (Fig. 13.3) [13] but was also the first to disappear regionally. In the United States, for example, wild poliovirus type 2 disappeared long before type 3 and finally type 1. By the mid-1980s, no wild type 2 polioviruses could be found in several large countries, including Brazil and China, where the other two serotypes were still endemic [170]. Only wild poliovirus type 1 was found in the Caribbean during the 1970s and 1980s, whereas both types 1 and 3 could be found in the larger island populations of the Philippines and Indonesia until the mid-1990s [289, 290].

With the introduction of OPV, OPV-like viruses of all three serotypes became ubiquitous in areas of high coverage. Unlike wild polioviruses, vaccine-related strains usually do

not persist. For example, in Cuba, where OPV was administered only twice a year in campaigns, vaccine-related viruses disappeared from the environment within 4 months of the second campaign round [291].

5.3 Seasonality

In temperate zones, circulation of polioviruses, like that of all enteroviruses, is seasonal [23]. The summer-fall seasonality of poliomyelitis outbreaks was clearly described in the early reports of epidemics in Europe and the United States [41, 45]. Seasonality was most pronounced in temperate zones and gradually decreased toward the equator, where intense poliovirus circulation could occur throughout the year [9, 34, 56]. In the tropics, the residual poliomyelitis seasonality was variable and circulation tended to increase during the rainy season. The typical summer-autumn wild poliovirus seasonality peak was offset by 6 months between the northern and southern temperate zones [9, 292]. Poliomyelitis outbreaks have occurred on rare occasions during the winter months [110].

Poliovirus seasonality is a reflection of the fluctuation in the number of transmission chains during the year [168, 193]. However, the underlying mechanisms for poliovirus seasonality are unknown. Seasonal patterns of human association do not appear to be major factors because the peaks of the poliovirus and rotavirus (another non-enveloped enteric virus) seasons are offset by 6 months in the United States [34]. One hypothesis for poliovirus seasonality is based on the observation that poliovirus is more stable when relative humidity is above 40 % [293]. In temperate zones, indoor relative humidity is highest in the summer, and in the tropics humidity is high during the rainy season and throughout the year in coastal areas, a pattern closely correlated with the observed patterns of poliovirus seasonality. However, seasonal patterns of human migration can also facilitate poliovirus dissemination. For example, the spread of wild poliovirus from the tropics to more temperate zones was correlated with the seasonal migration of underimmunized farm worker families moving with the harvest season. Reaching underimmunized children in migrant populations remains a key eradication strategy in developing countries [22].

Seasonality has been an important facilitating factor for the eradication of wild polioviruses in developing countries. Mass immunization campaigns in the cooler months, when the transmission chains of poliovirus (and potentially competing enteroviruses) are at their seasonal low [193], have been a mainstay of eradication efforts [294–296]. In the northern Andean region, polio circulation ceased in cities in the temperate highlands years before it ceased in cities of the tropical coastlands [164]. In temperate Bolivia, OPV coverage rates of only 50 % were sufficient to eradicate polio [70]. Sequence data showed that wild poliovirus was imported into the Bolivian highlands from tropical coastal Peru [170]. In Brazil, polio was rapidly controlled in the south, but the reservoirs persisted in the northeast, with its more tropical climate as well as poor sanitation [70]. Polio had already been eradicated from the temperate Southern Cone (Argentina, Chile, Paraguay, Uruguay) before the launch of the PAHO Polio Eradication Initiative [18]. In China, polio persisted in the provinces of the southeast but not in the coastal northeast [172, 297, 298]. One caveat is that the level of immunization (and OPV efficacy) is usually higher in temperate zones than in tropical zones.

5.4 Age and Sex

In endemic areas, children are chiefly responsible for maintaining poliovirus circulation. The primary ages of first infection (as indicated by appearance of cases) was 2 years or younger in the pre-vaccine era [9, 23, 28, 29, 66]. Older individuals may have added to poliovirus transmission, but their contributions were likely to have been relatively small because most would have had prior exposure to poliovirus and the effect of reexposure would have been to boost mucosal immunity and thus limit poliovirus excretion. In both developing and developed countries, the hygiene of children <2 years of age favors enteric virus dissemination, but in the pre-vaccine era the likelihood of exposure to wild poliovirus was much higher in settings with poor sanitation [29].

The age distribution of poliomyelitis cases has shifted dramatically to older age groups over the past century [9, 23, 28, 29, 66]. During the 1916 epidemic in New York, 80 % of cases were among children <5 years of age. By the mid-1950s, peak cases were in children 5–9 years of age and two-thirds of deaths were in patients >15 years of age [23]. In developing countries, poliomyelitis remained a disease of younger children, with >75 % of cases <2 years of age and >95 % of cases <5 years of age [299, 300].

In the endemic phase, which represented all countries before the late nineteenth century, many children would be infected by the then-prevalent wild polioviruses while still protected by maternal antibody, and thus could become immune without risk of paralytic disease. As sanitation improved, the first exposures to poliovirus were delayed to later in life, when the risk of severe disease is increased, and after protective maternal antibodies had waned. Delayed infection also resulted in expansion of the pool of nonimmune susceptible individuals, increasing the potential for explosive outbreaks once wild poliovirus was reintroduced into the population. Paul had first proposed this process to explain the sudden appearance of epidemic poliomyelitis, first in the most developed parts of Europe and North America and then elsewhere [1]. Paul's hypothesis has been repeatedly confirmed [23, 34], and it predicted an irreversible shift from the endemic to the epidemic phase. This had been the pattern in all countries until poliomyelitis was finally brought under control through immunization.

It has been known for decades that males are more susceptible than females to paralytic poliomyelitis and to more severe forms of the disease [9, 23, 66, 301]. The reasons for this are unknown, although it has been suggested that greater physical exertion by boys might be a factor [23]. A majority of the paralytic cases occurred in males in recent outbreaks in Namibia (89 %) [80], Republic of Congo (68 %) [302], and Tajikistan (66 %) [178].

5.5 Occurrence in Families and Contact Groups

Polioviruses, like other enteroviruses, are highly communicable [23]. In the pre-vaccine era, virus was spread efficiently by young children to other family members, many of whom had prior exposure and were not susceptible to disease. In the vaccine era, most cases of contact vaccine-associated paralytic poliomyelitis (VAPP; Sect. 9.1.4) were within the family unit [303, 304]. More recently, concerns have focused on extended family units in developing countries, especially among groups that refuse immunization and who could represent an interconnected, socially defined reservoir of poliovirus transmission within an otherwise adequately immunized population. Attention is also being given to nomads and other mobile populations who could facilitate dissemination of poliovirus originating from fixed reservoir communities [22].

5.6 Epidemiologic Patterns of Poliomyelitis

5.6.1 Epidemiologic Patterns in Developed Countries in Temperate Zones

The shift from endemic to epidemic phase was first observed in countries with the highest standards of community sanitation and personal hygiene [23, 28, 29, 33, 34]. As outlined above, the epidemic phase appears to have been the consequence of delaying poliovirus exposure to later age groups who are prone to more severe paralytic disease and to the accumulation of nonimmune susceptible populations poised for large outbreaks. As sanitation conditions continued to improve, the poliomyelitis outbreaks steadily shifted in size and severity and peaked in increasingly older age groups. In some settings the shift was gradual; in others it was abrupt [23].

The mechanism proposed by Paul [1] for the shift from endemicity to epidemicity received further support by the observation that families with the highest socioeconomic advantages were at highest risk for severe poliomyelitis, whereas less advantaged families living in communities with poor sanitation were at reduced risk [23]. Indeed, the last outbreaks in the United States in the epidemic pre-vaccine era included parents in more advantaged families whose children carried wild poliovirus into the home [305].

5.6.2 Poliomyelitis in the Vaccine Era

The high-income countries of North America, Western Europe, and the southwest Pacific were quick to adopt widespread immunization with IPV. The impact was rapid and dramatic. In the United States, for example, the incidence of paralytic poliomyelitis fell from 13,850 in 1955 to 829 in 1961 (Fig. 13.7) [308]. With the availability of OPV in 1961, more countries adopted immunization against poliomyelitis, and the downward trend continued. In the United States, Canada, Australia, and New Zealand, combined cases fell >700-fold from 44,378 in 1951–1955 to 62 in 1968 [8], and in Europe cases fell >50-fold from 28,359 in 1951–1955 to 529 in 1968 [8]. Dramatic progress was also made in the Soviet Union as some republics reported no cases by 1968 [8]. Poliomyelitis cases in Japan, which did not use IPV and introduced OPV in 1961 [309], fell >120-fold from 2414 in 1951–1955 to 20 in 1968 [8]. Apart from Singapore, the picture for the rest of Asia was not encouraging. Several countries in Latin America (Costa Rica, Uruguay, Chile, and Argentina) reduced cases by 4- to 50-fold [8], and Cuba eradicated indigenous polioviruses in 1962 [8, 310], as did Jamaica in 1968 [8]. However, many other countries in Latin America and the Caribbean made little progress [8]. Only Israel in the Middle East made evident progress (>35-fold reduction by 1968), and poliomyelitis in Africa remained virtually uncontrolled [8].

The downward trends continued in the United States and other higher-income countries through the 1970s (Fig. 13.7) [9]. Circulation of wild polioviruses indigenous to the United States apparently ceased after the 1970 outbreak along the Texas-Mexico border [163, 311]. Subsequent sporadic poliomyelitis cases (and a small outbreak in 1972) were usually associated with wild type 1 polioviruses imported from Mexico [163]. An outbreak associated with type 1 virus originating in Turkey spread to underimmunized religious communities in the Netherlands and Canada, and to the Amish community in the United States in 1978–1979 [147], was followed in August 1979 by one last sporadic case associated with wild type 1 poliovirus imported from Mexico [163]. A similar picture emerged in Europe. Outbreaks associated with imported wild polioviruses occurred in the Netherlands in 1971 (type 1) [312, 313], 1978 (type 1) [163, 312, 313] and 1992 (type 3) [314, 315]; in Sweden in 1977 (type 2) [105]; in Spain in 1983 (type 3) [180]; in Finland in 1984 (type 3) [110, 316]; and repeatedly in the Balkans and the southern Republics of the Soviet Union (types 1 and 3) [180, 317]. Also, a large outbreak in Taiwan in 1982 (1,031 cases) from wild type 1 poliovirus (probably imported from Indonesia) highlighted the risk to poliomyelitis-free countries in Asia of reinfection by importation from neighboring countries [318].

In many developed countries using OPV, the only cases of poliomyelitis each year were from VAPP (Sect. 9.1.4) [24, 319–323]. However, the risks of importation of wild polioviruses

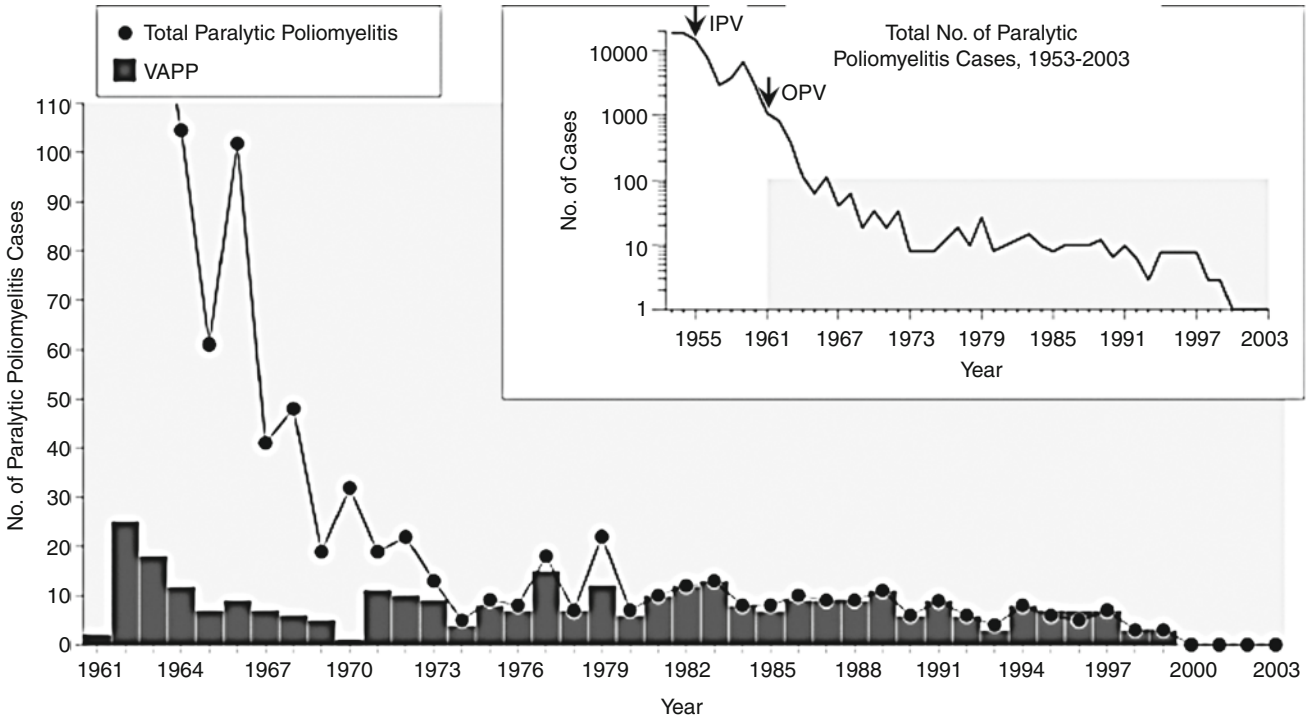

Fig. 13.7 Reported cases of poliomyelitis, United States, 1953–2003. Arrows indicate years of introduction of inactivated poliovirus vaccine (IPV; 1955) and oral poliovirus vaccine (OPV; 1961–62). The last wild poliovirus (type 1) case occurred in August 1979 [163, 306]. Bars indicate cases of vaccine-associated paralytic poliomyelitis (VAPP). New VAPP cases stopped after the shift to IPV in 2000 [307], but vaccine-derived poliovirus (VDPV) infections occurred in Minnesota in 2005 (type 1) [132] and a separate immunodeficiency-associated VDPV (iVDPV) case occurred in Minnesota in 2008 (type 2) [223]. Note that abscissa of inset is a logarithmic scale and abscissa of main graph is a linear scale (Source: Centers for Disease Control and Prevention (CDC). Reproduced from reference Alexander [307])

remained, and the sharp divergence between the poliomyelitis-free and poliomyelitis-endemic worlds was unsustainable.

5.6.3 Poliomyelitis in Developing Countries

By 1980, many developing countries had made little progress in controlling poliomyelitis in the nearly two decades since the widespread availability of OPV [9, 324]. With their growing populations, the problem of epidemic poliomyelitis in developing countries, well recognized by the mid-1950s [292], was increasing [98, 324]. In addition, lameness surveys revealed that the incidence of poliomyelitis in Africa and other developing countries was far higher than originally believed [97, 98, 319, 325]. Poliomyelitis cases in much of Africa and Asia had been grossly underreported [324], with the actual global incidence being tenfold greater than the officially reported counts. For example, lameness surveys in 1981–1982 suggested that India had half of the estimated world total of 400,000 poliomyelitis cases per year [326, 327]. Throughout the 1980s, the city of Mumbai alone reported ~1,000 cases per year [285, 286], more than 100 times the rate (from VAPP) for the United States over the same time period (Fig. 13.7) [303, 304]. Moreover, large outbreaks, some recurring, occurred in many developing countries as they steadily shifted to the epidemic phase [180]. Clearly, remedial action was urgently needed.

6 Mechanisms and Routes of Transmission

As with other enteroviruses, poliovirus is spread by person-to-person contact via two routes of transmission, fecal–oral and respiratory [23, 29]. The relative importance of these two routes varies by setting. Fecal–oral is the more efficient route because fecal shedding continues for up to 6 weeks, and the quantities of virus shed in stool may be as high as 300 million infectious particles per day [328]. Under experimental conditions, vaccine virus was shown to spread to contacts even when administered in gelatin capsules, thereby bypassing throat infection and respiratory transmission [329]. In areas of poor sanitation and hygiene, fecal–oral transmission (via contaminated fingers, food or water, utensils, or toys) is most likely the dominant route, as young children are continually exposed to unsanitary conditions and live in close proximity to contaminated soil and open sewers. Respiratory transmission probably played a more important role in developed countries with high standards of hygiene and sanitation, and where in the pre-vaccine era exposure to poliovirus in higher socioeconomic communities was typically delayed by several years. The effectiveness of IPV, which blocks oropharyngeal but not intestinal infection [330], to stop wild poliovirus transmission is evidence of the

importance of respiratory transmission in settings where fecal–oral transmission is less prominent.

There is no evidence for an extrahuman reservoir for poliovirus, apart from virus stored in laboratory freezers [331]. Sequence analyses show close genetic relatedness among the polioviruses obtained in the same locales from clinical cases, stool surveys, and sewage sampling [111, 113]. Virus imported over short or long distances can consistently be linked by molecular epidemiologic methods to infections recently occurring in the source communities [10, 11, 163, 193, 332].

On very rare occasions, poliomyelitis infections and outbreaks have started by mechanisms other than direct person-to-person transmission. In the spring of 1955, 204 vaccine-associated cases occurred in the United States following injection of children with IPV preparations which contained residual infectious wild poliovirus (the Cutter incident) [333, 334]. The majority of case isolates were derived from the neurovirulent type 1 Mahoney strain used in IPV production [1, 333, 334]. A small number of cases in Uttar Pradesh, India, in 2000 and again in 2002–2003 were found to be associated with the type 2 reference strain, MEF-1, found in contaminated lots of OPV [335, 336].

7 Pathogenesis and Immunity

7.1 Pathogenesis

Studies on the pathogenesis of poliomyelitis in humans date to the nineteenth century [1]. The availability of a primate model for pathogenesis in 1909 accelerated the pace of pathogenesis research up to the mid-1950s [1, 242, 243]. Interest in the pathogenesis of poliomyelitis waned with the availability of poliovirus vaccines and the increased focus on molecular virology, even though pathology studies continued to address key unanswered questions [337, 338]. Two major models of pathogenesis, one by Bodian [242] (Fig. 13.8) and the other by Sabin [243], were proposed in 1955 and 1956. The principal difference between the two models is whether the major primary sites of poliovirus replication are in lymphatic tissues (Bodian) or mucosal tissues (Sabin) [244]. According to Bodian's model, orally ingested poliovirus first replicates locally in lymphatic tissues at the sites of initial virus implantation (tonsils, intestinal microfold epithelial [M] cells of the Peyer's patches in the ileum). Within 1–2 days virus spreads via lymphatic pathways from the small intestine to the mesenteric lymph nodes and from the tonsils and adenoids to deep cervical lymph nodes.

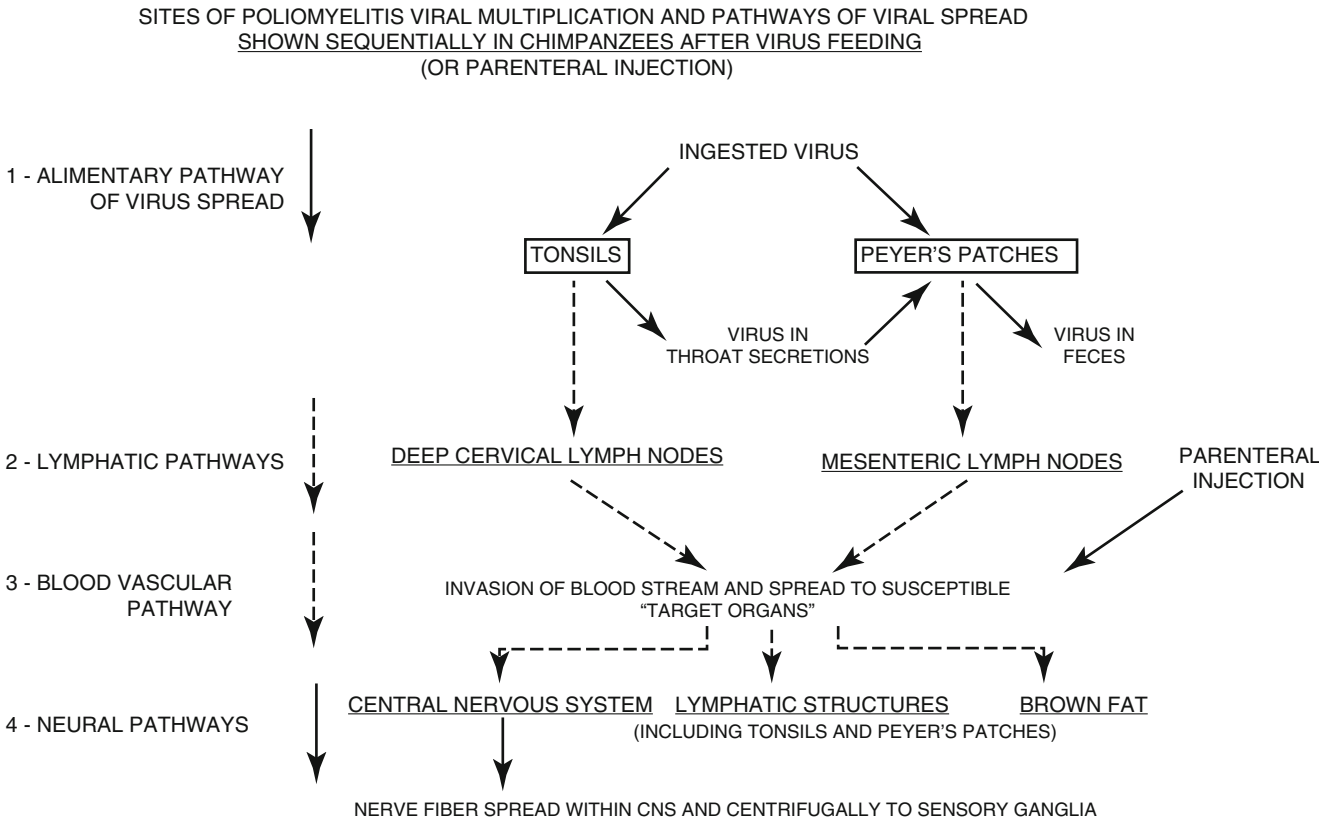

Fig. 13.8 David Bodian's scheme of the pathogenesis of poliovirus infection based on studies in monkeys, chimpanzees, and humans. Reproduced with permission from the original article by Bodian [241]. (Copyright 1955, American Association for the Advancement of Science (http://www.sciencemag.org).) *CNS* central nervous system

By days 1–3, virus appears in the feces and throat. In the next viremic phase, virus invades the bloodstream and infects other susceptible "target organs," including further spread to systemic lymph nodes, brown fat, and occasionally motor neurons of the CNS. The first 1–2 days of infection are asymptomatic. During viremia, all tissues are exposed to virus, and about 10 % of infected persons experience "minor illness" at days 3–4, with malaise and fever accompanying systemic viral infection. Clinical signs subside after day 4. Viremia ends with the appearance of antibody by day 6, and virus bound to antibody can be detected for a few days longer [320]. Incubation periods (time from exposure to disease onset) vary in different individuals, but they are usually from 7 to 17 days but range from 2 to 35 days [23]. Virus replicates in the oropharynx for 1–2 weeks and is shed in the stool for 3–6 weeks [23, 321].

Progression to "major illness," including nonparalytic poliomyelitis (aseptic meningitis) and paralytic poliomyelitis, occurs within 8–30 days of exposure [28]. The biphasic nature of the disease, with minor illness followed by major illness, has been termed the "Dromedary" form [322] to imply two humps (even though the dromedary is a one-humped camel) [1]. Invasion of the CNS may occur by either penetration of the blood–brain barrier or by retrograde axonal transport. Paralytic illness follows directly from the lytic infection of motor neurons, constituting the gray matter of the spinal cord. In fatal cases, virus replication in motor neurons rises sharply the day preceding paralysis, peaks to high titers at day 3, and disappears by day 7 [29]. In spinal poliomyelitis, motor neurons in the anterior horn of the spinal cord are rapidly destroyed, with the severity of paralysis correlated with the extent of neuronal destruction [29]. In bulbar poliomyelitis, motor neurons in the medulla in the brainstem are primarily affected. Lesions may also occur in the reticular formation in the brainstem and in the thalamus and hypothalamus just above the brainstem. Lesions in the brain occur in the roof nuclei of the cerebellum and in the precentral gyrus of the motor cortex [23, 29]. Other regions of the cortex are usually resistant to infection [264]. Some motor neurons may lose function because of edema, but the damage is temporary and motor function is restored once the inflammation subsides [23]. Local secretory IgA immunity can block poliovirus replication in tonsils and the intestinal tract, and neutralizing IgG and IgM antibodies can prevent virus spread to motor neurons of the CNS.

Poliovirus pathogenesis has enjoyed a renaissance [26, 232, 244, 323, 338] after cloning and identification of the PVR, CD155 [208], and the development of transgenic mice expressing CD155 [340–342]. The transgenic mice are susceptible to poliovirus infection upon intraspinal, intracerebral, intravenous, intranasal, intraperitoneal, or intramuscular inoculation and exhibit clinical signs and neural lesions typical of human poliomyelitis [232]. However, the transgenic mice are usually resistant to infection by the oral route [232, 340, 343]. It has been found in both humans and transgenic mice that CD155 RNA and protein are expressed in a wide variety of tissues, including tissues that do not support poliovirus replication [323]. Therefore, CD155 expression is necessary but not sufficient for poliovirus replication in vivo, and a stage in the replication cycle after initiation of translation determines tissue tropism [323]. One potential mechanism for the observed patterns of tissue tropism is that a barrier of innate immunity in extraneural tissues blocks poliovirus replication [232]. In support of this view, it was found that in PVR-transgenic mice deficient in the interferon-α/β receptor, poliovirus replication occurred not only in the CNS but also in extraneural tissues such as the liver, pancreas, and small intestine [343, 344].

The mechanism of axonal retrograde transport has been reexamined in transgenic mice [345]. At the molecular level, the cytoplasmic domain of CD155 was found to specifically bind to Tctex-1, a light chain of the dynein motor complex (a driver for retrograde transport), and that the rate of Tctex-1 transport is similar to the rate of poliovirus ascent along nerve fibers [232, 346].

New developments in the pathogenesis of poliomyelitis have been the subject of several recent reviews [26, 232, 244, 323, 338].

7.2 Immunity

Neutralizing antibody protects against paralytic disease [57]. Type-specific immunity from natural infection is lifelong [85], and immunity also appears to be permanent for individuals who have produced neutralizing antibodies after receipt of OPV or IPV. Infants are protected against disease during the first few months of life by maternal antibody, which has a half-life of approximately 28 days and falls to undetectable levels after 6 months [347]. In the endemic period, children <6 months of age rarely contracted paralytic disease, but could induce protective immunity when infected with wild poliovirus [28]. It has long been known that even low levels of circulating antibodies are protective [57], because virus titers during viremia are low [58].

Neutralizing IgM and IgG antibodies appear within a few days of exposure to virus. IgM titers decline after 10 days, but IgG titers continue to rise for at least 2 months [348]. Mucosal immunity in the gastrointestinal tract and oropharynx, in the form of secretory IgA, rises more slowly and persists for at least several months, but the duration of mucosal immunity to poliovirus has not been intensively studied [7, 349]. Children who have had tonsillectomy are more susceptible to poliomyelitis than children with intact oropharyngeal lymphoid tissue, a finding that points to the importance of oropharyngeal mucosal immunity in protection from disease [350–352]. OPV induces both serum antibody and intestinal immunity, whereas intestinal immunity from IPV is low (Sect. 9.1.5) [7, 348].

Additional evidence for the critical importance of neutralizing antibody to immunity is that individuals with primary

(B-cell) immunodeficiencies are at a 3,000-fold higher risk than immunocompetent individuals of contracting VAPP when exposed to OPV (Sect. 9.1.4) [353], and some may develop prolonged poliovirus infections [354]. Patients with primary immunodeficiencies are treated with intravenous immunoglobulin (IVIG), and preparations used in the United States must meet minimum neutralization titers to poliovirus [7]. However, IVIG neutralizing titers become undetectable after 3–5 weeks [23], and several immunodeficient individuals with chronic poliovirus infections have contracted poliomyelitis despite IVIG treatment, presumably after titers of neutralizing antibodies fell below protective levels (see Sect. 10.8.2) [186, 223]. Newly developed chimpanzee–human neutralizing monoclonal antibodies may hold promise for the availability of high-titer preparations for immunotherapy of immunodeficient patients with prolonged poliovirus infections [228].

8 Patterns of Host Response and Diagnosis

8.1 Clinical Features

The patterns of host response were described in Sect. 7.1 in the context of the pathogenesis of poliomyelitis. Paul recognized four categories of response to poliovirus infection in susceptible individuals: (1) inapparent (asymptomatic) infections, (2) abortive poliomyelitis, (3) nonparalytic poliomyelitis (aseptic meningitis), and (4) paralytic poliomyelitis [28]. Abortive poliomyelitis is described as minor illness, and nonparalytic and paralytic poliomyelitis constitute major illness. The proportion of each category of host response depends on the patient age, the poliovirus serotype, and other physiologic factors such as tonsillectomy, pregnancy, recent injections, trauma, or recent strenuous physical exertion [29].

8.1.1 Inapparent (Asymptomatic) Infections

Most (up to 95 %) wild poliovirus infections of nonimmune susceptible individuals are without fever or other symptoms. Infected individuals produce protective neutralizing antibodies and have permanent resistance to paralysis upon reinfection by the same poliovirus serotype. Infections that occur under the cover of maternal antibody are asymptomatic. Individuals with inapparent infections shed virus in their stool and can participate in the spread of infection.

8.1.2 Minor Illness: Abortive Poliomyelitis

Abortive poliomyelitis is the most frequent disease manifestation, occurring in about 8 % of poliovirus infections [23, 28]. Symptoms are indistinguishable from those of many other infections and include fever, malaise, drowsiness, headache, vomiting, and sore throat. Abortive poliomyelitis occurs during the viremic phase a few days after infection. Complete recovery occurs within a few days, although virus shedding may continue for up to 6 weeks postexposure.

8.1.3 Major Illness: Nonparalytic Poliomyelitis (Aseptic Meningitis)

Aseptic meningitis occurs in 1–2 % of infections. It may be preceded by minor illness and is characterized by signs of inflammation of the meninges, including severe headache, and stiffness and pain in the back and neck. Aseptic meningitis lasts 2–10 days and is usually followed by complete recovery. In rare instances, the disease progresses to paralytic poliomyelitis. Other viral infections, including those of many nonpolio enteroviruses (Chap. 11), may cause aseptic meningitis [23].

8.1.4 Major Illness: Paralytic Poliomyelitis

Paralytic poliomyelitis occurs in <1 % of wild poliovirus infections (Sect. 3.4) and is characterized by acute flaccid paralysis (AFP) usually accompanied by fever. It may be preceded by minor illness with apparent recovery followed a few days later by rapid onset of paralysis. Minor illness preceding paralysis may be absent in adolescents and adults, but pain in the affected extremities during onset of paralysis may be severe. Paralysis progresses rapidly and is descending (i.e., moving proximally to distally), with loss of deep reflexes but no sensory loss. Paralysis is usually asymmetric and affects the legs more frequently than the arms, especially in young children. Residual paralysis persists beyond 60 days. The affected muscles and severity of paralysis depends on the location and concentration of neuronal lesions [29]. Paralytic poliomyelitis is further divided into three categories based on the CNS lesions and the corresponding affected muscle groups.

Spinal Poliomyelitis

Spinal poliomyelitis (~79 % of paralytic poliomyelitis cases) occurs when lesions are localized to the spinal cord and cause weakness in the legs, arms, back, or abdominal muscles. Spinal poliomyelitis may also paralyze the diaphragm and intercostal muscles, causing respiratory failure.

Bulbar Poliomyelitis

Bulbar poliomyelitis (~2 % of paralytic poliomyelitis cases) occurs when lesions form in the cranial nerve or medulla and results in paralysis of the pharynx, vocal cords, and facial nerves. Bulbar poliomyelitis may rapidly progress to respiratory failure when the respiratory centers of the medulla are attacked.

Bulbospinal Poliomyelitis

Bulbospinal poliomyelitis (~19 % of paralytic poliomyelitis cases) is a combination of the above two forms and is most frequently seen in adults [29].

8.1.5 Post-Polio Syndrome

Post-polio syndrome is a recrudescence of weakness, pain, fatigue, and atrophy in the muscles originally affected by acute paralytic poliomyelitis [355]. Symptoms progress over a period of years and first appear on average 30–35 years after recovery from the initial acute attack [356]. It is estimated that

25–50 % of patients with acute paralytic poliomyelitis will develop some signs of post-polio syndrome, the risk being highest among survivors with more severe paralysis from the original attack. In the United States, over 440,000 survivors of paralytic poliomyelitis were recognized in 1994–1995, but no updated counts have been obtained. Post-polio syndrome is a neurologic condition whose underlying mechanisms are unknown. Current evidence indicates that the syndrome results from the denervation of the peripheral neuromuscular junctions that expanded during recovery to compensate for neuronal damage from the original infection [355]. It is thought that the neurologic dysfunction progresses subclinically for many years until a critical threshold is reached beyond which the remaining motor neurons cannot maintain the extended number of neuromuscular junctions [355]. Post-polio syndrome does not appear to result from persistence of the original poliovirus infection [357]. Polio eradication is the most effective way to prevent post-polio syndrome.

8.2 Diagnosis

Most clinicians trained since the late 1960s in the United States and other developed countries have never seen a case of poliomyelitis. Similarly, clinicians in most developing countries no longer see poliomyelitis, apart from rare sporadic cases of VAPP (Sect. 9.1.4). Nonetheless, the risk of importation of wild poliovirus (or cVDPVs; Sect. 10.8.1) and the potential for outbreaks remain as long as poliovirus circulation continues anywhere in the world [10, 12, 80–82, 132, 175–178, 181]. Diagnosis of paralytic poliomyelitis is primarily based on three criteria: (1) clinical signs and course, (2) virologic testing, and (3) residual neurologic deficit at 60 days after onset of paralysis [7].

8.2.1 Clinical Signs and Course

The most obvious sign of poliomyelitis is the appearance of AFP. However, AFP has multiple etiologies, including Guillain–Barré syndrome, transverse myelitis, enterovirus infections (especially EV71; Chap. 11), other viral infections, traumatic neuritis, Bell's palsy, hypokalemia, and toxin exposure [7]. Studies in the Americas comparing clinical findings with the isolation of wild poliovirus from stool specimens reported a sensitivity of 75 % and specificity of 73 % for poliomyelitis when the diagnostic criteria for AFP cases were age <6 years and fever at onset of paralysis [68]. Inclusion of progression to complete paralysis in <4 days increased sensitivity [68], whereas inclusion of specific patterns of paralysis (descending, asymmetric, absence of paralysis in all limbs) increased specificity but reduced sensitivity [7]. In India, which achieved AFP rates >25 per 100,000 children <15 years of age, residual paralysis at 60 days was most strongly correlated with wild poliovirus infection [358].

8.2.2 Virologic Testing

Virologic testing has high sensitivity provided that adequate specimens are collected soon after onset of paralysis and received by the laboratory in good condition (section "Clinical specimens"). Because wild polioviruses can be accurately identified by molecular methods, and contaminants ruled out by sequence analysis, specificities approach 100 %. Classification of cases of VAPP requires the inclusion of clinical, epidemiologic, and laboratory findings [303, 304, 307, 359]. In countries using OPV, most isolations of OPV-like virus from patients with AFP are independent events and do not signal the occurrence of VAPP. Laboratory detection of genetically divergent vaccine-derived polioviruses (VDPVs; Sect. 10.8) indicate either prolonged replication in an immunodeficient individual or community circulation of VDPVs [133]. The distinguishing genetic properties of the more divergent VDPVs, coupled with information about the rates of poliovirus vaccine coverage in the source community, are strong predictors of the VDPV category [133].

8.2.3 Residual Neurologic Deficit

The clinical case definition for paralytic poliomyelitis includes residual paralysis at 60 days after onset of paralysis [100, 133]. In less severe cases of paralytic poliomyelitis, residual paralysis may be difficult to discern because of functional compensation by intact muscles [7].

9 Control and Prevention of Poliomyelitis

9.1 Poliovirus Vaccines

Attempts to inactivate poliovirus infectivity through formalin treatment date to 1911, but when the results from leading investigators were consistently disappointing, interest in immunization against poliomyelitis waned [1]. Interest was briefly rekindled during the field trials of 1935, conducted by two groups who took different approaches to experimental poliovirus vaccines, one applying formalin inactivation [360] and the other using live virus that was claimed to be "attenuated" through treatment with detergent [361]. However, the appearance of poliomyelitis cases—including fatalities—linked to the field trials [362] cast a long shadow on further attempts to develop a poliovirus vaccine. The experimental poliovirus vaccines of 1935 were developed before the advent of cell culture, before the identification of three distinct poliovirus serotypes, before the outlines of poliovirus pathogenesis were firmly established, and, in the case of one investigator, a failure to distinguish between chemical inactivation and attenuation of neurovirulence through genetic selection [361]. Prospects for a poliovirus vaccine again brightened in 1948 with the successful immunization of

monkeys with formalin-inactivated poliovirus [363]. Moreover, the rising incidence of paralytic polio in developed countries in the postwar years greatly increased the urgency of developing and deploying effective poliovirus vaccines.

9.1.1 Inactivated Poliovirus Vaccine (IPV)

The IPV of Salk and colleagues was the first poliovirus vaccine to be licensed [4, 5]. Its development followed several key advances in virology, pathology, and immunology [5]: (1) the cultivation of poliovirus in nonneural cells [53], (2) the identification of three poliovirus serotypes [54, 55], (3) the finding that viremia precedes paralysis [58], and (4) the demonstration that administration of immune globulin protected against paralytic poliomyelitis [57]. IPV is prepared by formalin inactivation of three wild, virulent reference strains: Mahoney (type 1), MEF-1 (type 2), and Saukett (type 3). A less virulent type 1 strain, Brunenders [364], is used in IPV production in Sweden and Denmark. Although antigenic sites 1 (Fig. 13.5) of types 2 and 3 are modified by formalin inactivation [365], immunization with IPV can induce high titers of neutralizing antibodies protective against all poliovirus strains. After exposure of some IPV recipients with live wild poliovirus contained in production lots of incompletely inactivated vaccine (the Cutter incident) [333, 334], conditions for IPV manufacture were modified, resulting in a reduction in the immunogenicity of IPV preparations [23]. However, improvements in cell culture technology in the 1970s led to development of an enhanced-potency IPV (eIPV), similar in immunogenicity to the original product [5, 35, 366], which has replaced the second-generation IPV.

IPV was licensed for use in the United States, Canada, and Western Europe in 1955 and was the only poliovirus vaccine available until licensure of OPV in 1961–1962. IPV use in the United States declined after the introduction of OPV, but it has been used continuously by some countries in Western Europe (Finland, Iceland, Sweden, and the Netherlands) and some provinces of Canada [5, 35]. In 1997, in response to the eradication of wild polioviruses in the Americas and the continuing occurrence of cases of VAPP (Sect. 9.1.4), the United States shifted from an all-OPV immunization schedule to a sequential IPV/OPV schedule in 1997, which was replaced in 2000 by an all-IPV schedule [5, 307].

9.1.2 Oral Poliovirus Vaccine (OPV)

Early attempts to produce live-virus vaccines date to the work of Jenner, who "vaccinated" with cowpox virus to protect against smallpox in the 1790s, and Pasteur, who developed an "attenuated" rabies vaccine in the 1880s [35]. Experimental live poliovirus vaccines were tested in monkeys as early as 1910 [29]. In the 1930s, Theiler demonstrated that an effective attenuated yellow fever vaccine could be produced by serial passage of virus in chick embryo tissues [367]. Theiler applied this basic approach to develop an experimental attenuated variant of the poliovirus type 2 Lansing strain in 1946 [368]. In 1952, Enders, Weller, and Robbins developed the attenuated poliovirus type 1 Brunenders strain by serial passage in cultured cells [364]. Two years later, Li and Schaeffer developed a highly attenuated derivative of the neurovirulent type 1 Mahoney strain, LS-c, by passage at 35 °C in cultured rhesus or cynomolgus monkey kidney cells and in monkey skin [369]. The LS-c strain was further treated by three consecutive single-plaque passages by Albert Sabin to produce the LS-c, 2ab strain, generally known as Sabin type 1 (Sabin 1) [370]. Sabin also developed attenuated strains for serotypes 2 and 3. The Sabin 2 OPV strain (P712, Ch, 2ab) was derived from virus (P712) that was isolated from a healthy child and shown to have low neurovirulence [370]. In contrast, the attenuated Sabin 3 strain (Leon 12 a_1b) was produced by rapid passage in monkey kidney cell culture of a highly neurovirulent strain isolated from the spinal cord of a child who had died of bulbospinal poliomyelitis [370]. In addition to Sabin, two other groups, led by Koprowski [329] and Cox [371], developed attenuated poliovirus vaccine strains for each serotype. All three sets were carefully evaluated for low neuropathogenicity for monkeys, immunogenicity, genetic stability on human passage, safety (inability to cause paralysis in man), and restricted capacity to spread [372, 373]. The Sabin strains, which had the lowest neuropathogenicity and an excellent safety record from large-scale field trials [6, 60], were approved for worldwide distribution. In the United States, OPV was licensed sequentially (Sabin 1, August, 1961; Sabin 2, October, 1961; Sabin 3, March, 1962). Licensure of Sabin 3 was delayed because of concerns about its undesirable genetic instability and relatively low immunogenicity [1]. OPV was initially administered serially in monovalent form, frequently in mass immunization campaigns (SOS; "Sabin on Sunday"), but successful tOPV field trials in Canada led to licensure of a trivalent formulation in 1963 [7]. OPV and its application to poliomyelitis eradication has been recently reviewed [7].

OPV was developed in the 1950s using the well-established empirical approach of rapid passage of virus at suboptimal temperatures in cells and tissues of nonhuman origin. It would be another two decades after licensure of OPV that the molecular basis of OPV attenuation became amenable to systematic investigation.

9.1.3 Genetic Determinants of Attenuation of the Sabin OPV Strains

Identification of the genetic determinants of attenuation of the Sabin OPV strains has been comprehensively reviewed [207, 209, 268, 374]. The first reports of the sequences of complete poliovirus genomes in the early 1980s [375, 376] and the development of infectious poliovirus cDNA clones

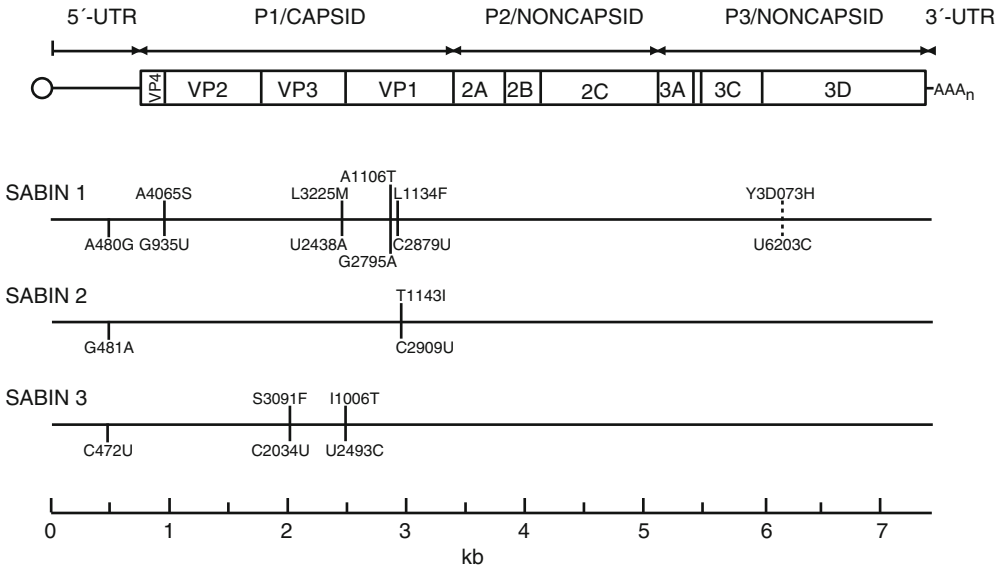

Fig. 13.9 Location of principal attenuating nucleotide (*lower bars*) and amino acid (*upper bars*) substitutions in each of the three Sabin OPV strains. Abbreviations of nucleotide residues: *A* adenine, *C* cytosine, *G* guanine, *U* uracil. Abbreviations of amino acid residues: *A* alanine, *C* cysteine, *F* phenylalanine, *H* histidine, *I* isoleucine, *L* leucine, *M* methionine, *S* serine, *T* threonine, *Y* tyrosine. Substitutions are shown as nonattenuated parent–position–Sabin strain; nucleotide positions are numbered consecutively from residue 1 of the RNA genome; amino acid positions are indicated by the abbreviated name of the viral protein (4, VP4; 2, VP2; 3, VP3; 1, VP1; 3D, 3D-polymerase) and numbered consecutively from residue 1 of each protein. For example, a guanine (Mahoney) → uracil (Sabin 1) substitution at RNA position 935 (G935U) encodes an alanine (Mahoney) → serine (Sabin 1) replacement at residue 65 of VP4 (A4065S). The Y3D073H substitution in Sabin 1 and S3091F substitution in Sabin 3 are important determinants of temperature sensitivity (Figure summarizes findings from references [379–384] (Redrawn from reference Kew et al. [94])

[377] opened the way for systematic investigation of the critical mutations responsible for the attenuated and temperature-sensitive phenotypes of the Sabin OPV strains. A common feature of the Sabin strains is the presence of nucleotide substitutions in the IRES, which in serotypes 1 and 3 have been clearly shown to be critical attenuating mutations. Additional mutations encoding amino acid substitutions in the capsid region contribute to and stabilize the attenuated phenotype.

Sabin 1

The 57 nucleotide substitutions distinguishing the Sabin 1 strain from its neurovirulent parent, Mahoney, are scattered throughout the genome [144]. Six map to the 5′-UTR, 49 map to the coding region (21 of which encode amino acid substitutions), and 2 map to the 3′-UTR. Infectious cDNA constructs containing different combinations of blocks of Sabin 1 and Mahoney sequences were tested for neurovirulence in monkeys or transgenic mice expressing the CD155 receptor, for temperature sensitivity, and for other phenotypic properties distinguishing the two strains [378, 379]. The single most important determinant of the attenuated phenotype of Sabin 1 was the A → G substitution at position 480 (abbreviated A480G) in the IRES [380]. Four other substitutions contributing to the attenuated phenotype mapped to the capsid region (one in VP4, one in VP3, and two in VP1), and a substitution contributing to the temperature-sensitive phenotype (but not to the attenuated phenotype) mapped to the $3D^{pol}$ region encoding the RdRP (Fig. 13.9) [7, 378, 379, 385].

Sabin 2

Only two nucleotide substitutions (G481A in the IRES and C2909U encoding a threonine → isoleucine substitution at position 143 of VP1) appear to be main determinants of the attenuated phenotype of Sabin 2 (Fig. 13.9) [381, 382]. Because P712 has inherently low neurovirulence [370], identification of critical attenuating sites in Sabin 2 involved determination of the effects of introduction of sequences derived from a minimally divergent neurovirulent revertant of Sabin 2 (obtained from a case of VAPP) into infectious cDNA constructs derived from Sabin 2 [381, 382]. The neurovirulence of Sabin 2 is remarkably stable in routine tests of OPV lots in monkeys, because the G481A substitution (not found in the IRES sequences of wild type 2 polioviruses) does not increase neurovirulence in monkeys, as it does in transgenic mice [386].

Sabin 3

Detailed analysis of the attenuated phenotype of Sabin 3 has been possible because the neurovirulent parental strain, Leon, differs from Sabin 3 by only ten nucleotide substitutions

[387]. In addition, numerous neurovirulent revertants of the Sabin 3 strain have been isolated from patients with VAPP [383] and from healthy OPV recipients [388]. Only three substitutions (C472U in the IRES, C2034U encoding a serine → phenylalanine substitution at position 91 of VP3, and U2493C encoding an isoleucine → threonine substitution at position 6 of VP1) appear to be the main determinants of the attenuated phenotype (Fig. 13.9) [207, 383, 388].

In all three Sabin strains, the attenuated phenotype is determined by multiple substitutions. The substitutions in the IRES, which alter stem-loop structures [207, 389–391] and reduce the efficiency of initiation of translation of the poliovirus RNA template [390, 392], contribute most to the attenuated phenotype of the Sabin 1 and Sabin 3 strains. Mutations restoring the original stem-loop structure in the IRES (Sabin 1, G480A [back mutation] or U525C [suppressor]; Sabin 2, A481G; Sabin 3, U472C) are frequently found in vaccine-related isolates from healthy OPV recipients [389, 393] and patients with VAPP [207] as well as from the environment [109]. In Sabin 3, the critical C472U substitution reduces the efficiency of binding of the polypyrimidine tract-binding protein (PTB), required for initiation of translation, to the IRES [394]. The translational deficit for Sabin 3 is moderate in intestinal cells, where PTB levels are high, but severe in neurons, where PTB levels are low. The precise mechanisms by which the capsid mutations contribute to the attenuated phenotype are less clear. Impairment of the efficiency of binding to the CD155 receptor [379] and reductions in the stability of the capsid [215] may play a role.

Sabin and other developers of OPV strains struck a balance between low neuropathogenicity, good immunogenicity, and acceptable levels of genetic stability [373]. The high genetic stability of the Sabin type 1 strain is probably attributable to the greater number of substitutions contributing to the attenuated phenotype. This property is especially important for the Sabin 1 vaccine strain, because wild type 1 polioviruses typically have high paralytic attack rates and can spread over wide geographic areas in explosive outbreaks [10, 170, 175–177, 180, 181]. Sabin 2 may revert more rapidly, but its immunogenicity is very high [7, 373] and the paralytic attack rates of wild type 2 polioviruses are low (Sect. 3.4) [34, 101]. Sabin 3 is associated with the highest rates of VAPP (Sect. 9.1.4), which is probably a consequence of low genetic stability of the critical attenuating substitution [275], relatively low immunogenicity [395], and an intermediate paralytic attack rate for type 3 polioviruses [34, 101]. Nonetheless, all three Sabin strains normally have very low pathogenic potentials, and incidence of VAPP in countries with high rates of OPV coverage [304] are several orders of magnitude lower than the incidence of paralytic poliomyelitis in areas with circulating wild polioviruses.

9.1.4 Vaccine-Associated Paralytic Poliomyelitis (VAPP)

After over 40 years of use and many billions of doses distributed worldwide, OPV has been associated with few adverse events. The most commonly recognized adverse event is VAPP, which is clinically indistinguishable from poliomyelitis caused by wild polioviruses. The first cases of VAPP were recognized within a year of licensure of OPV, and most of the early cases were associated with the Sabin 3 strain [396]. The Sabin 3 association was unambiguous because OPV had been delivered in monovalent form [7, 301, 396]. VAPP rates are very low and similar worldwide [7, 359, 397–400]. In the United States, the risk of VAPP in first-dose OPV recipients is about 1 case per 1.4 million children immunized [7, 301, 303, 304, 307, 401]. In immunologically normal recipients, the risk of VAPP decreases sharply (>tenfold) for subsequent doses. VAPP cases are sporadic and occur in both OPV recipients and their unimmunized household and non-household contacts. A small proportion (~7 %) of VAPP cases in the United States are classified as "community-acquired," indicating no known exposure to OPV. VAPP in OPV recipients and household contacts is most frequently associated with Sabin 3 (71 % of cases), followed by Sabin 2 (26 % of cases) [304]. VAPP in non-household contacts and in community-acquired cases is most frequently associated with Sabin 2 (50 % of cases), followed by Sabin 3 (33 % of cases) [304]. Sabin 1 is rarely associated with VAPP in immunocompetent individuals when administered in tOPV [303, 304, 398].

Poliovirus isolates from immunocompetent VAPP case-patients show only limited genetic divergence from the parental OPV strains, although the key substitutions conferring the attenuated phenotype have frequently reverted [393, 402]. Many isolates from VAPP cases, AFP cases with incidental isolation of vaccine-related virus (much more frequent than VAPP cases), and healthy OPV recipients are vaccine/vaccine recombinants [161, 279, 403, 404]. The biological and genetic properties of viruses isolated from healthy OPV recipients/contacts are often indistinguishable from viruses isolated from patients with VAPP [393, 405].

Persons with primary B-cell immunodeficiencies (Sect. 10.8.3) [406] should not be given OPV because they are at a much higher (~3,000-fold) risk for VAPP [353, 354]. However, some children have received OPV before their immunodeficiency was recognized. Immunodeficiency-associated VAPP (iVAPP) differs markedly from VAPP in immunocompetent individuals, as it is rarely associated with Sabin 3 (14 % of cases), and is more frequently associated with Sabin 2 (72 % of cases) and Sabin 1 (31 % of cases) (some patients were infected with more than one serotype) [354]. On the other hand, persons with T-cell immunodeficiencies, including those infected with HIV, do not appear to be at elevated risk for VAPP [304, 353, 354, 407].

Each case of VAPP is an independent event. It is estimated that 250–500 cases of VAPP occur worldwide, most in countries free of circulating wild poliovirus (Fig. 13.2a) [7]. VAPP is a direct clinical consequence of the genetic instability of the Sabin OPV strains, and the most effective means to prevent VAPP is to stop OPV use after cessation of wild poliovirus circulation [22, 307].

9.1.5 Sequential Use of IPV and OPV

The relative merits of IPV and OPV have been compared for many years [23, 408]. Key advantages of IPV are (1) it efficiently induces serum immunity protective against paralytic disease, (2) it is unaffected by interference by other enteroviruses or among vaccine components, (3) it can be used in combination with other injectable vaccines, and (4) it presents no risk for reversion to virulence. The main disadvantages to IPV are (1) it provides much reduced levels of intestinal immunity; (2) until recently, most preparations had been produced from virulent wild poliovirus strains; and (3) its costs of production and delivery are higher than OPV [24, 409]. Key advantages of OPV are (1) it is easily administered, (2) it confers both serum immunity and intestinal immunity (the latter important to limiting poliovirus transmission), (3) it can be easily used in mass campaigns in developing country settings, (4) it is suitable for use in outbreak control, and (5) it has low costs of production and delivery. Disadvantages to OPV include (1) it presents a continuous, low-level risk of VAPP, (2) it may have low per-dose efficacy rates of seroconversion in some high-risk settings, (3) it is subject to interference by other enteroviruses and among OPV strains (especially by the type 2 strain), (4) it has the potential to establish chronic infections in persons with primary immunodeficiencies, and (5) it has the potential to spread person-to-person and cause poliomyelitis outbreaks when used at low rates of coverage [23, 94, 133, 408, 410]. A sequential IPV/OPV immunization schedule can combine the major advantages of each vaccine and mitigate some disadvantages. A sequential schedule can virtually eliminate VAPP in OPV recipients and reduce it in OPV contacts, because shedding of OPV-related viruses is reduced. This was observed in the United States when it shifted to a sequential IPV/OPV schedule between 1997 and 2000, as no cases of VAPP were observed in persons using the sequential IPV/OPV schedule (Fig. 13.7) [307]. Mucosal immunity induced by an IPV/OPV schedule is much better than with IPV alone [411]. However, global implementation of any schedule using IPV will require much higher rates of routine immunization coverage than currently exist (Sect. 11.2.1) [22, 412].

9.2 Impact of Immunization

As described in Sect. 5.6.2, the high-income countries of North America, Western Europe, and the southwest Pacific were quick to adopt widespread immunization with IPV, with dramatic impact. In the United States, for example, the incidence of paralytic polio fell from 13,850 in 1955 to 829 in 1961 [308]. Although transition to OPV in the United States was not complete until the mid-1960s, the last peak year (>100 cases) for polio was 1966 (Fig. 13.7), and all domestic reservoirs for poliovirus circulation apparently had been eliminated after 1970 [163, 413]. Similar highly favorable results were achieved in other developed countries [8, 9, 309, 414, 415]. IPV was not widely used in developing countries (and some developed countries, such as Japan [309]), partly because of cost and also because of the challenges of administering an injectable vaccine to large populations. Thus, the possibility of widespread immunization against polio had to await the availability of OPV, where its key advantages were decisive. The synchronous induction of intestinal immunity through mass OPV campaigns efficiently blocks person-to-person transmission of wild poliovirus, thereby protecting both individual vaccine recipients and the wider community. Despite its advantages, OPV coverage in most developing countries of Asia, Africa, and the Americas remained low, and by 1970 a widening divide separated high-income countries where wild poliovirus circulation had stopped from most low-income countries where wild poliovirus circulation continued unabated [8, 9]. By 1985, poliomyelitis had all but disappeared in developed countries, while poliomyelitis crippled a child on average every 90 s in developing countries (Figs. 13.2 and 13.3).

10 The Global Polio Eradication Initiative (GPEI)

10.1 Prelude to the GPEI

A compelling case for global poliomyelitis eradication has been advanced for decades [294] and receives continued support from the perspectives of social equity [17, 416], economic benefit [417], and technical feasibility [12, 418]. However, the launch of a vertical program to eradicate poliomyelitis was controversial in the early 1980s [30] and remained so nearly two decades later [419]. Several key factors described below set the stage for the 1988 WHA resolution establishing the GPEI [30].

10.1.1 Poliomyelitis Eradication in Cuba, 1962

Sabin advocated mass OPV campaigns as the most effective means of polio control [294]. Cuba adopted his approach in 1962 and within a year had stopped all wild poliovirus circulation [295, 310], becoming the first country to eradicate poliomyelitis. The experience in Cuba conclusively demonstrated that mass campaigns were effective in a tropical developing country setting.

10.1.2 Smallpox Eradication, 1977

Smallpox was the first infectious disease to be eradicated. Global smallpox eradication was launched in 1966 following a WHA resolution to eradicate the disease by 1976. The last natural case of smallpox was reported in Somalia in 1977,

and global eradication was certified in 1980 [284]. In addition to the complete disappearance of a universally feared viral disease, the successful smallpox program accrued many other public health benefits. The program forged strong international cooperation in public health, trained a generation of international public health professionals who would lead the way to polio eradication, and set an irreversible precedent that infectious diseases could be controlled on a global scale [30]. In light of the smallpox eradication initiative, the Expanded Program on Immunization (EPI) was established by the WHO in 1974 to make immunization against six diseases (diphtheria, pertussis, tetanus, poliomyelitis, measles, and tuberculosis) available to every child in the world by 1990 [420]. The core EPI strategy was high routine immunization coverage, which was to prove insufficient to control poliomyelitis in some developing country settings.

10.1.3 National Immunization Days (NIDs) in Brazil, 1980

Brazil began routine immunization with OPV in the early 1960s [296]. The impact of immunization was initially unclear because poliomyelitis was not a notifiable disease until 1968. Although mass campaigns in 1971–1973 reduced poliomyelitis cases, they were replaced by routine immunization in 1974. Cases sharply increased until 1980, when Brazil reestablished mass campaigns in the form of biannual NIDs, targeting all children <5 years of age (regardless of prior immunizations) for immunization with OPV in a single day. Poliovirus circulation dropped sharply in Brazil after implementation of NIDs and set Brazil on the path to polio eradication [18, 296].

10.1.4 The PAHO Regional Polio Eradication Initiative, 1985

The dramatic success of mass campaigns in Cuba, Brazil, and Mexico led PAHO to resolve in 1985 to eradicate polio from the Americas by 1990 [14]. PAHO modeled their regional initiative on Brazil's successful strategy of NIDs and used poliomyelitis eradication as the vanguard of efforts to revitalize childhood immunization in the Americas. The PAHO approach of coordinated NIDs to supplement improved rates of routine OPV coverage eradicated indigenous wild polioviruses within 6 years [18]. PAHO set the standard for global efforts and built an infrastructure for the subsequent elimination of indigenous measles and rubella viruses from the Americas [421–423].

10.1.5 Rotary PolioPlus, 1985

Rotary International launched their global PolioPlus campaign in 1985, with the goal of helping eradicate poliomyelitis worldwide by 2005, the centennial of Rotary's founding. In their initial drives for support of PolioPlus, Rotarians more than doubled their target goal of $120 million for OPV and have raised more than $1.2 billion since 1985. Rotarians have been active in all phases of polio eradication, including active participation in mass campaigns, training of health workers, communications, regular engagement of national leaders, infrastructure development, support for surveillance and the GPLN, and support for research (http://www.endpolio.org/) [17, 30, 424].

10.2 The 1988 WHA Declaration to Eradicate Polio Worldwide

In view of the rapid progress attained in the Americas, the WHA resolved in 1988 to eradicate polio worldwide by the year 2000 [15], launching the WHO GPEI. At the time of the World Health Assembly resolution, wild polioviruses were circulating unabated in much of the developing world (Figs. 13.2 and 13.3). The Western Pacific Region set a goal of eradication of indigenous wild polioviruses by 1995, a goal reached in 1997 and certified in 2000 [425]. China, after years of outbreaks [426], adopted the strategy of NIDs and Subnational Immunization Days (SNIDs) which, when coupled with strengthened routine immunization, stopped wild poliovirus transmission by early 1994 [297, 427]. Other developing countries soon followed.

10.3 Biological Principles of Poliovirus Eradication

The key biological requirements for poliovirus eradication are the following: (1) the absence of a persistent carrier state, (2) virus is spread by person-to-person transmission, (3) immunization interrupts virus transmission, (4) the absence of any nonhuman reservoir hosts for the virus, and (5) finite virus survival time in the environment [230]. An additional important nonbiological requirement for any disease eradication effort is political will, arising from the perceived benefits of eradication, and expressed internationally through resolutions passed by the WHA. Essential to the success of the GPEI has been the strong alliance among the four "spearheading" partners, WHO (http://www.polioeradication.org/), UNICEF (http://www.unicef.org/health/index.html), Rotary International (http://www.endpolio.org/), and the United States Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/polio/), as well as strong support from national governments and new partners, including the Gates Foundation (http://www.gatesfoundation.org/What-We-Do/Global-Development/Polio) and the UN Foundation (http://www.unfoundation.org/news-and-media/press-releases/2013/fight-against-polio.html).

10.4 Basic Strategy for Global Polio Eradication

The four pillars of the GPEI strategy are (1) high routine immunization coverage of infants with OPV, (2) supplementary OPV immunization activities in the form of NIDs and

SNIDs, (3) targeted door-to-door "mop-up" OPV immunization in areas of focal transmission, and (4) sensitive surveillance for poliovirus [102]. Very high rates of routine OPV immunization are required to block poliovirus circulation in areas where the risk factors converge, conditions under which routine OPV coverage rates exceeding 90 % may be insufficient to block poliovirus circulation [7, 180, 296, 428]. Such rates are currently unattainable through routine immunization in the least developed countries. Supplementary immunization is the mainstay of polio eradication in developing countries and has been instrumental in raising population immunity rates above the thresholds required to block poliovirus transmission [429]. Supplementary immunization strategies are driven by poliovirus surveillance, which is used to guide the intensified SNIDs and mop-up campaigns to the reservoir communities where the chains of poliovirus transmission continue to survive and propagate.

10.4.1 AFP and Poliovirus Surveillance

Surveillance for circulating polioviruses has two arms: (1) AFP case investigations and (2) virologic studies of polioviruses obtained from clinical specimens or the environment. Even though most wild poliovirus infections are inapparent, over time, all effectively performing AFP surveillance systems can detect endemic poliovirus circulation. In suspected high-risk areas lacking effective AFP surveillance, supplementary surveillance activities, such as sampling community contacts of AFP cases, stool surveys of healthy children, or environmental sampling, have been implemented to increase sensitivity for detecting wild polioviruses [104, 106, 107, 111, 113, 116, 315].

10.5 Increasing Momentum Toward Eradication, 1988–2001

The global incidence of poliomyelitis was on a virtually unbroken downward trend between 1987 and 2001 (Figs. 13.2 and 13.3). Three WHO regions reported their last indigenous cases before 2000: the Americas (last indigenous case: Peru, 1991) [430], the Western Pacific Region (last indigenous case: Cambodia, 1997) [425], and the European Region (last indigenous case: Turkey, 1998) (Fig. 13.3) [431]. Wild poliovirus type 2 was last detected in October 1999 in Uttar Pradesh, India [13]. By 2001, the tide of global polio eradication, although delayed in some places, seemed unstoppable [432].

10.5.1 Polio Eradication in Conflict Countries

The sense of momentum was reinforced by control of poliomyelitis in countries with armed conflict such as Colombia, Peru, the Philippines, Sri Lanka, Cambodia, Angola, the Democratic Republic of the Congo, and Somalia [433, 434]. In many countries, conflict was suspended during "Days of Tranquility" allowing children to be immunized during NIDs and SNIDs. For example, Colombia and Peru were reservoirs for all three wild poliovirus serotypes in the early 1980s. Active cross-border migration of high-risk populations among the northern Andean countries facilitated wide dissemination of wild poliovirus [164, 170] and required close synchronization of NIDs among the affected countries [18]. A guerilla movement in the interior of Peru targeting health clinics and health personnel required active engagement by public health leaders to ensure access to unimmunized children. The final chains of wild poliovirus transmission in the Americas were broken in the interior of Peru in 1991 [18]. In Sri Lanka, major military campaigns in a devastating civil war were suspended during the NIDs, and wild poliovirus circulation stopped after 1993. In the 1990s, Vietnam and Cambodia were still recovering from decades of conflict. Detailed aerial mapping of the migratory at-risk populations in the water courses linking Cambodia and Vietnam (with active cross-border poliovirus transmission) set the stage for house-to-house and boat-to-boat immunization campaigns that stopped wild poliovirus transmission in 1997 [290, 435]. The Democratic Republic of the Congo and Angola have experienced shattering civil conflicts over the past two decades. Nonetheless, both countries eradicated their indigenous wild polioviruses in 2000 and 2002, respectively [11]. Both countries appear to have eradicated polio twice, the second time was a widely disseminated wild type 1 poliovirus imported from India around 2002 [177]. Similarly, Somalia eradicated the indigenous wild polioviruses in 2002 [436], and again eradicated wild poliovirus type 1 imported from Nigeria in 2005 [177, 437].

10.5.2 Polio Eradication in Very High-Risk Settings

Successful control of poliomyelitis in settings of intrinsically high biological risk demonstrated the feasibility of global polio eradication. However, conditions varied widely, and creative local solutions repeatedly had to be found and implemented [434]. The critical test case for the PAHO polio eradication initiative was Brazil, the most populous country in Latin America. Control of wild poliovirus transmission was more difficult in the tropical northeast (with its lower levels of hygiene and sanitation), and migration from the endemic northeast to the rapidly growing megacities of Rio de Janeiro and São Paulo presented the continuous risk of reinfection with wild polioviruses. Nonetheless, steady pressure from NIDs, SNIDs, and mop-up campaigns stopped all wild poliovirus transmission by early 1989 [75]. Success in the Americas did not necessarily presage success elsewhere because the Americas were often seen as having several key advantages: (1) greater resources and infrastructure than many developing countries elsewhere, (2) a tradition of strong public health leadership in many countries, (3) geographic isolation protective against re-importation of wild polioviruses, and (4) a cultural unity conducive to a common approach.

China, comprising 22 % of the world population, presented challenges of scale not encountered in the Americas [426]. Climate varied widely from the temperate north to the tropical south, and levels of infrastructure and sanitation also varied widely in this rapidly developing country. Population size and density across wide areas favored efficient wild poliovirus transmission, and massive economic migration from the countryside into the increasingly prosperous cities presented additional challenges of reaching unimmunized children. However, China had already established good coverage through routine immunization with tOPV, and a series NIDs and SNIDs in 1992–1993 broke the remaining chains of indigenous wild poliovirus transmission by early 1994 [297, 427].

Other successes in high-risk settings in Asia and Africa followed. Indonesia and the Philippines have humid tropical climates and very high population densities in their most populous islands. A combination of strengthened routine immunization and NIDs at high rates of coverage stopped wild poliovirus types 1 and 3 transmission in both countries by 1995 [289, 290]. Bangladesh has one of the highest population densities in the world, and wild poliovirus was deeply entrenched in multiple endemic reservoirs with poor infrastructure. High-quality NIDs built upon a solid national program of routine immunization stopped wild poliovirus transmission in 2000 [425]. Neighboring India has more children <5 years of age than any other country. Despite the biological challenges of high population densities, tropical conditions, and poor infrastructure in many areas, the southern states stopped indigenous wild poliovirus transmission through a combination of high rates of routine immunization coverage and mass campaigns. Progress was much slower in the large and populous states of Uttar Pradesh and Bihar (with a combined monthly birth cohort >500,000), but all wild poliovirus transmission stopped in India in 2011 [12, 91]. Eradication was especially challenging in these two Indian states because the per-dose efficacy of OPV was low, as many children with poliomyelitis had received multiple OPV doses. Mass campaigns (NIDs, SNIDs, and large-scale mop-ups) overcame weaknesses in routine immunization [438]. The most populous parts of Afghanistan (northern provinces) [184], Pakistan (Punjab province) [184], and Nigeria (southern states) [185] stopped wild poliovirus circulation several years ago, only to be subject to reinfection primarily from domestic endemic reservoirs.

10.5.3 Genotypic Indicators of Progress

Progress in polio eradication has been accompanied by a steady reduction in the genetic diversity of circulating wild polioviruses. Of the 20 wild poliovirus type 1 genotypes, 5 wild poliovirus type 2 genotypes, and 17 wild poliovirus type 3 genotypes found in 1988 [439], only 4 (or fewer) wild poliovirus genotypes remain (two type 1 and two type 3) (Figs. 13.10 and 13.11). Diversity within the surviving genotypes continues to decline as genetic clusters steadily disappear (Sect. 10.7), and wild poliovirus type 3 may be very near eradication in both Asia and Africa.

10.6 Resurgence of Wild Polioviruses, 2002–2011

After years of steady decline, poliomyelitis cases increased sharply from 483 cases in 2001 to 1918 cases in 2002 (Fig. 13.2) [436]. This increase was the result of a large epidemic in India (1,600 cases), primarily associated with wild poliovirus type 1, centered in Uttar Pradesh (78 % of total cases) [409], and a rise in reported cases in Nigeria [440]. Global cases fell to 784 in 2003, as the epidemic in India was controlled by large-scale NIDs and SNIDs [441], but wild poliovirus type 1 was beginning to spread from northern Nigeria to neighboring countries [442]. The upsurge in Nigeria followed suspension of NIDs and SNIDs in several northern states in 2003 and 2004 in response to false rumors about OPV safety [443]. By 2006, wild poliovirus type 1 had spread from northern Nigeria to 18 countries, across a wide importation belt in Africa from Guinea in the west to Somalia in the Horn of Africa, and into Asia from Saudi Arabia and Yemen in the west to Indonesia at the southeastern rim, sparking large outbreaks (>100 cases) in Sudan, Somalia, Yemen, and Indonesia [10, 176]. Wild poliovirus type 1 introduced into Angola from Uttar Pradesh caused cases that were first reported in 2005 [176], and Nepal experienced repeated importations from northern India [176]. The majority of cases in 2005 were associated with imported virus, but most of the outbreaks outside of the core endemic reservoirs of Nigeria, India, Pakistan, and Afghanistan were largely controlled by the end of 2005 (Fig. 13.2d, e) [176], except for Kenya and Uganda where virus originating from Nigeria persisted until 2011 [10]. By 2006, most wild type 1 poliovirus cases occurred in the core reservoir countries, but virus of Nigerian origin continued to circulate in Niger, Ethiopia, Yemen, and Kenya [444], and virus of Indian origin spread to Bangladesh and from Angola to Namibia [80] and the Democratic Republic of Congo [444]. Instrumental in rolling back the wave of imported wild poliovirus type 1 was the widespread use in NIDs and SNIDs of monovalent type 1 OPV (mOPV1), which has a higher type-specific per-dose efficacy than tOPV because interference from the robust type 2 component of OPV is eliminated [196, 410]. However, exclusive use of mOPV1 in successive campaigns led to the development of growing immunity gaps to poliovirus types 2 and 3.

In India, as wild poliovirus type 1 cases declined 87 % from 646 in 2006 to 87 in 2007, wild poliovirus type 3 cases surged from 28 in 2006 to 787 in 2007 [445]. Widespread

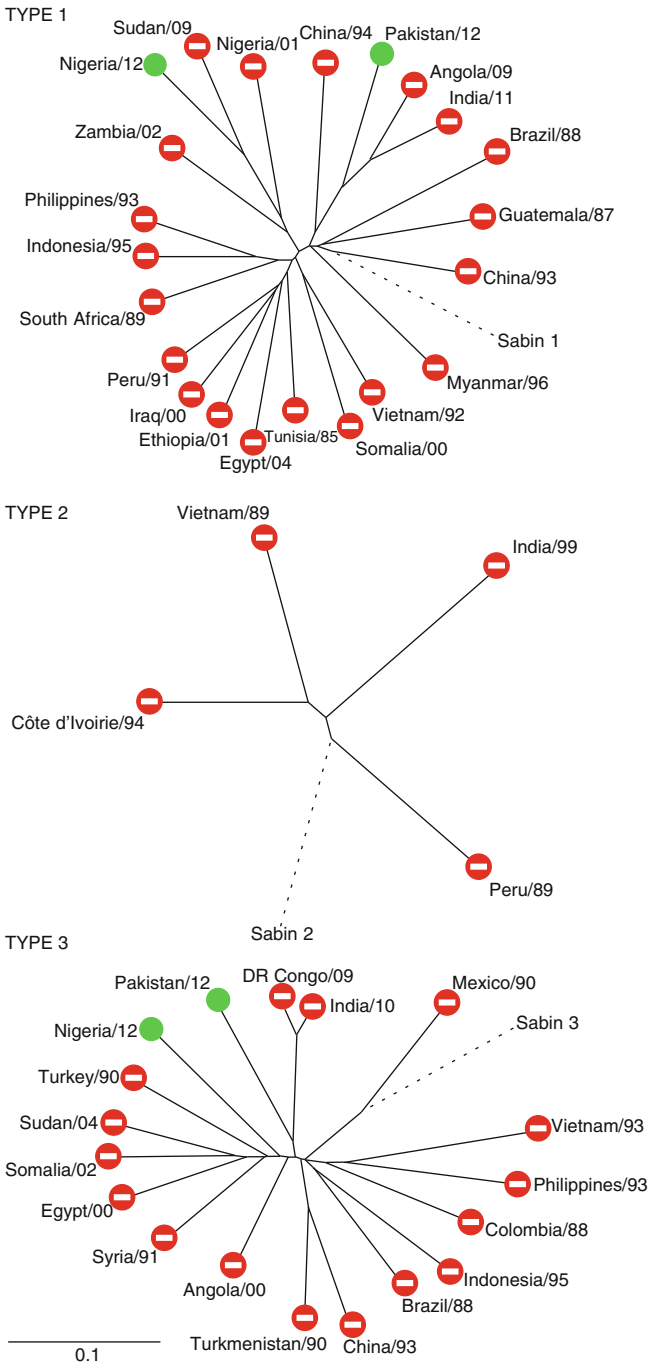

Fig. 13.10 Radial neighbor-joining trees of VP1 sequence relationships of representatives of wild poliovirus genotypes detected since the launch of polio eradication activities in the Americas in 1985. Genotypes believed to be extinct are represented by stop signs at the branch tips. Sequences representing the extinct genotypes usually are from the last known isolate of that genotype (Source: WHO Global Polio Laboratory Network. Modified from reference Chumakov and Kew [439])

circulation of wild poliovirus type 3 continued in Uttar Pradesh and Bihar through 2009 [438]. Wild poliovirus type 3 imported from Uttar Pradesh into Angola caused 24 cases in 2008 and spread to the Democratic Republic of Congo in 2008 and 2009 [10, 177]. During 2007–2011, wild poliovirus type 3 from Nigeria spread east to Chad, Cameroon, and the Central African Republic and north and west to Niger, Mali, Côte d'Ivoire, and Guinea [10, 177].

A second wave of wild poliovirus type 1 from Nigeria began in 2008 and spread to 14 countries, reinfecting many of the same countries that experienced the 2003–2006 outbreaks [177]. Wild poliovirus type 1 imported into Chad from northeastern Nigeria in 2010 caused a large outbreak that continued into 2012, resulting in reestablished transmission in Chad and further export of wild poliovirus type 1 into the Central African Republic [10]. However, effective NIDs and SNIDs in the countries of West Africa again rolled back the imported Nigerian virus [10, 16].

While wild poliovirus type 1 was disappearing from India, virus originating from Uttar Pradesh was associated in 2010 with a large outbreak (458 cases) in Tajikistan [178], with further spread to Turkmenistan, Kazakhstan, the Russian Federation, and probably Uzbekistan [181]. During 2010–2011, wild poliovirus type 1, originally imported several years earlier into Angola from Uttar Pradesh, sparked a large outbreak (~441 cases) in the Republic of the Congo with a very high CFR (Sect. 3.1) [81]. These outbreaks were controlled by aggressive mass immunization campaigns, and all wild polioviruses of Indian origin appear to have been eradicated worldwide.

Wild poliovirus type 1 circulation continued at much reduced levels in Pakistan and Afghanistan [184]. However, in 2011 wild poliovirus type 1 from southern Pakistan was imported into Xinjiang in western China, causing an outbreak of 21 cases [10, 82]. Sensitive surveillance in China detected the outbreak in its early stages, and a series of intensive mass immunization campaigns halted poliovirus circulation. Although China and the Western Pacific Region are again free of wild poliovirus circulation, the risk remains of re-importation from neighboring Pakistan even as poliomyelitis is in decline in that country.

10.7 Wild Poliovirus in Retreat, 2011–2012

A major milestone in global polio eradication was the eradication of indigenous wild poliovirus in India, once the world's most intense wild poliovirus reservoir [326, 327] and the source of repeated dissemination of wild poliovirus into countries in four continents [11, 170, 176, 177, 179, 315, 415, 446]. The last wild poliovirus type 3 in India was isolated in October 2010 and the last wild poliovirus type 1 was isolated in January 2011 (Figs. 13.10 and 13.11) [12, 91]. In neighboring Pakistan and Afghanistan, which constitute a common epidemiologic block, the diversity of wild poliovirus types 1 and 3 has been in steady decline, despite fluctuations in case counts (Fig. 13.12) [12].

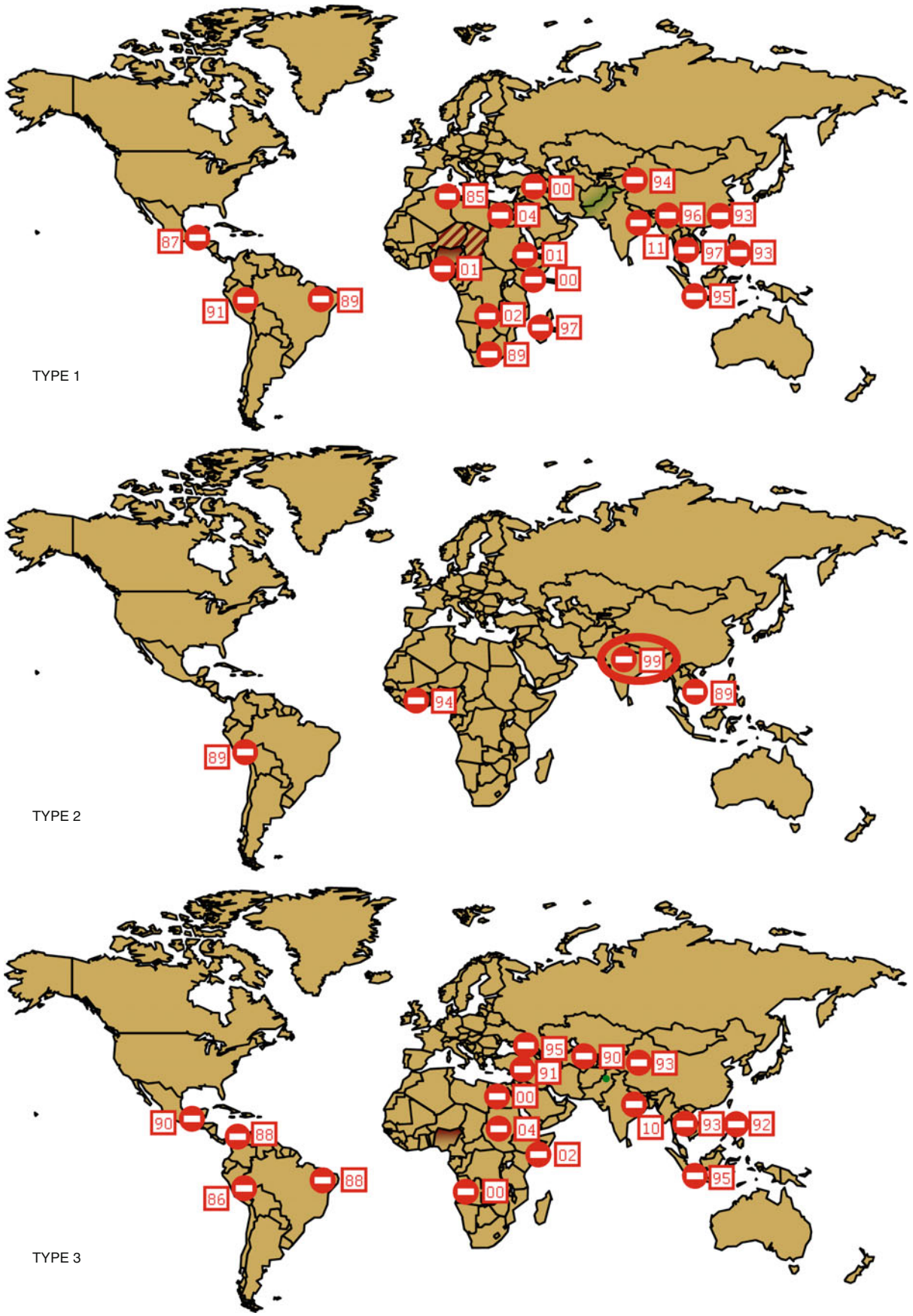

Fig. 13.11 Progressive eradication of wild poliovirus genotypes, 1986 to 2012. Stop signs indicate the year and location where the last isolate was obtained for each extinct genotype. Surviving type 1 and type 3 poliovirus genotypes are color-coded; hatch and shading patterns indicate, respectively, countries with reestablished transmission and sporadic importation of West Africa genotype viruses (Source: WHO Global Polio Laboratory Network. Modified from reference Kew and Pallansch [11])

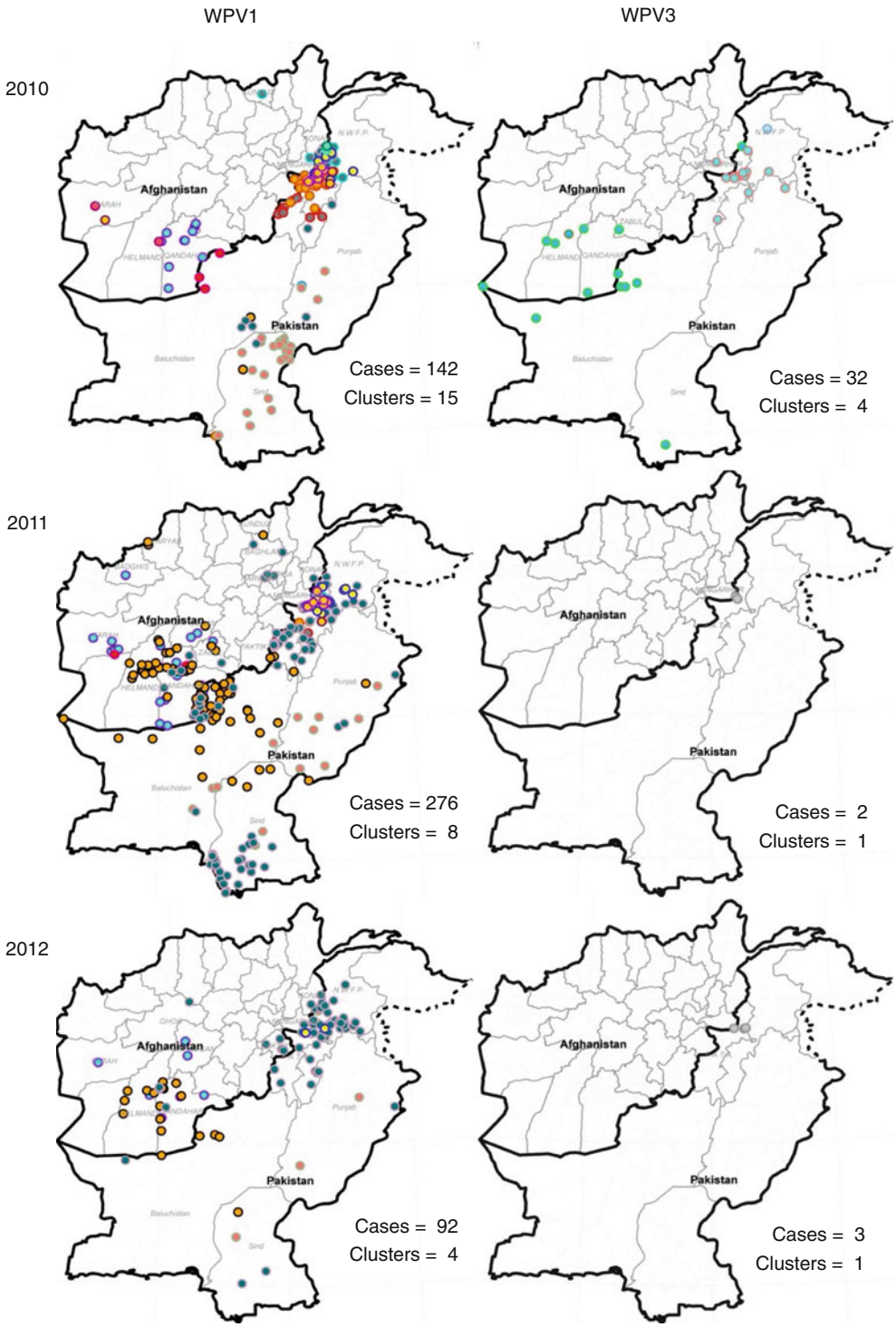

Fig. 13.12 Spot maps of genetic clusters based upon VP1 sequence relationships among isolates of wild poliovirus type 1 (*WPV1* left column) and wild poliovirus type 3 (*WPV3* right column), endemic to Pakistan and Afghanistan, 2010–2012. Isolates within a genetic cluster share ≥95 % VP1 nucleotide sequence identity, and the cluster count provides an indication of the genetic diversity of circulating wild polioviruses. Genetic clusters are color-coded to facilitate visualization of endemic reservoirs, transmission pathways (including cross-border transmission), and decline in genetic diversity. In 2011, poliovirus type 1 from southern Pakistan was imported into Xinjiang, China, and caused an outbreak of 21 cases [82]. (Map prepared by Elizabeth Henderson [CDC] based on sequence data produced by the Regional Polio Reference Laboratory at the National Institute of Health, Islamabad, Pakistan, and by CDC). As of April 2014, the last wild poliovirus type 3 isolate in Asia was from a child in the Federally Administered Tribal Area of Pakistan who had onset of AFP in April 2012

One measure of genetic diversity is the number of genetic "clusters," defined as isolates sharing ≥95 % VP1 nucleotide sequence identity (isolates within a genotype share ≥85 % VP1 nucleotide sequence identity [163]). Distinct clusters originate from different local endemic reservoirs, and in areas of sensitive surveillance, the disappearance of clusters within a genotype is an indication that source reservoirs within geographic area are being cleared of wild polioviruses. Just as the stepwise disappearance of genotypes is a measure of global progress toward eradication (Figs. 13.10 and 13.11), the stepwise disappearance of clusters is a measure of regional and local progress (Figs. 13.12, 13.13, and 13.14). Poliovirus genetic clusters are color-coded and mapped to facilitate visualization of patterns of circulation and to monitor progress toward eradication.

The number of wild poliovirus type 1 clusters in Pakistan and Afghanistan fell from 15 in 2010 to 4 in 2012 (Fig. 13.12).

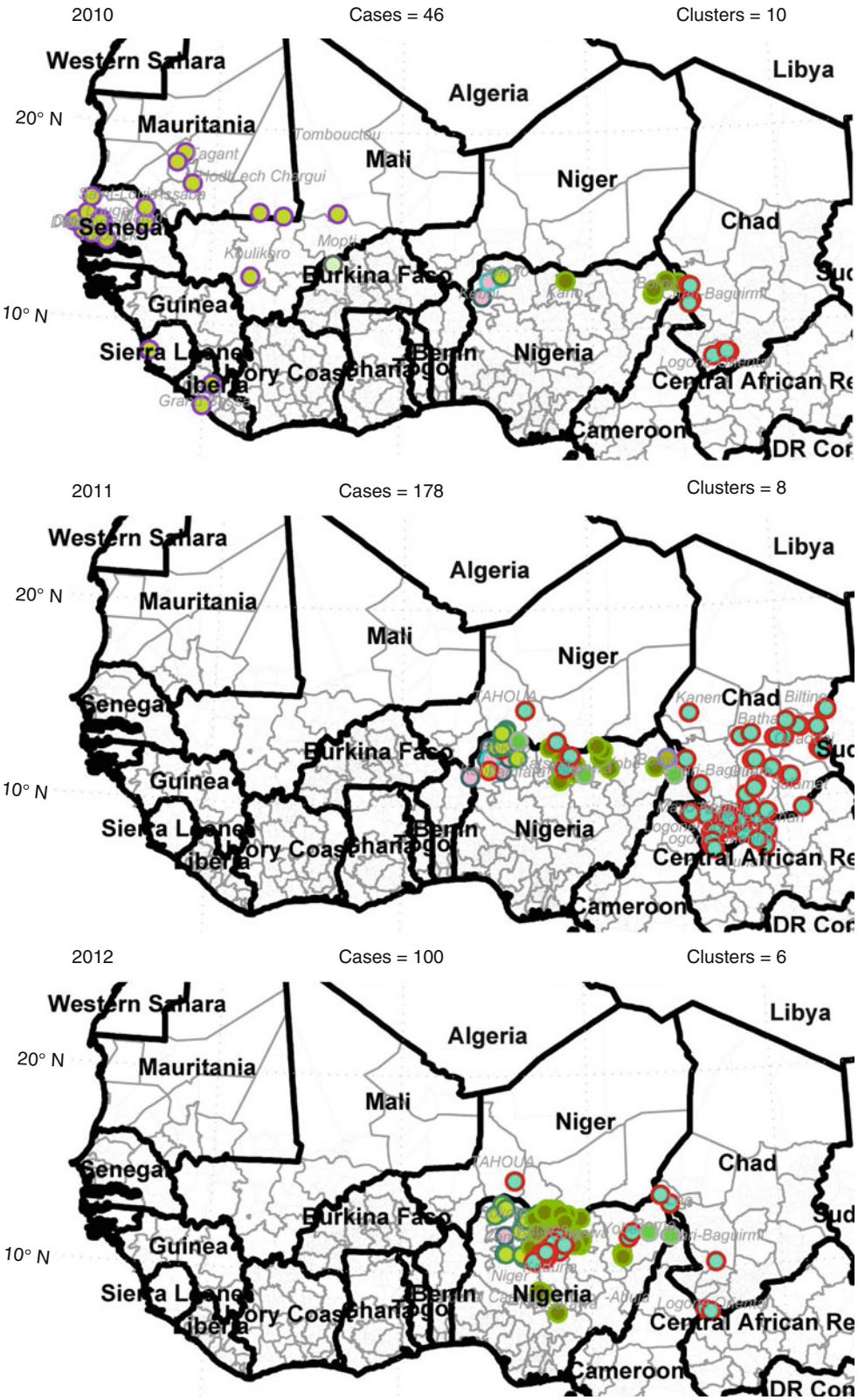

Fig. 13.13 Spot maps showing spread and retreat of genetic clusters of wild poliovirus type 1 from Nigerian reservoirs to neighboring countries in West and Central Africa, 2010–2012 (Map prepared by Elizabeth Henderson (CDC) based on sequence data produced by the Regional Polio Reference Laboratory at the National Institute for Communicable Diseases, Johannesburg, South Africa, and CDC). As of April 2014, the last wild poliovirus type 3 isolate in Asia was from a child in the Federally Administered Tribal Area of Pakistan who had onset of AFP in April 2012

Wild poliovirus type 3 clusters decreased from 4 in 2010 to 1 in 2012 (Fig. 13.12), and the last case associated with wild poliovirus type 3 in Pakistan (and in Asia; Fig. 13.2c) was reported in April 2012 [12]. By the end of 2012, reservoirs within Afghanistan appear to have been cleared of wild polioviruses.

Although progress in Nigeria has been less dramatic than in Pakistan and Afghanistan, wild poliovirus type 1 clusters

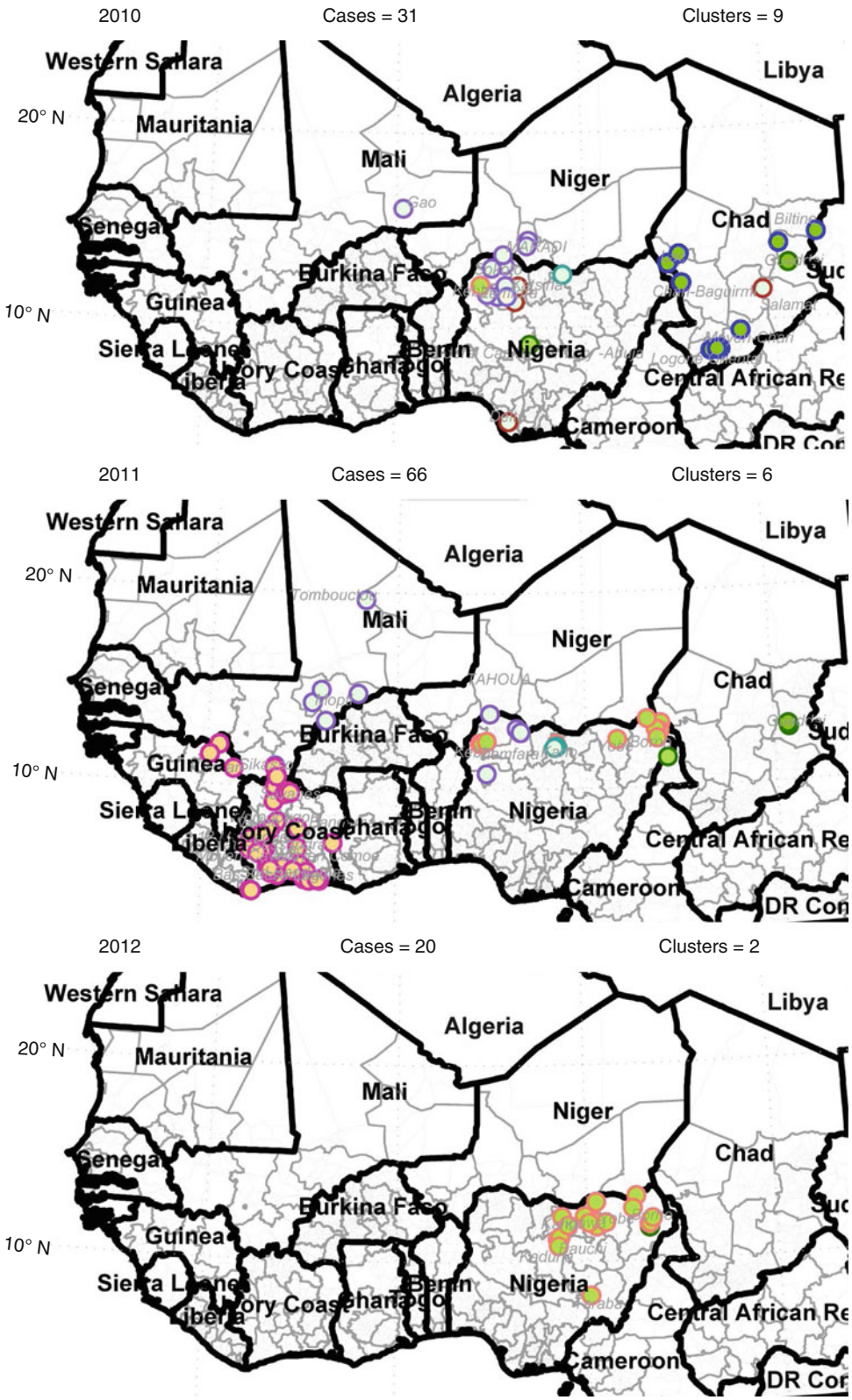

Fig. 13.14 Spot maps showing spread and retreat of genetic clusters of wild poliovirus type 3 from Nigerian reservoirs to neighboring countries in West and Central Africa, 2010–2012 (Map prepared by Elizabeth Henderson based on sequence data produced by the Regional Polio Reference Laboratory at the National Institute for Communicable Diseases, Johannesburg, South Africa, and CDC). As of April 2014, the last wild poliovirus type 3 isolate in the world was from a child in northeastern Nigeria who had onset of AFP in November 2012

Table 13.2 AFP and wild poliovirus cases by WHO region and globally, 2001–2012

	AFP cases (virologically confirmed wild poliovirus cases) reported[a]						
	WHO region[b]						
Year	AFR	AMR	EMR	EUR	SEAR	WPR	Global
2001	8,542 (69)	2,207 (0)	3,865 (143)	1,764 (3)[c]	10,612 (268)	6,529 (0)	33,519 (483)
2002	8,587 (208)	2,168 (0)	4,625 (110)	1,717 (0)	12,900 (1,600)	6,835 (0)	36,832 (1,918)
2003	8,181 (446)	2,229 (0)	5,290 (113)	1,529 (0)	11,289 (225)	6,397 (0)	34,915 (784)
2004	9,719 (934)	2,309 (0)	6,176 (187)	1,516 (0)	16,270 (134)	6,521 (0)	42,511 (1,255)
2005	11,683 (879)	2,213 (0)	8,849 (727)	1,479 (0)	31,530 (373)	6,680 (0)	62,434 (1,979)
2006	12,472 (1,189)	2,151 (0)	8,739 (107)	1,481 (0)	36,665 (701)	7,011 (0)	68,519 (1,997)
2007	12,080 (367)	2,111 (0)	9,394 (58)	1,449 (0)	46,124 (894)	6,237 (0)	77,395 (1,315)
2008	14,256 (912)	2,063 (0)	10,799 (174)	1,360 (0)	50,509 (565)	6,417 (0)	85,404 (1,651)
2009	15,127 (691)	1,873 (0)	10,611 (176)	1,363 (0)	54,962 (741)	6,291 (0)	90,227 (1,604)
2010	16,500 (657)	2,006 (0)	11,338 (169)	2,087 (478)[d]	60,456 (48)	6,401 (0)	98,788 (1,352)
2011	16,636 (350)	1,704 (0)	11,742 (278)	1,544 (0)	65,331 (1)	7,303 (21)[e]	104,260 (650)
2012	18,110 (128)	2,437 (0)	11,100 (95)	1,529 (0)	66,176 (0)	7,585 (0)	106,937 (223)

Source: WHO (http://apps.who.int/immunization_monitoring/en/diseases/poliomyelitis/afpextract.cfm). Starting in 2001, all wild poliovirus case counts were based on virologic confirmation by the GPLN

[a]cVDPV outbreaks not included

[b]WHO regions: *AFR* Africa, *AMR* Americas, *EMR* Eastern Mediterranean, *EUR* Europe, *SEAR* South-East Asia, *WPR* Western Pacific

[c]Importations of wild poliovirus type 1 into Bulgaria (from Pakistan) and Georgia (from India) [11]

[d]Outbreaks in Tajikistan, Turkmenistan, Kazakhstan, and Russian Federation following importation of wild poliovirus type 1 from India [178]

[e]Outbreak in Xinjiang, China, following importation of wild poliovirus type 1 from Pakistan [82]

fell from 10 in 2010 to 6 in 2012 (Fig. 13.13) and wild poliovirus type 3 clusters fell from 9 in 2010 to 2 in 2012 (Fig. 13.14). In addition, the surge of wild polioviruses from Nigeria was again rolled back between 2010 and 2012 (Figs. 13.13 and 13.14). In 2012, only Chad (5 cases in 2012 compared with 132 cases in 2011) and Niger (1 case in 2012 compared with 5 cases in 2011) had residual circulation of the wild poliovirus type 1 imported from Nigeria [12]. Although wild poliovirus transmission in Africa is localized to northern Nigeria, the risk for resurgence remains until immunization activities in key northern states are intensified [12].

Important factors in improving OPV coverage have been intensified community engagement, targeting mobile and migrant populations for immunization, and high-resolution mapping of underserved communities [12, 22]. An additional critical factor for eradication of wild polioviruses has been the widespread use of monovalent OPV type 1 (mOPV1) and mOPV3 in NIDs, SNIDs, and mop-ups. However, in countries where both wild poliovirus types 1 and 3 were co-circulating, balancing the use of mOPV1, mOPV3, and tOPV in NIDs and SNIDs was difficult. Priority was usually given to the use of mOPV1 because of the higher risk of wild poliovirus type 1 spread compared with wild poliovirus type 3 [10, 176, 177, 180, 181] and the greater risk of large outbreaks [181]. Routine OPV immunization exclusively uses tOPV, but coverage rates in the core reservoirs remain low [12], such that immunity gaps to types 2 and 3 could develop with near exclusive use of mOPV1 [447, 448]. The introduction of bivalent OPV (bOPV; types 1 and 3) [92, 449, 450] in late 2009 and early 2010 sharply reduced wild poliovirus type 3 circulation in areas where coverage rates in mass campaigns were high (Figs. 13.2c, 13.12, and 13.14) [12, 16, 82, 451]. However, a remaining challenge is to prevent widening immunity gaps to poliovirus type 2.

In 2012, more than two billion doses of OPV were administered to 448 million people, primarily to children <5 years of age in NIDs and SNIDs in 46 countries [12]. Only 223 cases of poliomyelitis associated with wild poliovirus infection were reported in 2012, an historic low, from a total of 106,937 reported cases of AFP, the most ever investigated (Table 13.2) [12]. The number of wild poliovirus cases in 2012 fell below the estimated number of worldwide VAPP cases (250–500) (Fig. 13.2a). For the first time, the risk of poliomyelitis from the use of OPV exceeded the annual case burden from wild poliovirus infection.

10.8 Emergence of Vaccine-Derived Polioviruses (VDPVs), 2000–2012

In principle, all clinical and environmental vaccine-related poliovirus isolates are VDPVs. However, as described in section "Categories of poliovirus isolates", for the purposes of poliovirus surveillance, vaccine-related isolates have been classified into two broad categories: (1) OPV-like isolates, which have limited divergence from their parental OPV strains and are ubiquitous wherever OPV is used, and (2) VDPV isolates, whose higher level of VP1 sequence divergence from their parental OPV strains (>1 % [types 1 and 3] or >0.6 % [type 2]) indicates prolonged replication (or

transmission) of the vaccine virus. VDPVs are further categorized as (1) circulating VDPVs (cVDPVs), (2) immunodeficiency-associated VDPVs (iVDPVs), and 3) ambiguous VDPVs (aVDPVs) [133].

Although the vast majority of vaccine-related isolates are OPV-like, VDPVs are of particular interest because of their implications for current and future strategies for global polio eradication [22, 94, 133, 428, 450]. VDPVs can cause paralytic polio in humans and have the potential for sustained circulation. The clinical signs and severity of paralysis associated with VDPV and wild poliovirus infections are indistinguishable. VDPVs resemble wild polioviruses phenotypically [133] and differ from the majority of vaccine-related poliovirus isolates by having genetic properties consistent with prolonged replication or transmission. Because poliovirus genomes evolve at a rate of approximately 1 % per year (Sect. 3.6.3), vaccine-related viruses that differ from the corresponding OPV strain by >1 % of VP1 nucleotide positions are estimated to have replicated for at least 1 year in one or more persons after administration of an OPV dose. This is substantially longer than the normal period of 4–6 weeks of vaccine virus replication in an OPV recipient [321]. The demarcation for type 2 VDPVs was lowered to 0.6 % to increase sensitivity for early detection of type 2 cVDPV outbreaks [133, 168, 452].

It seems likely that many OPV-like isolates have recovered the capacity for higher neurovirulence and possibly increased transmissibility. The small number of substitutions controlling neurovirulence (Sect. 9.1.3; Fig. 13.9) was found to have reverted among many OPV-like isolates, especially among isolates of types 2 and 3 [382, 388, 393]. Because the critical attenuating mutations in the 5′-UTRs of the Sabin strains also affect fitness for virus replication in the human intestine [393], it appears possible that revertants at these sites would have a higher fitness for person-to-person spread. However, spread is normally limited by high OPV coverage, and the VDPVs represent viruses whose potentials for prolonged replication or transmission have been clearly actualized, as demonstrated by their genetic prioperties.

The three categories of VDPVs differ in their public health importance. Circulating VDPVs pose the same public health threat as wild polioviruses because they have recovered the biological properties of wild polioviruses, have the potential to circulate for years in settings where poliovirus vaccination coverage to prevent that particular type is low, and require the same control measures. Immunodeficiency-associated VDPVs may be excreted by persons with certain primary immunodeficiencies for many (>10) years with no apparent paralytic signs [223, 453]. Persons infected with iVDPVs without paralysis are at risk of developing paralytic poliomyelitis [186, 223, 454] and may infect others with poliovirus, presenting the potential risk of outbreaks in areas with low poliovirus vaccine coverage [132]. Ambiguous VDPVs are heterogeneous: some represent the initial isolates from cVDPV outbreaks, whereas others, such as highly divergent aVDPVs detected in sewage, are probably iVDPVs from inapparent chronic infections.

10.8.1 Outbreaks of Circulating VDPVs (cVDPVs)

Among the two well-defined categories of VDPVs, cVDPVs are of the greatest current public health concern [22, 450, 455]. Since 2000, cVDPV outbreaks have occurred in 18 countries, with the large majority (86.6 %) of reported cases associated with type 2 (Fig. 13.15; Table 13.3). Type 1 cVDPVs were associated with outbreaks in Hispaniola in 2000–2001 and Indonesia in 2005 [166, 183]. In contrast, type 3 cVDPV outbreaks are rare, accounting for only 1.7 % of known cVDPV cases, an unexpected finding because the type 3 OPV strain is a major contributor to VAPP in OPV recipients [94]. Because the case/infection ratio for poliovirus type 2 infections is low (Sect. 3.4), the number of type 2 cVDPV infections worldwide since 2000 is estimated at nearly one million [168, 461].

During 2005–2012, a polio outbreak of 411 cases associated with cVDPV2 was reported from 11 northern and 3 north central states of Nigeria (Fig. 13.16; Table 13.3) [447, 448, 455]. The outbreak peaked at 153 cases in 2009, but 27 cases were detected in 2010, 35 cases in 2011, and 7 cases in 2012. Genetic analysis resolved the outbreak into >20 independent type 2 VDPV emergences that occurred during 2004–2012, at least 7 of which established circulating lineages [168, 448]. The outbreak occurred in states where routine immunization coverage with tOPV is low and NIDs and SNIDs using tOPV were infrequent [447, 448]. Spread of the type 2 cVDPV from northern Nigeria has been very limited, with only six cases in Niger from multiple importations and one case in Chad from an importation in 2010 (Fig. 13.16; Table 13.3). The limited spread may indicate that surrounding areas have higher levels of population immunity to poliovirus type 2 (because of the high immunogenicity of Sabin 2 and its higher tendency to infect OPV contacts) and that poliovirus type 2 may have an intrinsically lower capacity to spread than types 1 and 3 (Figs. 13.13 and 13.14).

Most of the other recent outbreaks have also been associated with type 2 cVDPVs (Table 13.3), several of which (e.g., Afghanistan, the Democratic Republic of Congo, India, Pakistan, and Yemen) were associated with multiple independent emergences [133]. Other type 2 cVDPVs have emerged successively in the same geographic area [459]. In several settings, this reflects the continued weakness in routine immunization with tOPV and the extensive use of mOPV1 and bOPV in mass campaigns. However, it also reflects the greater tendency of the Sabin 2 OPV strain to revert and spread to contacts [94]. Three separate type 3 cVDPV emergences were detected in Ethiopia in 2009–2010, and a single type 1 cVDPV emergence was detected in Mozambique in 2011 (Table 13.3).

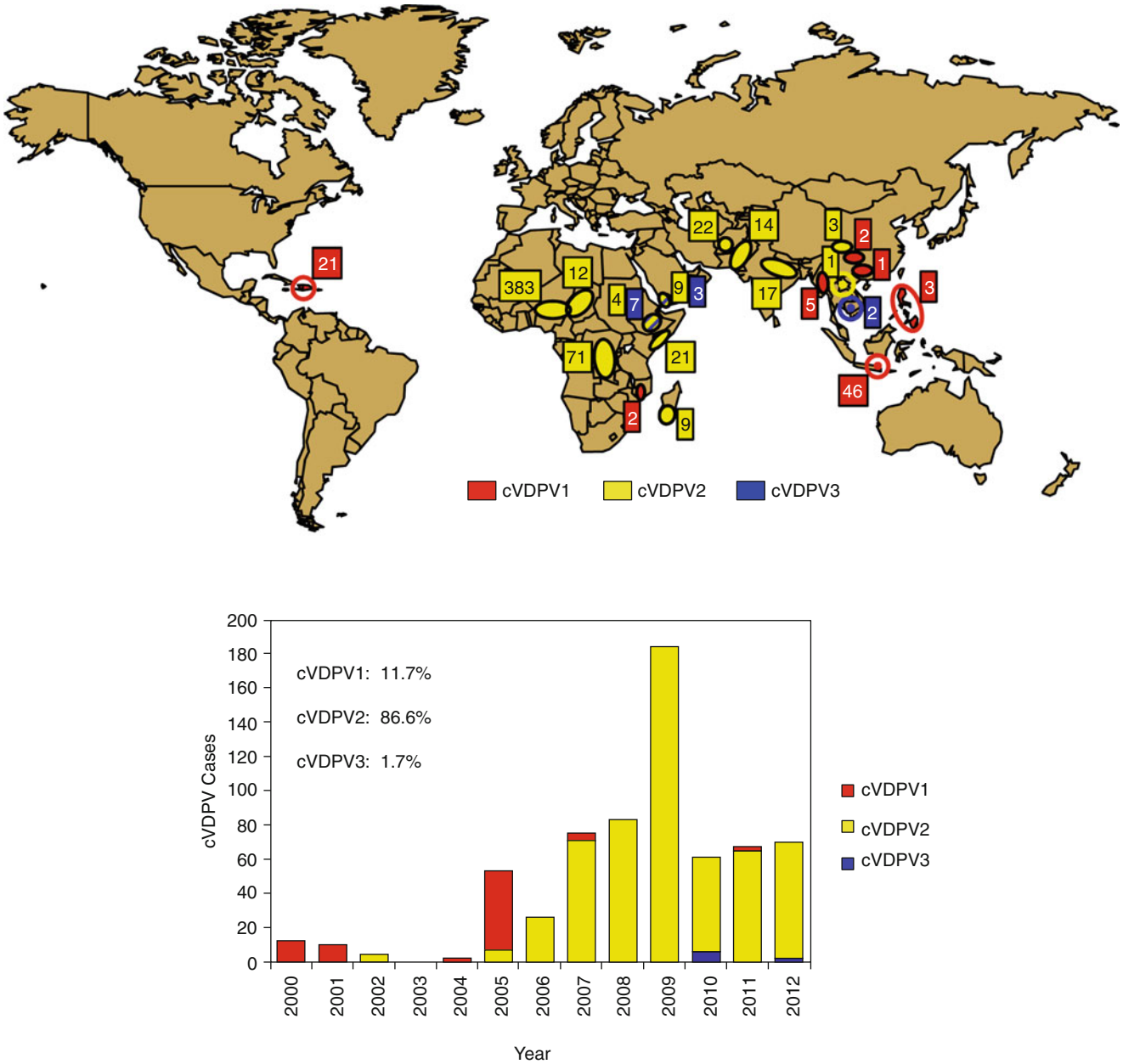

Fig. 13.15 Circulating vaccine-derived poliovirus (cVDPV) outbreaks, 2000–2012. Map: Location of cVDPV outbreaks, color-coded by serotype (*red* cVDPV type 1 [cVDPV1], *yellow* cVDPV2, *blue* cVDPV3). The independent emergences of cVDPV2 and cVDPV3 in Ethiopia and Yemen are indicated by *yellow* and *blue* diagonal patterns. Apart from the 2000–2001 cVDPV1 outbreak on the island of Hispaniola (Haiti and the Dominican Republic) and the limited spread of the cVDPV2 from Nigeria to Niger and Chad, all other outbreaks are independent events. Some countries had successive (e.g., Madagascar) or concurrent (e.g., Nigeria and D. R. Congo) cVDPV2 outbreaks. *Bar chart*: cases associated with cVDPV outbreaks, 2000–2012, color-coded by serotype (Modified from reference Kew [10])

In 2013, cVDPV2 from the outbreak in Chad continued with 4 new cases and spread to Cameroon (4 cases), Niger (1 case), and Nigeria (4 cases). The cVDPV2 outbreak in Pakistan continued with 47 new cases and spread to Afghanistan (4 cases in 2012–2013).

Key risk factors for cVDPV emergence and spread are (1) development of immunity gaps arising from low OPV coverage, (2) prior elimination of the corresponding wild poliovirus serotype, (3) emphasis on use mOPV and bOPV in NIDs and SNIDs [133, 447], and (4) insensitive AFP surveillance. Many of these factors exist in areas of insecurity and where rates of routine tOPV coverage remain low, such as in parts of northern Nigeria, Somalia, and Yemen. In this context, type 2 VDPVs present the greatest threat for emergence [133], and routine immunization should be strengthened and, for the immediate future, whenever possible regular

Table 13.3 Circulating vaccine-derived poliovirus (cVDPV) outbreaks, 2000–2012

Country	Sero-type	Years	Reported cases	% VP1 divergence	Estimated independent emergences	References
Haiti/Dominican Republic	1	2000–2001	21	1.9–2.6	1	[166]
Madagascar	2	2001–2002	4	2.5–3.0	1	[187]
Philippines	1	2001	3	3.1–3.5	1	[189]
China	1	2004	2	1.0–1.2	1	[456]
Laos	2	2004–2005	1 (2 contacts)	1.1–1.2	1	[94]
Cambodia	3	2005–2006	2	1.9–2.4	1	[457]
D R Congo	2	2005–2009	7	1.0	>10	[458]
Indonesia	1	2005	46	1.1–3.0	1	[183]
Madagascar	2	2005	5	1.1–2.7	1	[459]
Nigeria[a]	2	2005–2012	385	0.7–7.2	>20	[168, 447, 448]
China	1	2006	1 (6 contacts)	1.4–2.2	1	[190]
Myanmar	1	2006–2007	5	1.5–2.2	1	[457]
D R Congo	2	2008–2012	64	0.7–3.5	>10	[133, 455]
Ethiopia	2	2008–2009	4	1.3–3.1	2	[458]
Somalia[b]	2	2008–	18	0.7–4.0	4	[133, 455]
Ethiopia	3	2009–2010	7	1.1–1.2	1	[455]
India	2	2009–2010	17	1.3–1.6	5	[455]
Afghanistan	2	2010–	22	0.9–5.5	2	[455]
Madagascar	2	2011	0 (2 contacts)	3.3–3.7	1	[133]
Mozambique	1	2011	2	3.0–4.3	1	[133, 455]
China	2	2011–2012	3	0.7–1.8	1	[133]
Yemen	2	2011	9	0.6–1.6	4	[133]
Yemen	3	2011–	3	2.0–3.0	1	[460]
Chad	2	2012–	12	0.7–2.1	2	[460]
Pakistan[c]	2	2012–	14	0.7–2.9	2	[460]

[a]cVDPV2 from Nigeria was exported to Niger (2006, 2009–2012; 7 total cases) and Chad (2010 and 2012; 2 cases)
[b]cVDPV2 from Somalia was exported to Kenya (2012; 3 cases)
[c]cVDPV2 from Pakistan was exported to Afghanistan (2012; 3 cases)

immunization campaigns using tOPV should be conducted to close any immunity gaps.

All of the cVDPVs shown in Table 13.3 except those from China have vaccine/non-vaccine recombinant genomes, which are very rare among OPV-like and iVDPV isolates [94]. Recombination with other species-C enteroviruses frequently occurs during wild virus circulation and may be interpreted as an indication of person-to-person transmission. Whether recombination facilitates cVDPV emergence is unclear, because type 1 cVDPVs from China had nonrecombinant genomes [456] and recombination continues after emergence, similar to what is observed with wild polioviruses [94].

10.8.2 Immunodeficiency-Associated VDPVs (iVDPVs)

It has long been recognized that patients with primary B-cell immunodeficiencies (defects in antibody production) could become chronically infected when exposed to OPV [353]. Unambiguous demonstration that vaccine-related poliovirus isolates from immunodeficient patients had unusual sequence properties awaited the application of molecular tools, such as oligonucleotide fingerprinting [186, 462] and genomic sequencing [165, 186, 454], to poliovirus diagnostics. The extent of sequence divergence is correlated with the duration of the prolonged infection [165, 173, 186, 223, 454]. Not all isolates from immunodeficient patients are classified as iVDPVs. Some isolates are from specimens taken early in the prolonged infection and no subsequent specimens were taken. In other situations, either the prolonged infections had resolved spontaneously or the patient died from complications of the immunodeficiency (including fatal poliomyelitis) [354]. Prolonged iVDPV infections are independent events, and the isolates obtained from such infections trace separate pathways of divergence from the original OPV strains.

Since the introduction of OPV in 1961, >70 persons with primary immunodeficiencies have been found worldwide to be excreting iVDPVs; the majority of these immunodeficiencies were detected only after onset of AFP (Table 13.4). The extent of sequence divergence is correlated with the duration of the prolonged infection [165, 173, 186, 223, 454]. Four of the iVDPV isolates are highly divergent (>10 % VP1 sequence divergence from the parental OPV strain),

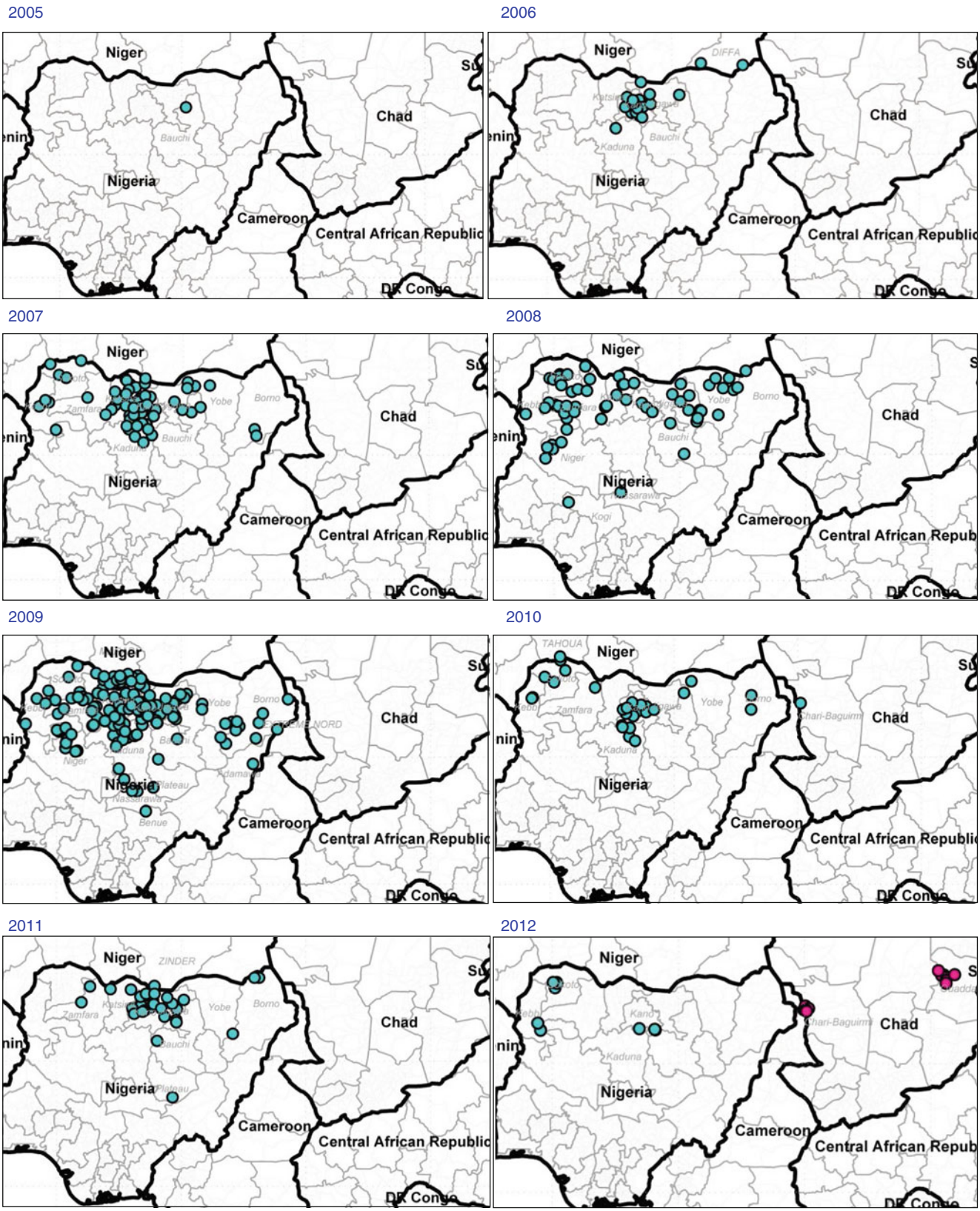

Fig. 13.16 Emergence and spread of type 2 cVDPV in Nigeria, 2005–2012. At least 20 independent emergences of type 2 cVDPVs (*green dots*) occurred in Nigeria between 2005 and 2012. Independent type 2 cVDPV emergences that occurred in Chad in 2012 are represented by *purple dots* (Map prepared by Elizabeth Henderson based on sequence data produced by CDC)

Table 13.4 Documented prolonged iVDPV excretors, 1962–2012

Country	Year detected	Immune deficiency[a]	Paralysis	Serotype	Maximum %VP1 divergence from Sabin	References
UK	1962	HGG	No	1	2.5	[463–465]
UK	1962	HGG	No	3	2.3	[165, 466]
Japan	1977	XLA	Yes	2	–	[462]
USA	1980	AGG	Yes	2	1.3	[354]
USA	1981	**CVID**	Yes	1	**10.0**	[186]
USA	1986	XLA	Yes	2	2.0	[467]
USA	1986	**CVID**	No	1	**5.4**	[467]
	1992			2	**11.8**	
UK	1987	**CVID**	No	2	**4.1**	[464, 468]
USA	1989	AGG	Yes	1	1.1	[354]
Germany	1990	**CVID**	Yes	1	**8.3**	[454]
USA	1990	SCID	Yes	2	1.9	[354]
USA	1991	**CVID**	Yes	2	**1.4**	[354]
Iran	1995	HGG	Yes	2	2.2	[469]
UK	1995	**CVID**	No	2	**12.9**	[453, 464]
USA	1995	SCID	Yes	2	2.1	[354]
Argentina	1998	XLA	Yes	1	2.8	[470]
Germany	2000	**CVID**	Yes	1	**3.5**	[467]
UK	2000	**CVID**	No	2	**6.3**	[464]
Taiwan	2001	**CVID**	Yes	1	**3.5**	[173]
Kazakhstan	2002	HGG	Yes	2	2.3	[467]
Kuwait	2002	MHC II def	No	2	2.0	[467]
UK	2002	ICF syndrome	No	2	2.5	[464]
Peru	2003	AGG	Yes	2	1.2	[467, 471]
Thailand	2003	HGG	Yes	2	2.2	[472]
China	2005	XLA	Yes	2; 3	4.2; 3.9	[457, 467]
Iran	2005	MHC II def	Yes	1; 2	1.1; 1.4	[469, 473]
Morocco[b]	2005	SCID	Yes	2	4	[467]
Syria	2005	HGG	Yes	2	1.3	[457, 467]
USA	2005	SCID	Yes	1	>2.3	[132]
Iran	2006	SCID	Yes	2	1.7	[469]
Iran	2006	XLA	Yes	3	2.1	[192, 469]
Kuwait	2006	SCID	Yes	3	1.2	[457]
Syria	2006	HCI	Yes	2	2.2	[457]
Tunisia[c]	2006	SCID	No	2	2.0	[457, 467]
Belarus	2007	HGG	Yes	2	1.9	[195]
Egypt	2007	SCID	Yes	3	1.1	[457]
Iran	2007	SCID	Yes	1; 2	1.7; 1.7	[469]
Iran	2007	XLA	Yes	3	2.0	[469]
Russia	2007	HGG	Yes	1	1.0	[195]
Iran	2008	XLA	Yes	2	1.2	[195]
Argentina	2009	HGG	Yes	1	3.8	[458]
Colombia	2009	AGG	Yes	2	1.5	[455]
India	2009	**CVID**	Yes	1	**6.2**	[455]
USA	2009	**CVID**	Yes	2	**12.3**	[223]
Algeria	2010	HLA-DR	Yes	2	1.8	[455]
China	2011	PID	Yes	3	2.0	[455]
China	2011	PID	Yes	2	1.9	[455]
India	2010	PID	Yes	2	1.6	[455]
Iraq	2010	PID	Yes	2	1.2	[455]

Table 13.4 (continued)

Country	Year detected	Immune deficiency[a]	Paralysis	Serotype	Maximum %VP1 divergence from Sabin	References
Sri Lanka	2010	SCID	No	2	1.3	[455, 474]
Egypt	2011	PID	No	2	1.4	[133]
Egypt	2011	AGG	Yes	1	2.1	[133]
Egypt	2011	PID	Yes	3	4.2	[133]
Iran	2011	SCID	Yes	2	2.0	[133]
Iran	2011	XLA	Yes	2	2.7	[133]
Iran	2011	PID	Yes	1; 2	2.7; 3.3	[133]
South Africa	2011	AGG	Yes	3	1.9	[475]
Sri Lanka	2011	**CVID**	Yes	3	**1.9**	[133, 474]
Turkey	2011	PID	No	2	1.8	[455]
West Bank	2011	SCID	No	2	1.2	[133]
China	2012	**CVID**	Yes	2; 3	**1.4**; **1.6**	[133]
Egypt	2012	PID	No	2	1.0	[460]
India	2012	HGG	Yes	2	2.8	[133]
Iran	2012	PID	Yes	2	1.4	[133]
Iran	2012	PID	Yes	2	1.1	[460]
Iraq	2012	PID	Yes	2	1.0	[460]

[a]*Ab deficiency* antibody deficiency, *AGG* agammaglobulinemia, *CVID* common variable immunodeficiency (**CVID** is shown in **bold font** because it is most frequently associated with chronic iVDPV excretion and highly divergent iVDPV isolates), *HCI* humoral and cellular immunodeficiency, *HGG* hypogammaglobulinemia, *HLA-DR* HLA-DR-associated immunodeficiency, *ICF* immunodeficiency-centromeric instability-facial abnormalities, *MHC II def* major histocompatability complex class II molecule deficiency, *SCID* severe combined immunodeficiency, *XLA* X-linked agammaglobulinemia
[b]Patient treated in Spain
[c]Patient treated in France

suggesting that the chronic poliovirus infections had persisted for ~10 years (Table 13.4). Patients with the most divergent isolates and the most prolonged (chronic) infections have common variable immunodeficiency (CVID), a group of late-onset immunodeficiencies that have multiple etiologies [476]. CVID is the most prevalent (~1 in 50,000) [476] of the primary immunodeficiencies, but only a small proportion of CVID patients exposed to OPV become chronically infected with iVDPVs.

Unlike cVDPV outbreaks, prolonged iVDPV infections cannot be prevented by high OPV coverage. Persons with prolonged iVDPV infections can transmit poliovirus to others, raising the risk for VDPV circulation in settings of low population immunity to the corresponding poliovirus serotype. So far, all reports of persistent iVDPV infections have been from countries with high to intermediate levels of development, where the rates of vaccine coverage are high and where the survival times of immunodeficient patients may be extended by treatment with intravenous immune globulin. The survival rates for persons with primary immunodeficiencies are probably very low in developing countries at highest risk for poliovirus spread. The population of prolonged iVDPV excretors is declining in developed countries because some patients have died (Table 13.4), some have cleared their infections, and no new iVDPV infections have been found in countries that have shifted to IPV. However, the prevalence of long-term iVDPV excretors might be higher than suggested by existing surveillance of persons with primary immunodeficiencies, and several aVDPVs have properties closely resembling iVDPVs (Sect. 10.8.3). The development of treatment for prolonged iVDPV infections might facilitate detection of and access to those with prolonged infections [133, 477].

Type 2 iVDPVs are the most prevalent (63 %), followed by type 1 (22 %) and type 3 (15 %). Some patients have heterotypic iVDPV infections, with the isolates having similar degrees of divergence from the parental OPV strains, consistent with a common OPV dose initiating the infections (Table 13.4). In addition to the occasional heterotypic infections, iVDPV isolates generally have distinguishing genetic properties, including heterogeneity at sites of nucleotide variability within serotypes (indicative of mixed virus populations), extensive antigenic variability, and either nonrecombinant or vaccine/vaccine recombinant genomes [94, 133].

10.8.3 Ambiguous VDPVs (aVDPVs)

Unlike the well-defined cVDPV and iVDPV categories, aVDPVs are a heterogeneous collection of isolates (Tables 13.5 and 13.6). Some aVDPVs have diverged by just over 1 % from the parental OPV strains, have no detected progeny, and may reflect the extremes of the usual distribution of

Table 13.5 Other vaccine-derived poliovirus (VDPV) with genetic evidence of community circulation, 1965–2012

Country	Serotype	Years	Reported cases (contacts)	% VP1 divergence	Estimated independent emergences	Reference
Byelorussia	2	1965–1966	0 (9)	1.6–2.1	3	[156]
Romania	1	2002	1 (7)	1.1–1.3	1	[479]
Laos	2	2004	1 (1)	1.1	1	[94]
United States	1	2005	0 (5)	2.3–2.6	1	[132]
China	1	2006	1 (6)	1.4–2.2	1	[190]
Madagascar	2	2011	0 (2)	3.3–3.7	1	[133]

Table 13.6 Highly divergent aVDPVs from the environment, 1998–2012

Country	Years isolated	Serotype	% VP1 divergence	Reference
Israel	2009–2012	1	8.0–13.8	[117, 133, 455, 460]
	1998–2012	2[a]	6.6–16.2	
	2006–2011	2[b]	10.7–11.2	
Finland	2008–2012	1	12.4–14.0	[133, 455, 460, 480]
	2008–2012	2	13.0–15.5	
	2008–2010	3	13.7–14.6	
Slovakia	2003–2004	2	13.4–15.0	[119]
Estonia	2008–2012	2	13.5–16.2	[455, 460]
	2002–2008	3	12.6–14.9	

[a]Group 1 aVDPV2 isolates from Israel are genetically distinct from Group 2 isolates, and are probably derived from two different chronic excretors
[b]Group 2

vaccine-related variants in countries using OPV [133]. Detection of other aVDPVs have foreshadowed subsequent cVDPV outbreaks [133, 478], others indicate limited person-to-person spread of OPV virus in small communities with gaps in OPV coverage [94, 132, 156, 190, 479], whereas others appear to indicate more prolonged circulation (Table 13.5) [133]. Most of these latter aVDPVs have vaccine/non-vaccine recombinant genomes typical of cVDPVs.

Many of the aVDPVs are isolated from the environment, and some have genetic properties (mixed VDPV populations, extensive antigenic changes, and nonrecombinant or vaccine/vaccine recombinant genomes) typical of iVDPVs. Highly divergent aVDPVs have been detected in Israel [117], Estonia [455], Slovakia [119], and Finland [120], countries with high rates of polio vaccination coverage that are unlikely to have extensive VDPV transmission (Table 13.6). The sequence divergence of some of these isolates is consistent with more than 15 years of replication from the original initiating OPV dose. It is likely that the sources of these viruses are immunodeficient persons with asymptomatic VDPV infections, although the infected persons remain to be identified.

10.9 Certification of Polio Eradication

The WHO GPEI has established a formal process for the certification of polio eradication to ensure that future immunization policy decisions are based on the best available evidence [481]. The certification process for polio is modeled on the International Smallpox Certification Commission, which certified in December 1979 that smallpox had been eradicated [482]. However, polio certification differs in important ways from the smallpox model. PAHO established its Regional Certification Commission (RCC) in 1990 and certified regional eradication of indigenous wild polioviruses in 1994 [112, 430], setting the standard for certification by individual regions and implicitly considering genetic evidence to distinguish indigenous wild polioviruses from imported virus. Because of the high proportion of inapparent wild poliovirus infections, regional certification requires a period of at least 3 years of sensitive surveillance since the last reported case associated with indigenous wild poliovirus. Small outbreaks from imported virus occurred on the eve of certification in the Western Pacific (in China in 1999 [483, 484]) and in Europe (in Bulgaria [485] and Georgia [11, 431] in 2001), but they were quickly controlled and followed by intensive surveillance. The certification of eradication of indigenous wild polioviruses by three WHO regions—the Americas in 1994 [112, 430], the Western Pacific in 2000 [486], and Europe in 2002 [431]—has stood the test of time. No indigenous wild poliovirus circulation has been detected in these regions post-certification. Cases and outbreaks associated with imported wild polioviruses and cVDPVs (in addition to cases of VAPP) have occurred subsequently (Sects. 10.6 and 10.8.1), but circulation has been promptly stopped by effective outbreak control measures [12, 82, 166, 178, 189, 190, 456]. The South-East Asia Region was certified by the RCC as polio-free in March 2014

(http://www.searo.who.int/entity/campaigns/polio-certification/en/). Key to certification was the observation that >3 years have passed since the last wild poliovirus case in India where surveillance has been maintained at high levels of sensitivity [12]. Similarly, documentation in all countries except Pakistan and Afghanistan has been accepted by the Eastern Mediterranean RCC. Several countries in the African Region have also prepared detailed documentation of polio-free status [22].

10.9.1 The Certification Process

Review of documentation to assess polio-free status is organized on three tiers [481]. National Certification Committees (NCCs) meet to critically assess national data and prepare detailed reports to Regional Certification Commissions (RCCs), who in turn report to the Global Certification Commission (GCC). The NCCs, RCCs, and GCC meet on a regular basis, and in the regions already certified as free of poliovirus circulation, the RCCs working with NCCs are primarily responsible to oversee and support activities to maintain the polio-free status. The NCC reports contain current information on the following: (1) national demographic data, including high-risk populations, (2) poliovirus vaccine coverage and any changes in vaccine policy, (3) surveillance data and indicators of surveillance sensitivity, (4) descriptions of any poliomyelitis cases, (5) information on the performance of laboratories serving each country, and (6) progress toward poliovirus containment. NCC, RCC, and GCC members are charged to serve as independent experts. Following certification of all WHO regions, the GCC will review all RCC reports and other relevant information to determine when the world can be declared free of all poliovirus circulation [481]. Special provisions are planned to recognize the global eradication of wild poliovirus type 2 (Sect. 11.2) [22]. An important prerequisite for certification is poliovirus containment.

10.9.2 Laboratory Containment of Polioviruses

Containment is integral to poliovirus eradication [487, 488]. Unlike smallpox, where very few facilities stored materials containing (virus stocks) or potentially containing (clinical specimens) smallpox virus (variola), many facilities worldwide store materials containing polioviruses. The purpose of containment is to minimize, as much as possible, the risk of reintroduction into the community of polioviruses stored in laboratories and vaccine production facilities. In 1998, the GCC approved the *WHO Global Action Plan for Laboratory Containment of Wild Polioviruses* [487] and subsequently established that satisfactory completion of containment activities be a requirement for regional certification. Containment will be implemented in three phases. During the Laboratory Inventory and Survey Phase (Phase 1) countries will (1) conduct national surveys of all biomedical laboratories to identify those storing materials containing (poliovirus stocks) or potentially containing (clinical specimens or environmental samples taken at locations during times of known poliovirus circulation) wild and vaccine-derived polioviruses, (2) encourage destruction of all unneeded materials, (3) develop inventories of laboratories retaining these materials and report to the RCC, (4) encourage implementation of biosafety level 2 (BSL-2) procedures in all enterovirus laboratories, and (5) plan for Phase 2. The Global Eradication Phase (Phase 2) will begin 1 year after detection of the last wild poliovirus or cVDPV at which time countries will (1) notify biomedical laboratories that wild poliovirus and cVDPV transmission has been stopped; (2) require laboratories on national inventories to select among the three options to (a) render materials noninfectious, (b) transfer all materials to high containment facilities, or (c) implement the appropriate laboratory procedures (BSL-2/polio or BSL-3/polio); and (3) document that all containment requirements have been met for global certification [487]. The Post-Global Certification Phase (Phase 3) was developed in response to the WHO goal of stopping all OPV use post-certification and proposes to minimize facility-associated poliovirus risk by destruction of both wild and vaccine-related polioviruses in all facilities, except for an absolute minimum needed for vaccine production, quality control, reference, and research [22].

Phase 1 containment was completed in the Americas several years after certification of polio-free status. In the United States, among the 105,356 laboratories surveyed, 180 laboratories in 122 institutions were found to be storing materials containing or potentially containing wild poliovirus; 87 held wild poliovirus stocks, 56 stored materials potentially containing wild polioviruses, and 37 stored both [489]. Europe completed Phase 1 containment in 2006 [96]. Among the 55,748 biomedical facilities surveyed, 265 laboratories in 164 institutions were found to be storing materials containing or potentially containing wild poliovirus; 116 held wild poliovirus stocks and 149 stored materials potentially containing wild polioviruses. In 20 European countries, 1 or more laboratories reported destroying all previously stored wild poliovirus materials during the survey. The Western Pacific Region completed Phase 1 containment in 2009 [490]. Of the 77,260 biomedical facilities surveyed, 45 laboratories (27 of which are members of the GPLN) retained materials containing or potentially containing wild polioviruses. Surveys have been completed in 155 of the 194 WHO member states, with the remaining surveys to be completed in countries in sub-Saharan Africa (these countries are thought unlikely to have many facilities with wild poliovirus materials). Worldwide ~550 facilities, including 6 IPV manufacturers, have been found to be storing wild poliovirus-infectious materials or potentially infectious materials [22]. VDPVs are stored in GPLN laboratories and a small number of collaborating laboratories.

11 Unresolved Problems

The immediate and crucial unresolved problem is to finish the task of eradicating wild polioviruses from the last remaining reservoir communities. The second unresolved problem is implementation of the "polio endgame" to ensure that the promise of polio eradication is forever secured. The third challenge is to build upon the legacy of polio eradication.

11.1 Eradication of Wild Polioviruses in Last Global Reservoirs

In May 2012, the WHA declared that polio eradication was a "programmatic emergency for global public health" and called for intensification of eradication activities in the remaining reservoir areas [22]. A Global Emergency Action Plan and National Emergency Action Plans (NEAPs) were developed and implemented in the remaining three reservoir countries [22]. All three countries face challenges of conflict and insecurity, as well as underperformance of immunization activities in high-risk areas. Afghanistan and Pakistan made clear improvements in 2012, but the pace of eradication has been slower in northern Nigeria.

11.1.1 Afghanistan

Polio has been controlled in most of Afghanistan except for the southern region, where intense armed conflict continues. The last case associated with wild poliovirus type 3 had onset in April 2010. Wild poliovirus type 1 had spread from the southern region provinces of Kandahar and Helmand in 2012 (Fig. 13.12), but the only 2013 cases were associated with wild poliovirus type 1 imported across the northeastern border from reservoirs in the insecure Tribal Areas of Pakistan. Only 2–5 % of children in southern Afghanistan were inaccessible in 2012 compared with 6–21 % in 2010 and 2011 [184]. In 2012, 65 % of case-patients were underimmunized and 35 % had received no OPV dose. The Afghanistan NEAP calls for reaching missed children through (1) improved detailed planning of mass campaigns; (2) better selection, training, monitoring, and support of vaccination teams; (3) enhanced community engagement; (4) robust response to poliomyelitis outbreaks; (5) expanded field staff in high-risk areas; and (6) use of permanent polio teams of trusted local people in insecure areas who visit homes and immunize children on a regular basis [22, 184].

11.1.2 Pakistan

Pakistan made dramatic progress in 2012 with implementation of the NEAP. Wild poliovirus type 3 was highly localized to the tribal areas in 2012 and was last detected in April 2012 from an AFP case, with no subsequent detections in the environment (Fig. 13.12) [184]. Several wild poliovirus type 1 reservoirs have been cleared and genetic diversity continues to decline. In recent years, >70 % of cases have been in the Pashtun minority, many of whom migrate widely within Pakistan and into Afghanistan. The NEAP focuses on (1) the implementation of an aggressive polio campaign schedule in the remaining reservoirs, (2) immunizing children of migrant and mobile Pashtun communities, (3) closer integration of immunization campaigns with community engagement, (4) closer civil–military cooperation in insecure areas, and (5) increased (>1,350) human resources for deployment in high-risk areas [22, 184]. Campaigns in some highest-risk areas (with ~165,000 children) have been suspended because of repeated fatal attacks on young women vaccinators from the community [184]. However, the attacks have been met by the young vaccinators with courage and resolve, and campaigns have continued elsewhere, with large numbers of children receiving OPV in the key reservoir areas.

Polio cases in Pakistan increased from 58 in 2012 to 93 in 2013; all cases in 2013 were associated with wild poliovirus type 1. Virus spread from Pakistan to Syria (33 cases in 2013) (http://www.polioeradication.org/), and to Egypt and Israel where wild poliovirus type 1 was detected in sewage [491].

11.1.3 Nigeria

All three poliovirus serotypes circulated in northern Nigeria in 2012: wild type 1, type 2 cVDPV, and wild type 3. In 2011–2012, 25 % of poliomyelitis case-patients had never received a dose of OPV [185]. Progress has been gradual in the northern states, and although polio campaign quality has improved, serious gaps remain in rural and hard-to-reach communities. In addition to insecurity in the northeast, 18 % of missed children are because their parents refuse them to be vaccinated because of anti-OPV rumors propagated by some clerics [22]. The Polio Emergency Action Plan calls for the following: (1) improved detailed planning of mass campaigns; (2) better selection, training, monitoring, and support of vaccination teams; (3) enhanced community engagement; (4) increased (~2,500) human resources for deployment in high-risk areas; (5) real-time data monitoring; (6) focused interventions in hard-to-reach rural and nomadic communities; (7) GIS mapping of settlements and GPS tracking of vaccination teams; and (8) strengthened surveillance [22, 185]. Full implementation of this plan should break all remaining chains of poliovirus transmission, as had been achieved a decade earlier in the more densely populated and tropical southern states. As in Pakistan, fatal attacks on young women vaccinators have been an impediment to complete implementation of the plan across the northern Nigerian states.

Polio cases in northern Nigeria decreased from 122 in 2012 to 53 in 2013; all cases in 2013 were associated with wild poliovirus type 1. Wild poliovirus type 1 spread from Nigeria to Somalia (194 cases in 2013), and from Somalia to Kenya (14 cases) and Ethiopia (9 cases). Wild poliovirus type 1 of Nigerian origin spread from Chad to Cameroon in 2013 (http://www.polioeradication.org/).

11.2 Implementation of the Polio Eradication Endgame

Compared with the straightforward smallpox eradication endgame, the polio eradication endgame is much more complex and includes the following elements, some operating on parallel tracks: (1) cessation of wild poliovirus transmission, (2) outbreak response (especially cVDPVs), (3) strengthened routine immunization, (4) IPV introduction and shift from tOPV to bOPV, (5) sequential IPV/bOPV in routine immunization, (6) completion of poliovirus containment (ultimately including vaccine-related isolates), (7) global certification, (8) cessation of bOPV use, and (9) mainstreaming of polio activities into national and global disease alert and response systems. Several of these activities are discussed above. A key new element is the worldwide withdrawal of use of the type 2 component of OPV.

11.2.1 Withdrawal tOPV

Since 1999, all poliomyelitis cases associated with poliovirus type 2 (apart from a few cases in India associated OPV contaminated with MEF-1 [335, 336]) have been associated with the continued use of tOPV. The rising incidence of type 2 cVDPV outbreaks (Sect. 10.8.1; Fig. 13.15), representing nearly 90 % of all reported cVDPV cases, prompted the WHO GPEI to plan for the logistically challenging step of worldwide withdrawal of tOPV and replacement with bOPV [22]. The tOPV–bOPV switch, targeted for 2016, would be predicated on the complete cessation of type 2 cVDPV transmission and will require continued sensitive AFP and poliovirus surveillance. Under the new strategic plan, by the end of 2014, polio-funded field staff will devote >50 % of their time assisting countries to strengthen routine immunization. Large stockpiles of mOPV2 (500 million doses; in addition to 300 million doses each of mOPV1 and mOPV3) will be maintained, and a robust surveillance and response capacity established. In addition, steps will be taken to secure affordable IPV and introduce at least one dose of IPV into the routine immunization programs of all countries by the end of 2015. Before tOPV withdrawal, the GCC must "validate" (certification will be for all poliovirus serotypes) the eradication of type 2 wild polioviruses and the elimination of type 2 cVDPV transmission. Also, Phase 2 containment of type 2 wild polioviruses and cVDPVs must be completed, along with Phase 1 containment of type 2 vaccine-related isolates. When all prerequisites are met, coordinated global cessation of tOPV use in routine immunization and mass campaigns will occur over a short time period, replaced with bOPV, the remaining tOPV stocks will be withdrawn worldwide, and the process carefully documented [22]. After the switch, the WHO will stockpile "stand-alone" IPV (IPV is usually formulated as a component of multivalent injectable vaccines) for emergency deployment to areas peripheral to outbreak communities. The tOPV–bOPV switch will precede the global bOPV cessation and withdrawal projected for 2019.

11.2.2 Transition to IPV

Many previously OPV-using countries, including the United States, have transitioned to exclusive use of IPV. Several countries have maintained a sequential IPV/OPV schedule (Fig. 13.17), similar to what the United States employed in 1997–1999 (Sect. 9.1.5). The WHO has obtained extensive experience in the introduction of new vaccines into low- and middle-income countries. However, worldwide use of IPV will be greatly facilitated by reducing the cost of IPV, which is substantially higher than OPV. Steps to reduce IPV costs could include (1) the use of intradermal fractional (1/5th) IPV doses [492], (2) development of new adjuvanted intramuscular IPV products, and (3) replacement of conventional IPV (using neurovirulent wild strains as seeds) with IPV based on the Sabin OPV strains [493–495], more suitable for production in developing countries [22]. New-generation biotechnology tools are being explored to develop genetically stable IPV seeds that would substantially enhance biological containment in IPV production facilities [496–499]. However, the many options for immunization products and schedules present increasingly complex policy decisions for national public health authorities [500].

11.3 The Legacy of Polio Eradication

The GPEI is the largest public health initiative in history, engaging communities, governments, health professionals, and private donors throughout the world. During the past quarter century, the GPEI has trained millions of volunteers, social mobilizers, and health workers to reach and immunize children in the most marginalized and vulnerable communities in the world [22]. In all but a few places, poliomyelitis, increasingly a disease of the poorest of the poor, has gone the way of smallpox. Moreover, other health interventions have directly benefited from polio immunization campaigns. For example, vitamin A supplements delivered during OPV campaigns are estimated to have prevented more than one million childhood deaths [22], and the administration of

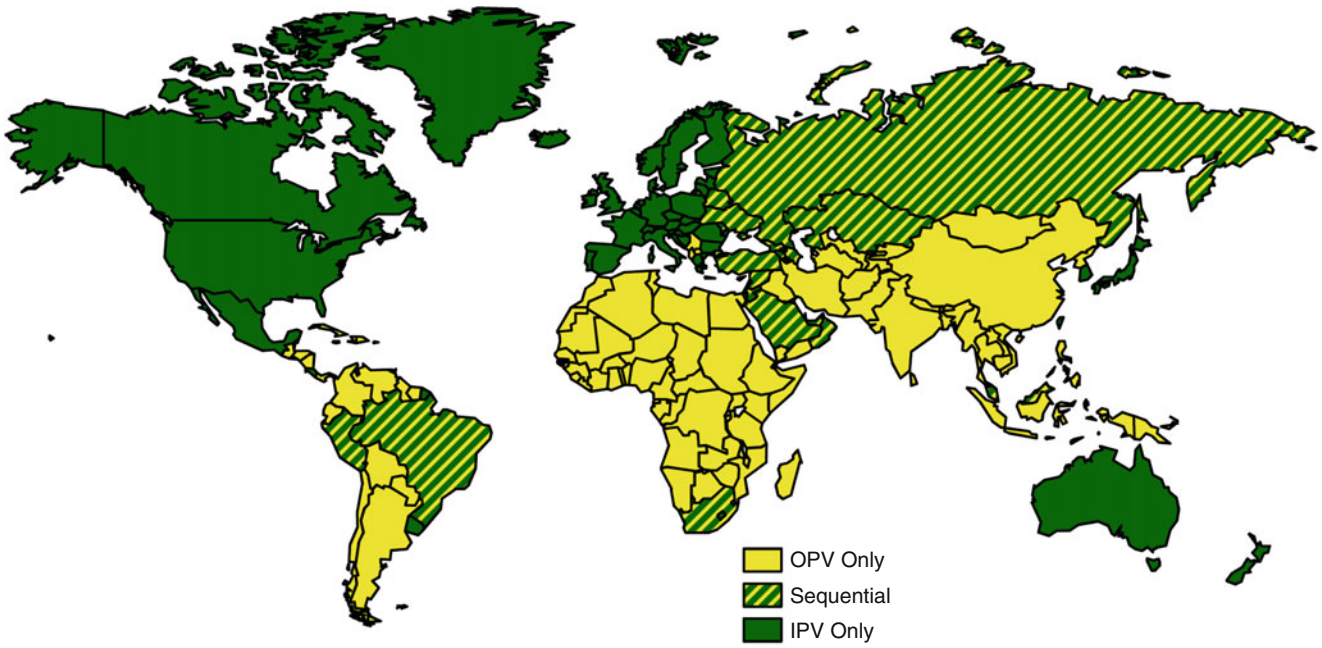

Fig. 13.17 Countries using exclusively oral poliovirus vaccine (*yellow* OPV), exclusively inactivated poliovirus vaccine (*green* IPV), or a combination of IPV and OPV (hatched pattern) (Modified from reference Sutter [7])

anti-helminthics and the distribution of bed nets have spared many more of disease and early death.

As the century-long struggle against poliomyelitis draws to a close, a new generation of highly experienced public health professionals—epidemiologists, virologists, and program managers—from around the world have built on the solid foundations of the GPEI to help guide the control of other vaccine-preventable diseases, including measles, rubella, rotaviral gastroenteritis, tetanus, invasive bacterial diseases, yellow fever, Japanese encephalitis, and hepatitis B [22, 422, 423, 501–503]. The model of close integration of field and laboratory surveillance with programmatic action has been replicated in other vaccine-preventable disease programs. The GPLN, the first laboratory network with global reach, established the blueprint for other vaccine-preventable disease laboratory networks [130, 131], some of which have grown to be more extensive than the GPLN [129]. These new networks are guided by laboratory network coordinators who are experienced in ensuring high technical performance and working in close cooperation with epidemiologists. Another important legacy of the GPEI, building on the models of smallpox eradication and the EPI, is a permanent worldwide change in public perception and expectation. Outbreaks of vaccine-preventable diseases, and the huge costs they inflict on individuals and communities, are no longer acceptable, and public demand for their control has steadily grown. As poliomyelitis fades into history, the legacy of its eradication will help shape global efforts to control infectious diseases for many years to come.

References

1. Paul JR. A history of poliomyelitis. New Haven: Yale University Press; 1971.
2. Melnick JL. Portraits of viruses: the picornaviruses. Intervirology. 1983;20:61–100.
3. Oshinsky DM. Polio: an American story. New York: Oxford University Press; 2005.
4. Salk JE, Krech U, Youngner JS, Bennett BL, Lewis LJ, Bazeley PL. Formaldehyde treatment and safety testing of experimental poliomyelitis vaccines. Am J Public Health. 1954;44:563–70.
5. Vidor E, Plotkin SA. Poliovirus vaccine—inactivated. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. London: W.B. Saunders; 2013. p. 573–97.
6. Sabin AB. Oral poliovirus vaccine: history of its development and use, and current strategies to eliminate poliomyelitis from the world. J Infect Dis. 1985;151:420–36.
7. Sutter RW, Kew OM, Cochi SL, Aylward RB. Poliovirus vaccine—live. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. London: W.B. Saunders; 2013. p. 598–645.
8. Cockburn WC, Drozdov SG. Poliomyelitis in the world. Bull World Health Organ. 1970;42:405–17.
9. Paccaud MF. World trends in poliomyelitis morbidity and mortality, 1951–1975. World Health Stat Q. 1979;32:198–224.
10. Kew O. Reaching the last one per cent: progress and challenges in global polio eradication. Curr Opin Virol. 2012;2:188–98.
11. Kew OM, Pallansch MA. The mechanism of polio eradication. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: ASM Press; 2002. p. 481–91.
12. World Health Organization. Progress towards global interruption of wild poliovirus transmission, January 2012–March 2013. Wkly Epidemiol Rec. 2013;88:181–7.
13. Centers for Disease Control and Prevention. Apparent global interruption of wild poliovirus type 2 transmission. MMWR Morb Mortal Wkly Rep. 2001;50:222–4.

14. Pan American Health Organization. Director announces campaign to eradicate poliomyelitis from the Americas by 1990. Bull Pan Am Health Organ. 1985;19:21–35.
15. World Health Assembly. Polio eradication by the year 2000. Geneva: World Health Organization; 1988.
16. World Health Organization. Progress towards interrupting wild poliovirus transmission worldwide: January 2010–March 2011. Wkly Epidemiol Rec. 2011;86:199–204.
17. Pigman HA. Conquering polio. Evanston: Rotary International; 2005.
18. de Quadros CA, Andrus JK, Olivé JM, Guerra de Macedo C, Henderson DA. Polio eradication from the Western Hemisphere. Ann Rev Public Health. 1992;13:239–52.
19. Aylward B, Yamada T. The polio endgame. N Engl J Med. 2011;364:2273–5.
20. Arita I, Francis DP. Safe landing for global polio eradication: a perspective. Vaccine. 2011;29:8827–34.
21. Donaldson L, Toole M, El Sayed N, et al. Polio's last stand? Sixth report of the Independent Monitoring Board of the Global Polio Eradication Initiative, November 2012. (http://www.polioeradication.org/imb.aspx)2012.
22. Polio eradication and endgame strategic plan (2013–2018). 2013. at http://www.polioeradication.org/portals/0/document/resources/strategywork/endgamestratplan_20130414_eng.pdf.
23. Melnick JL. Poliovirus and other enteroviruses. In: Evans AS, Kaslow RA, editors. Viral infections of humans, epidemiology and control. 4th ed. New York: Plenum; 1997. p. 583–663.
24. Brown B, Oberste MS, Maher K, Pallansch M. Complete genomic sequencing shows that polioviruses and members of human enterovirus species C are closely related in the non-capsid coding region. J Virol. 2003;77:8973–84.
25. Knowles NJ, Hovi T, Hyypiä T, et al. Picornaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier; 2011. p. 855–80.
26. Mueller S, Wimmer E, Cello J. Poliovirus and poliomyelitis: a tale of guts, brains, and an accidental event. Virus Res. 2005;111:175–93.
27. Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010.
28. Paul JR. Epidemiology of poliomyelitis. In: Poliomyelitis, vol. World Health Organization monograph series no. 26. Geneva: World Health Organization; 1955. p. 9–29.
29. Bodian D, Horstmann DM. Polioviruses. In: Horsfall Jr FL, Tamm I, editors. Viral and rickettsial infections of man. 4th ed. Philadelphia: J.B. Lippincott; 1965. p. 430–73.
30. Dowdle WR, Cochi SL. Global eradication of poliovirus: history and rationale. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: ASM Press; 2002. p. 473–80.
31. Eggers HJ. History of poliomyelitis and poliomyelitis research. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: American Society for Microbiology; 2002. p. 3–14.
32. Robbins FC. The history of polio vaccine development. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th ed. Philadelphia: W.B. Saunders Company; 2004. p. 17–30.
33. Smallman-Raynor MR, Cliff AD. Poliomyelitis: a world geography: emergence to eradication. New York: Oxford University Press; 2006.
34. Nathanson N, Kew OM. From emergence to eradication: the epidemiology of poliomyelitis deconstructed. Am J Epidemiol. 2010;172:1213–29.
35. Plotkin SL, Plotkin SA. A short history of vaccination. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. London: W.B. Saunders; 2013. p. 1–13.
36. Underwood M. A treatise on the diseases of children. London: J. Mathews; 1789.
37. Heine J. Spinale Kinderlähmung, 2. umgearbeitete und vermehrte Auflage. Stuttgart: J.G. Cotta'scher Verlag; 1860.
38. Medin O. Über eine Epidemie von spinaler Kindelähmung. Verhandl. d. 10. Internat Med Kongr. Berlin;1891. p.37–47.
39. Trevelyan B, Smallman-Raynor M, Cliff AD. The spatial dynamics of poliomyelitis in the United States: from epidemic emergence to vaccine-induced retreat, 1910–1971. Ann Assoc Am Geogr. 2005;95:269–93.
40. Caverly CS. Notes of an epidemic of acute anterior poliomyelitis. J Am Med Assoc. 1896;26:1–5.
41. Wickman I. Die akute Poliomyelitis bzw. Heine-Medinsche Krankheit. Berlin: Julius Springer; 1911.
42. Landsteiner K, Popper E. Übertragung der Poliomyelitis acuta auf Affen. Z Immunitätsforsch exp Ther. 1909;2:377–90.
43. Flexner S, Lewis PA. The transmission of epidemic poliomyelitis to monkeys: a further note. J Am Med Assoc. 1909;53:1913.
44. Kling C, Petterson A, Wernstedt W. Experimental and pathological investigation. I. The presence of the microbe of infantile paralysis in human beings (Trans: Rosen AV). Commun State Med Inst. 1912;3:12.
45. Lavinder CH, Freeman AW, Frost WH. Epidemiologic studies of poliomyelitis in New York City and the northeastern United States during the year 1916. Public Health Bulletin No 91. Washington, D.C: Government Printing Office; 1918.
46. United States Public Health and Marine-Hospital Service. Poliomyelitis in the United States. Public Health Rep. 1910;25:1347–50.
47. Drinker P, Shaw LA. An apparatus for the prolonged administration of artificial respiration: I. A design for adults and children. J Clin Invest. 1929;7:229–47.
48. Burnet FM, Macnamara J. Immunological differences between strains of poliomyelitic virus. Br J Exp Pathol. 1931;12:57–61.
49. Armstrong C. Successful transfer of Lansing strain of poliomyelitis virus from the cotton rat to the white mouse. Public Health Rep. 1939;54:2302–5.
50. Trask JD, Paul JR, Vignec AJ. I. Poliomyelitic virus in human stools. J Exp Med. 1940;71:751–63.
51. Trask JD, Paul JR. Periodic examination of sewage for the virus of poliomyelitis. J Exp Med. 1942;75:1–6.
52. Sabin AB, Ward R. The natural history of human poliomyelitis. I. Distribution of virus in nervous and non-nervous tissues. J Exp Med. 1941;73:771–93.
53. Enders J, Weller T, Robbins F. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. Science. 1949;109:85–7.
54. Bodian D, Morgan IM, Howe HA. Differentiation of types of poliomyelitis viruses. III. The grouping of fourteen strains into three basic immunologic types. Am J Hyg. 1949;49:234–45.
55. Committee on Typing of the National Foundation for Infantile Paralysis. Immunologic classification of poliomyelitis viruses: a cooperative program for the typing of one hundred strains. Am J Hyg. 1951;54:191–204.
56. Serfling RE, Sherman IL. Poliomyelitis distribution in the United States. Public Health Rep. 1953;68:943–55.
57. Hammon WM, Coriell LL, Wehrle PF, Stokes Jr J. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. IV. Final report of results based on clinical diagnoses. J Am Med Assoc. 1953;151:1272–85.
58. Horstmann DM, McCollum RW, Mascola AD. Viremia in human poliomyelitis. J Exp Med. 1954;99:355–69.
59. Francis Jr T, Korns RF, Voight RB, et al. An evaluation of the 1954 poliomyelitis vaccine trials. Am J Public Health. 1955;45:1–63.
60. Chumakov MP, Voroshilova MK, Drozdov SG, et al. Some results of the work on mass immunization in the Soviet Union with live

poliovirus vaccine prepared from Sabin strains. Bull World Health Organ. 1961;25:79–91.
61. United States Public Health Service. Public Health Rep 1910–1951.
62. Dauer CC. National morbidity reporting–revised system. Public Health Rep. 1952;67:21–5.
63. Centers for Disease Control and Prevention. Morbid Mortal Wkly Rep. 1952–1972.
64. Centers for Disease Control and Prevention. Annual poliomyelitis surveillance summaries for years 1960–1973. Atlanta 1961–1974.
65. International Symposium on Poliomyelitis Control. Poliomyelitis: papers and discussions presented at the First through Fifth International Poliomyelitis Conferences. Philadelphia: Lippincott; 1949, 1952, 1955, 1958, 1961.
66. Freyche M-J, Nielsen J. Incidence of poliomyelitis since 1920. In: Poliomyelitis, vol. World Health Organization monograph series no. 26. Geneva: World Health Organization; 1955. p. 59–106.
67. World Health Organization. Poliomyelitis surveillance. Wkly Epidemiol Rec. 1970;45:189–94.
68. Andrus JK, de Quadros C, Olivé JM, Hull HF. Screening of cases of acute flaccid paralysis for poliomyelitis eradication: ways to improve specificity. Bull World Health Organ. 1992;70:591–6.
69. Pinheiro FP, Kew O, Hatch MH, Silveira CM, de Quadros CA. Eradication of wild polioviruses from the Americas. Part II. Wild poliovirus surveillance: laboratory issues. J Clin Microbiol. 1997;175 Suppl 1:S43–9.
70. Pan American Health Organization. EPI Newsl. 1985–1994; 7(3)–16(4).
71. World Health Organization. Wkly Epidemiol Rec. 1985–.
72. Centers for Disease Control and Prevention. Morbid Mortal Wkly Rep. 1985–.
73. United States Public Health Service. Poliomyelitis (infantile paralysis)—the accuracy of diagnosis under epidemic conditions. Public Health Rep. 1916;31:3291.
74. Birmingham ME, Linkins RW, Hull BP, Hull HF. Poliomyelitis surveillance: the compass for eradication. J Infect Dis. 1997;175 Suppl 1:S146–50.
75. de Quadros CA, Hersh BS, Olive JM, Andrus JK, da Silveira CM, Carrasco PA. Eradication of wild poliovirus from the Americas: acute flaccid paralysis surveillance, 1988–1995. J Infect Dis. 1997;175 Suppl 1:S37–42.
76. Sabin AB. Paralytic consequences of poliomyelitis infection in different parts of the world and in different population groups. Am J Public Health. 1951;41:1215–30.
77. Doshi SJ, Sandhu HS, Venczel LV, et al. Poliomyelitis-related case-fatality ratio in India, 2002–2006. Clin Infect Dis. 2011;53:13–9.
78. Prevots DR, Ciofi degli Atti ML, Sallabanda A, et al. Outbreak of paralytic poliomyelitis in Albania, 1996: high attack rate among adults and apparent interruption of transmission following nationwide mass vaccination. Clin Infect Dis. 1998;26:419–25.
79. World Health Organization. Outbreak of poliomyelitis, Cape Verde: August–October 2000. Wkly Epidemiol Rec. 2000;75:401.
80. World Health Organization. Outbreak of type-1 wild poliovirus in adults, Namibia, 2006. Wkly Epidemiol Rec. 2006;81:425–30.
81. Patel MK, Konde MK, Didi-Ngossaki BH, et al. An outbreak of wild poliovirus in the Republic of Congo, 2010-2011. Clin Infect Dis. 2012;55:1291–8.
82. World Health Organization. Progress towards global interruption of wild poliovirus transmission, January 2011–March 2012. Wkly Epidemiol Rec. 2012;87:195–200.
83. Paul JR. Development and use of serum surveys. In: Paul JR, White C, editors. Serological epidemiology. New York: Academic Press; 1973. p. 1–13.
84. Aycock WL, Kramer SD. Immunity to poliomyelitis in normal individuals in urban and rural communities as indicated by the neutralization test. J Prev Med. 1930;4:189–200.
85. Paul JR, Riordan JT, Melnick JL. Antibodies to three different antigenic types to poliomyelitis virus in sera from North Alaskan Eskimos. Am J Hyg. 1951;54:275–85.
86. Melnick JL, Ledinko N. Development of neutralizing antibodies against the three types of poliomyelitis virus during an epidemic period. The ratio of inapparent infection to clinical poliomyelitis. Am J Hyg. 1953;58:207–22.
87. Poland JD, Plexico K, Flynt JW, Chin TD. Poliovirus neutralizing antibody levels among preschool children. Public Health Rep. 1968;83:507–12.
88. Melnick JL. Periodic serological surveillance. In: Paul JR, White C, editors. Serological surveillance. New York: Academic Press; 1973. p. 143–54.
89. Sutter RW, Patriarca PA, Brogan S, et al. An outbreak of paralytic poliomyelitis in Oman: evidence for widespread transmission among fully vaccinated children. Lancet. 1991;338:715–20.
90. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis. 1991;13:926–39.
91. World Health Organization. Progress towards eradicating poliomyelitis: India, January 2010–September 2011. Wkly Epidemiol Rec. 2011;86:501–7.
92. Sutter RW, John TJ, Jain H, et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. Lancet. 2010;376:1682–8.
93. Aylward RB, Porta D, Fiore L, Ridolfi B, Chierchini P, Forastiere F. Unimmunized Gypsy populations and implications for the eradication of poliomyelitis in Europe. J Infect Dis. 1997;175 Suppl 1:S86–8.
94. Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. Annu Rev Microbiol. 2005;59:587–635.
95. El-Sayed N, Al-Jorf S, Hennessey KA, et al. Survey of poliovirus antibodies during the final stage of polio eradication in Egypt. Vaccine. 2007;25:5062–70.
96. Habib M, Soofi S, Ali N, et al. A study evaluating poliovirus antibodies and risk factors associated with polio seropositivity in low socioeconomic areas of Pakistan. Vaccine. 2013;31:1987–93.
97. Bernier RH. Some observations on poliomyelitis lameness surveys. Rev Infect Dis. 1984;6 Suppl 2:S371–5.
98. Ofosu-Amaah S. The challenge of poliomyelitis in tropical Africa. Rev Infect Dis. 1984;6 Suppl 2:S318–20.
99. Basu R, Sokhey J. Prevalence of poliomyelitis in India. Indian J Pediatr. 1984;51:515–9.
100. World Health Organization. Field guide for supplementary activities aimed at achieving polio eradication, 1996 revision. Geneva: World Health Organization; 1997.
101. Nathanson N, Martin JR. The epidemiology of poliomyelitis: enigmas surrounding its appearance, epidemicity, and disappearance. Am J Epidemiol. 1979;110:672–92.
102. Hull HF, Ward NA, Milstien JB, de Quadros C. Paralytic poliomyelitis: seasoned strategies, disappearing disease. Lancet. 1994;343:1331–7.
103. Melnick JL. Poliomyelitis virus in urban sewage in epidemic and in nonepidemic times. Am J Hyg. 1947;45:240–53.
104. Pöyry T, Stenvik M, Hovi T. Viruses in sewage waters during and after a poliomyelitis outbreak and subsequent nationwide oral poliovirus vaccination campaign in Finland. Appl Environ Microbiol. 1988;54:371–4.
105. Böttiger M, Herrstrom E. Isolation of polioviruses from sewage and their characteristics: experience over two decades in Sweden. Scand J Infect Dis. 1992;24:151–5.
106. van der Avoort HG, Reimerink JH, Ras A, Mulders MN, van Loon AM. Isolation of epidemic poliovirus from sewage during the 1992–3 type 3 outbreak in The Netherlands. Epidemiol Infect. 1995;114:481–91.

107. Manor Y, Handsher R, Halmut T, et al. Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian Authority. J Clin Microbiol. 1999;37:1670–5.
108. World Health Organization. Tracking progress towards global polio eradication, 2010–2011. Wkly Epidemiol Rec. 2012;87: 153–60.
109. Yoshida H, Horie H, Matsuura K, Kitamura T, Hashizume S, Miyamura T. Prevalence of vaccine-derived polioviruses in the environment. J Gen Virol. 2002;83:1107–11.
110. Hovi T, Cantell K, Huovalainen A, Kinnunen E. Outbreak of paralytic poliomyelitis in Finland: widespread circulation of antigenically altered poliovirus type 3 in a vaccinated population. Lancet. 1986;1:1427–32.
111. Tambini G, Andrus JK, Marques E, et al. Direct detection of wild poliovirus transmission by stool surveys of healthy children and analysis of community wastewater. J Infect Dis. 1993;168:1510–4.
112. Robbins FC, de Quadros CA. Certification of the eradication of indigenous transmission of wild poliovirus in the Americas. J Infect Dis. 1997;175 Suppl 1:S281–5.
113. El Bassioni L, Barakat I, Nasr E, et al. Prolonged detection of indigenous wild polioviruses in sewage from communities in Egypt. Am J Epidemiol. 2003;158:807–15.
114. Centers for Disease Control and Prevention. Progress towards poliomyelitis eradication—Egypt, 2003–2004. MMWR Morb Mortal Wkly Rep. 2004;53:820–2.
115. Hovi T, Blomqvist S, Nasr E, et al. Environmental surveillance of wild poliovirus circulation in Egypt–balancing between detection sensitivity and workload. J Virol Methods. 2005;126:127–34.
116. Deshpande JM, Shetty SJ, Siddiqui ZA. Environmental surveillance system to track wild poliovirus transmission. Appl Environ Microbiol. 2003;69:2919–27.
117. Shulman LM, Manor Y, Sofer D, et al. Neurovirulent vaccine-derived polioviruses in sewage from highly immune populations. PLoS One. 2006;1:e69.
118. Blomqvist S, Savolainen C, Laine P, et al. Characterization of a highly evolved vaccine-derived poliovirus type 3 isolated from sewage in Estonia. J Virol. 2004;78:4876–83.
119. Cernáková B, Sobotová Z, Rovný I, Bláhova S, Roivainen M, Hovi T. Isolation of vaccine-derived polioviruses in the Slovak Republic. Eur J Clin Microbiol Infect Dis. 2005;24:438–9.
120. Roivainen M, Blomqvist S, Al-Hello H, et al. Highly divergent neurovirulent vaccine-derived polioviruses of all three serotypes are recurrently detected in Finnish sewage. Euro Surveill. 2010;15:pii/19566.
121. Más Lago P, Gary Jr HE, Pérez LS, et al. Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. Int J Epidemiol. 2003;32:772–7.
122. Huang QS, Greening G, Baker MG, et al. Persistence of oral polio vaccine virus after its removal from the immunisation schedule in New Zealand. Lancet. 2005;366:394–6.
123. Hull BP, Dowdle WR. Poliovirus surveillance: building the Global Polio Laboratory Network. J Infect Dis. 1997;175 Suppl 1:S113–6.
124. World Health Organization. Polio laboratory manual. 4th ed. Geneva: World Health Organization; 2004.
125. Melnick JL, Wenner HA, Phillips CA. Enteroviruses. In: Lennette EH, Schmidt NJ, editors. Diagnostic procedures for viral, rickettsial, and chlamydial infections. Washington, D.C.: American Public Health Association; 1979. p. 471–534.
126. Pallansch MA, Oberste MS. Enteroviruses and parechoviruses. In: Specter S, Hodinka RL, Young SA, Wiedbrauk DL, editors. Clinical virology manual. 4th ed. Washington, D.C.: ASM Press; 2009. p. 249–82.
127. World Health Organization. Polio Laboratory Network Quarterly Update 1995–2007.
128. World Health Organization. Informal consultation of the Global Polio Laboratory Network—June 2008. Wkly Epidemiol Rec. 2008;83:261–8.
129. Featherstone DA, Rota PA, Icenogle J, et al. Expansion of the global measles and rubella laboratory network 2005-09. J Infect Dis. 2011;204 Suppl 1:S491–8.
130. Centers for Disease Control and Prevention. Expanding poliomyelitis and measles surveillance networks to establish surveillance for acute meningitis and encephalitis syndromes—Bangladesh, China, and India, 2006–2008. MMWR Morb Mortal Wkly Rep. 2013;61:1008–11.
131. Centers for Disease Control and Prevention. Building laboratory capacity to support the Global Rotavirus Surveillance Network. MMWR Morb Mortal Wkly Rep. 2013;62:409–12.
132. Alexander JP, Ehresmann K, Seward J, et al. Transmission of imported vaccine-derived poliovirus in an under-vaccinated community—Minnesota, USA. J Infect Dis. 2009;199:391–7.
133. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, April 2011–June 2012. MMWR Morb Mortal Wkly Rep. 2012;61:741–6.
134. Gary Jr HE, Sanders R, Pallansch MA. A theoretical framework for evaluating the sensitivity of surveillance for detecting wild poliovirus: I. Factors affecting detection sensitivity in a person with acute flaccid paralysis. J Infect Dis. 1997;175 Suppl 1:S135–40.
135. Nolan JP, Wilmer BH, Melnick JL. Poliomyelitis: its highly invasive nature and narrow stream of infection in a community of high socioeconomic level. N Engl J Med. 1955;253:945–54.
136. McAllister RM, Mclnyk J, Finklestein JZ, Adams ECJ, Gardner MB. Cultivation in vitro of cells derived from a human rhabdomyosarcoma. Cancer. 1969;24:520–6.
137. Wood DJ, Hull B. L20B cells simplify culture of polioviruses from clinical samples. J Med Virol. 1999;58:188–92.
138. Nadkarni SS, Deshpande JM. Recombinant murine L20B cell line supports multiplication of group A coxsackieviruses. J Med Virol. 2003;70:81–5.
139. Nakano JH, Hatch MH, Thieme ML, Nottay B. Parameters for differentiating vaccine-derived and wild poliovirus strains. Prog Med Virol. 1978;24:78–206.
140. Crainic R, Couillin P, Blondel B, Cabau N, Boué A, Horodniceanu F. Natural variation of poliovirus neutralization epitopes. Infect Immun. 1983;41:1217–25.
141. Minor PD, John A, Ferguson M, Icenogle JP. Antigenic and molecular evolution of the vaccine strain of type 3 poliovirus during the period of excretion by a primary vaccinee. J Gen Virol. 1986;67:693–706.
142. Osterhaus ADME, van Wezel AL, Hazendonk TG, UytdeHaag FG, van Asten JA, van Steenis B. Monoclonal antibodies to polioviruses. Comparison of intratypic strain differentiation of poliovirus type 1 using monoclonal antibodies versus cross-absorbed antisera. Intervirology. 1983;20:129–36.
143. van Wezel AL, Hazendonk AG. Intratypic serodifferentiation of poliomyelitis virus by strain-specific antisera. Intervirology. 1979;11:2–8.
144. Nomoto A, Omata T, Toyoda H, et al. Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome. Proc Natl Acad Sci U S A. 1982;79:5793–7.
145. van der Avoort HGAM, Hull BP, Hovi T, et al. A comparative study of five methods of intratypic differentiation of polioviruses. J Clin Microbiol. 1995;33:2562–6.
146. Ferguson M, Magrath DI, Minor PD, Schild GC. WHO collaborative study on the use of monoclonal antibodies for the intratypic differentiation of poliovirus strains. Bull World Health Organ. 1986;64:239–46.
147. Nottay BK, Kew OM, Hatch MH, Heyward JT, Obijeski JF. Molecular variation of type 1 vaccine-related and wild

polioviruses during replication in humans. Virology. 1981;108: 405–23.
148. Minor PD. Characterization of strains of type 3 poliovirus by oligonucleotide mapping. J Gen Virol. 1982;59:307–17.
149. De L, Nottay BK, Yang C-F, Holloway BP, Pallansch MA, Kew O. Identification of vaccine-related polioviruses by hybridization with specific RNA probes. J Clin Microbiol. 1995;33:562–71.
150. De L, Yang C-F, da Silva E, et al. Genotype-specific RNA probes for the direct identification of wild polioviruses by blot hybridization. J Clin Microbiol. 1997;35:2834–40.
151. Yang C-F, De L, Holloway BP, Pallansch MA, Kew OM. Detection and identification of vaccine-related polioviruses by the polymerase chain reaction. Virus Res. 1991;20:159–79.
152. Yang C-F, De L, Yang S-J, et al. Genotype-specific *in vitro* amplification of sequences of the wild type 3 polioviruses from Mexico and Guatemala. Virus Res. 1992;24:277–96.
153. Kilpatrick DR, Yang C-F, Ching K, et al. Rapid group-, serotype-, and vaccine strain-specific identification of poliovirus isolates by real-time reverse transcription-PCR using degenerate primers and probes containing deoxyinosine residues. J Clin Microbiol. 2009;47:1939–41.
154. Balanant J, Guillot S, Candréa A, Delpeyroux F, Crainic R. The natural genomic variability of poliovirus analyzed by a restriction fragment polymorphism assay. Virology. 1991;184:645–54.
155. Cherkasova E, Laassri M, Chizhikov V, et al. Microarray analysis of evolution of RNA viruses: evidence of circulation of virulent highly divergent vaccine-derived polioviruses. Proc Natl Acad Sci U S A. 2003;100:9398–403.
156. Korotkova EA, Park R, Cherkasova EA, et al. Retrospective analysis of a local cessation of vaccination against poliomyelitis: a possible scenario for the future. J Virol. 2003;77:12460–5.
157. Kilpatrick DR, Nottay B, Yang C-F, et al. Group-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residues at positions of codon degeneracy. J Clin Microbiol. 1996;34:2990–6.
158. Kilpatrick DR, Nottay B, Yang C-F, et al. Serotype-specific identification of polioviruses by PCR using primers containing mixed-base or deoxyinosine residues at positions of codon degeneracy. J Clin Microbiol. 1998;36:352–7.
159. Kilpatrick DR, Ching K, Iber J, et al. Identification of vaccine-derived polioviruses using dual-stage real-time RT-PCR. J Virol Meth. 2014;197:25–8.
160. Centers for Disease Control and Prevention. Laboratory surveillance for wild and vaccine-derived polioviruses—worldwide, January 2008–June 2009. MMWR Morb Mortal Wkly Rep. 2009;58:950–4.
161. Kilpatrick DR, Ching K, Iber J, et al. Multiplex PCR method for identifying recombinant vaccine-related polioviruses. J Clin Microbiol. 2004;42:4313–5.
162. Minor PD. Antigenic structure of picornaviruses. Curr Top Microbiol Immunol. 1990;161:121–54.
163. Rico-Hesse R, Pallansch MA, Nottay BK, Kew OM. Geographic distribution of wild poliovirus type 1 genotypes. Virology. 1987;160:311–22.
164. Jorba J, Campagnoli R, De L, Kew O. Calibration of multiple poliovirus molecular clocks covering an extended evolutionary range. J Virol. 2008;82:4429–40.
165. Martín J, Dunn G, Hull R, Patel V, Minor PD. Evolution of the Sabin strain of type 3 poliovirus in an immunodeficient patient during the entire 637-day period of virus excretion. J Virol. 2000;74:3001–10.
166. Kew OM, Morris-Glasgow V, Landaverde M, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science. 2002;296:356–9.
167. Jegouic S, Joffret M-L, Blanchard C, et al. Recombination between polioviruses and co-circulating Coxsackie A viruses: role in the emergence of pathogenic vaccine-derived polioviruses. PLoS Pathog. 2009;5:e1000412.
168. Burns C, Shaw J, Jorba J, et al. Multiple independent emergences of type 2 vaccine-derived polioviruses during a large outbreak in northern Nigeria. J Virol. 2013;87:4907–22.
169. Manor Y, Handsher R, Halmut T, et al. A double-selective tissue culture system for isolation of wild-type poliovirus from sewage applied in a long-term environmental surveillance. Appl Environ Microbiol. 1999;65:94–7.
170. Kew OM, Mulders MN, Lipskaya GY, da Silva EE, Pallansch MA. Molecular epidemiology of polioviruses. Semin Virol. 1995;6:401–14.
171. Gavrilin GV, Cherkasova EA, Lipskaya GY, Kew OM, Agol VI. Evolution of circulating wild poliovirus and of vaccine-derived poliovirus in an immunodeficient patient: a unifying model. J Virol. 2000;74:7381–90.
172. Liu H-M, Zheng D-P, Zhang L-B, Oberste MS, Pallansch MA, Kew OM. Molecular evolution of a type 1 wild-vaccine poliovirus recombinant during widespread circulation in China. J Virol. 2000;74:11153–61.
173. Yang C-F, Chen H-Y, Jorba J, et al. Intratypic recombination among lineages of type 1 vaccine-derived poliovirus emerging during chronic infection of an immunodeficient patient. J Virol. 2005;79:12623–34.
174. Odoom JK, Yunus Z, Dunn G, Minor PD, Martín J. Changes in population dynamics during long-term evolution of Sabin type 1 poliovirus in an immunodeficient patient. J Virol. 2008;82:9179–90.
175. Centers for Disease Control and Prevention. Wild poliovirus importations—West and Central Africa, January 2003–March 2004. MMWR Morb Mortal Wkly Rep. 2004;53:433–5.
176. Centers for Disease Control and Prevention. Resurgence of wild poliovirus type 1 transmission and consequences of importation—21 countries, 2002–2005. MMWR Morb Mortal Wkly Rep. 2006;55:145–50.
177. Centers for Disease Control and Prevention. Wild poliovirus type 1 and type 3 importations—15 countries, Africa, 2008–2009. MMWR Morb Mortal Wkly Rep. 2009;58:357–62.
178. World Health Organization. Poliomyelitis in Tajikistan—update. Wkly Epidemiol Rec. 2010;85:165–6.
179. Mulders MN, Lipskaya GY, van der Avoort HGAM, Koopmans MPG, Kew OM, van Loon AM. Molecular epidemiology of wild poliovirus type 1 in Europe, the Middle East, and the Indian Subcontinent. J Infect Dis. 1995;171:1399–405.
180. Patriarca PA, Sutter RW, Oostvogel PM. Outbreaks of paralytic poliomyelitis, 1976–1995. J Infect Dis. 1997;175 Suppl 1:S165–72.
181. Centers for Disease Control and Prevention. Outbreaks following wild poliovirus importations—Europe, Africa, and Asia, January 2009–September 2010. MMWR Morb Mortal Wkly Rep. 2010;59:1393–9.
182. Afif H, Sutter RW, Fontaine RE, et al. Outbreak of poliomyelitis in Gizan, Saudi Arabia: co-circulation of wild type 1 polioviruses from three separate origins. J Infect Dis. 1997;175 Suppl 1:S71–5.
183. Estívariz C, Watkins MA, Handoko D, et al. A large vaccine-derived poliovirus outbreak on Madura Island—Indonesia, 2005. J Infect Dis. 2008;197:347–54.
184. World Health Organization. Progress towards eradicating poliomyelitis: Afghanistan and Pakistan, January 2011–August 2012. Wkly Epidemiol Rec. 2012;87:381–8.
185. World Health Organization. Progress towards poliomyelitis eradication, Nigeria, January 2011–September 2012. Wkly Epidemiol Rec. 2012;87:439–46.
186. Kew OM, Sutter RW, Nottay B, et al. Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. J Clin Microbiol. 1998;36:2893–9.

187. Rousset D, Rakoto-Andrianarivelo M, Razafindratsimandresy R, et al. Recombinant vaccine-derived poliovirus in Madagascar. Emerg Infect Dis. 2003;9:885–7.
188. Yang C-F, Naguib T, Yang S-J, et al. Circulation of endemic type 2 vaccine-derived poliovirus in Egypt, 1983 to 1993. J Virol. 2003;77:8366–77.
189. Shimizu H, Thorley B, Paladin FJ, et al. Circulation of type 1 vaccine-derived poliovirus in the Philippines in 2001. J Virol. 2004;78:13512–21.
190. Yan D, Li L, Zhu S, et al. Emergence and localized circulation of a vaccine-derived poliovirus in an isolated mountain community in Guangxi, China. J Clin Microbiol. 2010;48:3274–80.
191. Martín J, Odoom K, Tuite G, et al. Long-term excretion of vaccine-derived poliovirus by a healthy child. J Virol. 2004;78:13839–47.
192. Shahmahmoodi S, Parvaneh N, Burns C, et al. Isolation of a type 3 vaccine-derived poliovirus (VDPV) from an Iranian child with X-linked agammaglobulinemia. Virus Res. 2008;137:168–72.
193. Shulman LM, Handsher R, Yang S-J, et al. Resolution of the pathways of poliovirus type 1 transmission during an outbreak. J Clin Microbiol. 2000;38:945–52.
194. World Health Organization. Progress towards poliomyelitis eradication in Egypt, 2001. Wkly Epidemiol Rec. 2002;77:70–4.
195. Centers for Disease Control and Prevention. Laboratory surveillance for wild and vaccine-derived polioviruses—worldwide, January 2007–June 2008. MMWR Morb Mortal Wkly Rep. 2008;57:967–70.
196. El-Sayed N, El-Gamal Y, Abbassy AA, et al. Monovalent type 1 oral poliovirus vaccine in newborns. N Engl J Med. 2008;359:1655–65.
197. Mohammed AJ, AlAwaidy S, Bawikar S, et al. Fractional doses of inactivated poliovirus vaccine in Oman. N Engl J Med. 2010;362:2351–9.
198. John TJ, Jain H, Ravishankar K, et al. Monovalent type 1 oral poliovirus vaccine among infants in India: report of two randomized double-blind controlled clinical trials. Vaccine. 2011;29:5793–801.
199. Estívariz CF, Jafari H, Sutter RW, et al. Immunogenicity of supplemental doses of poliovirus vaccine for children aged 6–9 months in Moradabad, India: a community-based, randomised controlled trial. Lancet Infect Dis. 2012;12:128–35.
200. Resik S, Tejeda A, Sutter RW, et al. Priming after a fractional dose of inactivated poliovirus vaccine. N Engl J Med. 2013;368:416–24.
201. Pallansch MA, Roos RP. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, et al., editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2007. p. 839–93.
202. Racaniello VR. *Picornaviridae*: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, et al., editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2007. p. 795–838.
203. Hogle JM, Chow M, Filman DJ. The three-dimensional structure of poliovirus at 2.9 Å resolution. Science. 1985;229:1358–65.
204. Toyoda H, Kohara M, Kataoka Y, et al. Complete nucleotide sequences of all three poliovirus serotype genomes: implication for genetic relationship, gene function and antigenic determinants. J Mol Biol. 1984;174:561–85.
205. Guillot S, Caro V, Cuervo N, et al. Natural genetic exchanges between vaccine and wild poliovirus strains in humans. J Virol. 2000;74:8434–43.
206. Liu H-M, Zheng D-P, Zhang L-B, Oberste MS, Kew OM, Pallansch MA. Serial recombination during circulation of type 1 wild-vaccine recombinant polioviruses in China. J Virol. 2003;77:10994–1005.
207. Minor PD, Almond JW. Poliovirus vaccines: molecular biology and immune response. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: ASM Press; 2002. p. 381–90.
208. Mendelsohn CL, Wimmer E, Racaniello VR. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell. 1989;56:855–65.
209. Gromeier M, Nomoto A. Determinants of poliovirus pathogenesis. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: ASM Press; 2002. p. 367–79.
210. Hogle JM. Poliovirus cell entry: common structural themes in viral cell entry pathways. Annu Rev Microbiol. 2002;56:677–702.
211. Le Bouvier GL. The D to C change in poliovirus particles. Br J Exp Pathol. 1959;40:605–20.
212. Wallis C, Menick JL. Cationic stabilization—a new property of enteroviruses. Virology. 1962;16:504–6.
213. Melnick JL, Ashkenazi A, Midulla VC, Wallis C, Bernstein A. Immunogenic potency of magnesium chloride-stabilized oral poliovaccine. J Am Med Assoc. 1963;185:406–8.
214. Rueckert RR. Picornaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, editors. Fields virology. 3rd ed. Philadelphia: Lippincott-Raven Publishers; 1996. p. 609–54.
215. Minor PD, Dunn G, Evans DMA, et al. The temperature sensitivity of the Sabin type 3 vaccine strain of poliovirus: molecular and structural effects of a mutation in the capsid protein VP3. J Gen Virol. 1989;70:1117–23.
216. Lentz KN, Smith AD, Geisler SC, et al. Structure of poliovirus type 2 Lansing complexed with antiviral agent SCH48973: comparison of the structural and biological properties of three poliovirus serotypes. Structure. 1997;5:961–78.
217. Rueckert RR, Pallansch MA. Preparation and characterization of encephalomyocarditis (EMC) virus. Methods Enzymol. 1981;78:315–25.
218. Page GS, Mosser AG, Hogle JM, Filman DJ, Rueckert RR, Chow M. Three-dimensional structure of poliovirus serotype 1 neutralizing determinants. J Virol. 1988;62:1781–94.
219. Filman DJ, Syed R, Chow M, Macadam AJ, Minor PD, Hogle JM. Structural factors that control conformational transitions and serotype specificity in type 3 poliovirus. EMBO J. 1989;8:1567–79.
220. Huovilainen A, Kinnunen L, Ferguson M, Hovi T. Antigenic variation among 173 strains of type 3 poliovirus isolated in Finland during the 1984 to 1985 outbreak. J Gen Virol. 1988;69:1941–8.
221. Shulman L, Manor J, Handsher R, et al. Molecular and antigenic characterization of a highly evolved derivative of the type 2 oral poliovaccine strain isolated from sewage in Israel. J Clin Microbiol. 2000;38:3729–34.
222. Yakovenko ML, Cherkasova EA, Rezapkin GV, et al. Antigenic evolution of vaccine-derived polioviruses: changes in individual epitopes and relative stability of the overall immunological properties. J Virol. 2006;80:2641–53.
223. DeVries AS, Harper J, Murray A, et al. Vaccine-derived poliomyelitis 12 years after infection in Minnesota. N Engl J Med. 2011;364:2316–23.
224. Harber J, Bernhardt G, Lu H-H, Sgro JY, Wimmer E. Canyon rim residues, including antigenic determinants, modulate serotype-specific binding of polioviruses to mutants of the poliovirus receptor. Virology. 1995;214:559–70.
225. He Y-N, Bowman VD, Mueller S, et al. Interaction of the poliovirus receptor with poliovirus. Proc Natl Acad Sci U S A. 2000;97:79–84.
226. Melnick JL. Antigenic crossings within poliovirus types. Proc Soc Exp Biol Med. 1955;89:131–3.
227. Uhlig J, Wiegers K, Dernick R. A new antigenic site of poliovirus recognized by an intertypic cross-neutralizing monoclonal-antibody. Virology. 1990;178:606–10.
228. Chen ZC, Chumakov K, Dragunsky E, et al. Chimpanzee-human monoclonal antibodies for treatment of chronic poliovirus

excretors and emergency postexposure prophylaxis. J Virol. 2011;85:4354–62.
229. Roizman B, Mayer MM, Roane Jr PR. Immunochemical studies of poliovirus. IV. Alteration of the immunologic specificity of purified poliomyelitis virus by heat and ultraviolet light. J Immunol. 1959;82:19–25.
230. Dowdle WR, Birmingham ME. The biologic principles of poliovirus eradication. J Infect Dis. 1997;175 Suppl 1:S286–92.
231. Allmond BW, Froeschle JE, Guilloud NB. Paralytic poliomyelitis in large laboratory primates: virologic investigation and report on the use of oral poliomyelitis virus (OPV) vaccine. Am J Epidemiol. 1967;85:229–39.
232. Koike S, Nomoto A. Poliomyelitis. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 339–51.
233. Howe HA, Bodian D. Poliomyelitis in the chimpanzee: a clinical-pathologic study. Bull Johns Hopkins Hosp. 1941;69:149–69.
234. Morbeck ME, Zihlman AL, Sumner DRJ, Galloway A. Poliomyelitis and skeletal asymmetry in Gombe chimpanzees. Primates. 1991;32:77–91.
235. Howe HA, Bodian D. Poliomyelitis in the cynomolgus monkey following oral inoculation. Am J Hyg. 1948;48:99–106.
236. Melnick JL, Von Magnus H. Comparative susceptibility of cynomolgus and other monkey species to poliomyelitis virus by the intracerebral and oral routes. Am J Hyg. 1948;48:107–12.
237. Sabin AB, Hennessen WA, Winsser J. Studies of variants of poliomyelitis virus. J Exp Med. 1954;99:551–76.
238. Crotty S, Lohman BL, Lu FX-S, Tang S, Miller CJ, Andino R. Mucosal immunization of cynomolgus macaques with two serotypes of live poliovirus vectors expressing simian immunodeficiency virus antigens: stimulation of humoral, mucosal, and cellular immunity. J Virol. 1999;73:9485–95.
239. Ida-Hosonuma M, Sasaki Y, Toyoda H, et al. Host range of poliovirus is restricted to simians because of a rapid sequence change of the poliovirus receptor gene during evolution. Arch Virol. 2003;148:29–44.
240. Khan S, Peng X, Yin J, Zhang P, Wimmer E. Characterization of the New World monkey homologues of human poliovirus receptor CD155. J Virol. 2008;82:7167–79.
241. Shelokov A, Habel K, McKinstry DW. Relation of poliomyelitis virus types to clinical disease and geographic distribution: a preliminary report. Ann N Y Acad Sci. 1955;61:998–1004.
242. Bodian D. Emerging concept of poliomyelitis infection. Science. 1955;122:105–8.
243. Sabin AB. Pathogenesis of poliomyelitis: reappraisal in light of new data. Science. 1956;123:1151–6.
244. Minor PD. Poliomyelitis. In: Nathanson N, editor. Viral pathogenesis. Philadelphia: Lippincott-Raven; 1997. p. 555–74.
245. Armstrong C. The experimental transmission of poliomyelitis to the Eastern cotton rat, *Sigmodon hispidus hispidus*. Public Health Rep. 1939;54:1719–21.
246. Li CP, Habel K. Adaptation of Leon strain of poliomyelitis to mice. Proc Soc Exp Biol Med. 1951;78:233–8.
247. Li CP, Schaeffer M. Adaptation of type 1 poliovirus to mice. Proc Soc Exp Biol Med. 1953;82:477–81.
248. Roca-Garcia M, Koprowski H, Jervis GA, Norton TW, Nelson TL, Cox HR. Immunization of humans with a chick embryo adapted strain of MEF1 poliomyelitis virus. J Immunol. 1956;77:123–31.
249. Holland JJ, McLaren LC, Syverton JT. The mammalian cell-virus relationship. IV. Infection of naturally insusceptible cells with enterovirus ribonucleic acid. J Exp Med. 1959;110:65–80.
250. Miller DA, Miller OJ, Dev VG, et al. Human chromosome 19 carries a poliovirus receptor gene. Cell. 1974;1:167–73.
251. Maier MK, Seth S, Czeloth N, et al. The adhesion receptor CD155 determines the magnitude of humoral immune responses against orally ingested antigens. Eur J Immunol. 2007;37:2214–25.
252. De Jesus N. Epidemics to eradication: the modern history of poliomyelitis. Virol J. 2007;4:70.
253. Arita M, Koike S, Aoki J, Horie H, Nomoto A. Interaction of poliovirus with its purified receptor and conformational alteration of the virion. J Virol. 1998;72:3578–86.
254. Belnap DM, McDermott BM, Filman DJ, et al. Three-dimensional structure of poliovirus receptor bound to poliovirus. Proc Natl Acad Sci U S A. 2000;97:73–8.
255. He Y, Mueller S, Chipman PR, et al. Complexes of poliovirus serotypes with their common cellular receptor, CD155. J Virol. 2003;77:4827–35.
256. Bergelson JM. Receptors. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 73–86.
257. Levy H, Bostina M, Filman DJ, Hogle JM. Cell entry: a biochemical and structural perspective. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 87–104.
258. Palmenberg A, Neubauer D, Skern T. Genome organization and encoded proteins. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 3–17.
259. Wimmer E, Paul A. The making of a picornavirus genome. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 33–55.
260. Martínez-Salas E, Ryan MD. Translation and protein processing. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 141–61.
261. Dougherty JD, Park N, Gustin KE, Lloyd RE. Interference with cellular gene expression. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 165–80.
262. Kirkegaard K, Semler BL. Genome replication II: the process. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 127–40.
263. Rozovics JM, Semler BL. Genome replication I: the players. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 107–25.
264. Bodian D. Histopathologic basis of clinical findings in poliomyelitis. Am J Med. 1949;6:563–78.
265. Jenkins GM, Rambaut A, Pybus OG, Holmes EC. Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. J Mol Evol. 2002;54:156–65.
266. de la Torre JC, Giachetti C, Semler BL, Holland JJ. High frequency of single- base transitions and extreme frequency of precise multiple-base reversion mutations in poliovirus. Proc Natl Acad Sci U S A. 1992;89:2531–5.
267. Drake JW. Rates of spontaneous mutation among RNA viruses. Proc Natl Acad Sci U S A. 1993;90:4171–5.
268. Wimmer E, Hellen CU, Cao X. Genetics of poliovirus. Annu Rev Genet. 1993;27:353–436.
269. Wells VR, Plotch SJ, DeStefano JJ. Determination of the mutation rate of poliovirus RNA-dependent RNA polymerase. Virus Res. 2001;74:119–32.
270. Arnold JJ, Cameron CE. Poliovirus RNA-dependent RNA polymerase ($3D^{pol}$): pre-steady-state kinetic analysis of ribonucleotide incorporation in the presence of Mg^{2+}. Biochemistry. 2004;43:5126–37.
271. Runckel C, Westesson O, Andino R, DeRisi JL. Identification and manipulation of the molecular determinants influencing poliovirus recombination. PLoS Pathog. 2013;9:e1003164.
272. Domingo E, Escarmís C, Sevilla N, et al. Basic concepts in RNA virus evolution. FASEB J. 1996;10:859–64.
273. Agol VI. Picornavirus genetics: an overview. In: Semler BL, Wimmer E, editors. Molecular biology of picornaviruses. Washington, D.C.: ASM Press; 2002. p. 269–84.

274. Domingo E. Virus evolution. In: Knipe DM, Howley PM, editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2007. p. 389–421.
275. Chumakov KM, Norwood LP, Parker ML, Dragunsky EM, Ran YX, Levenbook IS. RNA sequence variants in live poliovirus vaccine and their relation to neurovirulence. J Virol. 1992;66:966–70.
276. Minor PD, Ferguson M, Evans DMA, Almond JW, Icenogle JP. Antigenic structure of polioviruses of serotypes 1, 2, and 3. J Gen Virol. 1986;67:1283–91.
277. Cooper PD. Genetics of picornaviruses. In: Fraenkel-Conrat H, Wagner R, editors. Comprehensive virology. New York: Plenum Press; 1977. p. 133–207.
278. Tolskaya EA, Romanova LI, Kolesnikova MS, Agol VI. Intertypic recombination in poliovirus: genetic and biochemical studies. Virology. 1983;124:121–32.
279. Cammack N, Phillips A, Dunn G, Patel V, Minor PD. Intertypic genomic rearrangements of poliovirus strains in vaccinees. Virology. 1988;167:507–14.
280. Arita M, Zhu SL, Yoshida H, Yoneyama T, Miyamura T, Shimizu H. A Sabin 3-derived poliovirus recombinant contained a sequence homologous with indigenous human enterovirus species C in the viral polymerase coding region. J Virol. 2005;79:12650–7.
281. Escarmís C, Dávila M, Charpentier N, Bracho A, Moya A, Domingo E. Genetic lesions associated with Muller's ratchet in an RNA virus. J Mol Biol. 1996;264:255–67.
282. Agol VI. Picornaviruses as a model for studying the nature of RNA recombination. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 239–52.
283. Combelas N, Holmblat B, Joffret M-L, Colbère-Garapin F, Delpeyroux F. Recombination between poliovirus and coxsackie A viruses of species C: a model of viral genetic plasticity and emergence. Viruses. 2011;3:1460–84.
284. Fenner F, Henderson DA, Arita I, Ježek Z, Ladnyi ID. Smallpox and its eradication. Geneva: World Health Organization; 1988.
285. Kim-Farley RJ, Dave KH, Sokhey J, Mandke VB. Poliomyelitis surveillance and vaccine efficacy in Bombay, 1982–87. Bull World Health Organ. 1989;67:663–7.
286. Mandke BV, Pawar RM, Naik DD, Torne VP, Naiksatam AM. Review of poliomyelitis in Bombay (1982–1989): observations on the improved immunisation coverage. Indian J Public Health. 1990;34:200–8.
287. Peart AF, Rhodes AJ. An outbreak of poliomyelitis in Canadian Eskimos in wintertime. Can J Public Health. 1949;40:405–19.
288. Moses SH. Report on the epidemic of anterior poliomyelitis in the Nicobar Islands. Ind Med Gaz. 1948;83:355–63.
289. World Health Organization. Progress towards poliomyelitis eradication, Indonesia, 1995. Wkly Epidemiol Rec. 1996;71:253–5.
290. World Health Organization. Final stages of polio eradication WHO Western Pacific Region, 1997–1998. Wkly Epidemiol Rec. 1999;74:20–4.
291. Más Lago P, Cáceres VM, Galindo MA, et al. Persistence of vaccine-derived poliovirus following a mass vaccination campaign in Cuba: implications for stopping polio vaccination after global eradication. Int J Epidemiol. 2001;30:1029–34.
292. Gear JHS. Poliomyelitis in under-developed areas of the world. In: Poliomyelitis, vol. World Health Organization monograph series no. 26. Geneva: World Health Organization; 1955. p. 31–58.
293. Hemmes J, Winkler K, Kool S. Virus survival as a seasonal factor in influenza and poliomyelitis. Antonie Van Leeuwenhoek. 1962;28:221–33.
294. Sabin AB, Ramos-Alvarez M, Alvarez-Amezquita J, et al. Live, orally given poliovirus vaccine. Effects of rapid mass immunization on population under conditions of massive enteric infection with other viruses. J Am Med Assoc. 1960;173:1521–6.
295. Cruz RR. Cuba: mass polio vaccination program, 1962–1982. Rev Infect Dis. 1984;6 Suppl 2:S408–12.
296. Risi JB. The control of poliomyelitis in Brazil. Rev Infect Dis. 1984;6 Suppl 2:S400–3.
297. Wang K-A, Zhang L-B, Otten Jr MW, et al. Status of the eradication of indigenous wild poliomyelitis in the People's Republic of China. J Infect Dis. 1997;175 Suppl 1:S105–12.
298. Zhang J, Zhang LB, Otten Jr MW, et al. Surveillance for polio eradication in the People's Republic of China. J Infect Dis. 1997;175 Suppl 1:S122–34.
299. Onadeko MO, Familusi JB. Observations on the age and spatial distribution of paralytic poliomyelitis in Ibadan, Nigeria. Ann Trop Paediatr. 1990;10:133–8.
300. Singh J, Khare S, Bhatia R, Jain DC, Sokhey J. Epidemiological characteristics of poliomyelitis in Delhi, 1997. Indian Pediatr. 1999;36:1211–9.
301. Schonberger LB, McGowan Jr JE, Gregg MB. Vaccine-associated poliomyelitis in the United States, 1961-1972. Am J Epidemiol. 1976;104:202–11.
302. Centers for Disease Control and Prevention. Notes from the field: poliomyelitis outbreak—Republic of the Congo, September 2010–February 2011. MMWR Morb Mortal Wkly Rep. 2011;60:312–3.
303. Nkowane BM, Wassilak SGF, Orenstein WA, et al. Vaccine-associated paralytic poliomyelitis, U.S.A. 1973–1984. J Am Med Assoc. 1987;257:1335–40.
304. Strebel PM, Sutter RW, Cochi SL, et al. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. Clin Infect Dis. 1992;14:568–79.
305. Melnick JL, Ledinko N. Social serology: antibody levels in a normal young population during an epidemic of poliomyelitis. Am J Hyg. 1951;54:354–82.
306. Kew OM, Nottay BK. Molecular epidemiology of polioviruses. Rev Infect Dis. 1984;6 Suppl 2:S499–504.
307. Alexander LN, Seward JF, Santibanez TA, et al. Vaccine policy changes and epidemiology of poliomyelitis in the United States. J Am Med Assoc. 2004;292:1696–701.
308. Schonberger LB, Sullivan-Bolyai JZ, Bryan JA. Poliomyelitis in the United States. Adv Neurol. 1978;19:217–27.
309. Shimojo H. Poliomyelitis control in Japan. Rev Infect Dis. 1984;6 Suppl 2:S427–30.
310. Más Lago P. Eradication of poliomyelitis in Cuba: a historical perspective. Bull World Health Organ. 1999;77:681–7.
311. Schonberger LB, Kaplan J, Kim-Farley R, Moore M, Eddins DL, Hatch M. Control of paralytic poliomyelitis in the United States. Rev Infect Dis. 1984;6 Suppl 2:S424–6.
312. Bijkerk H. Surveillance and control of poliomyelitis in the Netherlands. Rev Infect Dis. 1984;6 Suppl 2:S451–6.
313. Mulders MN, Reimerink JHJ, Koopmans MPG, van Loon AM, van der Avoort HGAM. Genetic analysis of wild-type poliovirus importation into the Netherlands (1979-1995). J Infect Dis. 1997;176:617–24.
314. Oostvogel PM, van Wijngaarden JK, van der Avoort HGAM, et al. Poliomyelitis outbreak in an unvaccinated community in the Netherlands. Lancet. 1994;344:665–70.
315. Mulders MN, van Loon AM, van der Avoort HGAM, et al. Molecular characterization of the wild poliovirus type 3 epidemic in the Netherlands (1992-93). J Clin Microbiol. 1995;33:3252–6.
316. Pöyry T, Kinnunen L, Kapsenberg J, Kew O, Hovi T. Type 3 poliovirus/Finland/1984 is genetically related to common Mediterranean strains. J Gen Virol. 1990;71:2535–41.
317. Lipskaya GY, Chervonskaya EA, Belova GI, et al. Geographic genotypes (geotypes) of poliovirus case isolates from the Former Soviet Union: relatedness to other known poliovirus genotypes. J Gen Virol. 1995;76:1687–99.

318. Kim-Farley RJ, Rutherford G, Lichfield P, et al. Outbreak of paralytic poliomyelitis, Taiwan. Lancet. 1984;2:1322–4.
319. Heymann DL. House-to-house and school lameness surveys in Cameroon: a comparison of two methods for estimating the prevalence and annual incidence of paralytic poliomyelitis. Rev Infect Dis. 1984;6 Suppl 2:S376–8.
320. Melnick JL, Proctor RO, Ocampo AR, Diwan AR, Ben-Porath E. Free and bound virus in serum after administration of oral poliovirus vaccine. Am J Epidemiol. 1966;84:329–42.
321. Alexander Jr JP, Gary Jr HE, Pallansch MA. Duration of poliovirus excretion and its implications for acute flaccid paralysis surveillance: a review of the literature. J Infect Dis. 1997;175:S176–82.
322. Draper G. Acute poliomyelitis. Philadelphia: Blackiston's; 1917.
323. Racaniello VR. One hundred years of poliovirus pathogenesis. Virology. 2006;344:9–16.
324. World Health Organization. Poliomyelitis 1980, Parts 1 and 2. Wkly Epidemiol Rec. 1981;56:329–32, 37-41.
325. LaForce FM, Lichnevski MS, Keja J, Henderson RH. Clinical survey techniques to estimate prevalence and annual incidence of poliomyelitis in developing countries. Bull World Health Organ. 1980;58:609–20.
326. Basu RN. Magnitude of problem of poliomyelitis in India. Indian Pediatr. 1981;18:507–11.
327. John TJ. Poliomyelitis in India: prospects and problems of control. Rev Infect Dis. 1984;6:S438–41.
328. Melnick JL, Rennick V. Infectivity titers of enterovirus as found in human stools. J Med Virol. 1980;5:205–20.
329. Koprowski H, Norton TW, Jervis GA, et al. Clinical investigations on attenuated strains of poliomyelitis virus; use as a method of immunization of children with living virus. J Am Med Assoc. 1956;160:954–66.
330. Ogra PL, Karzon DT. Formation and function of poliovirus antibody in different tissues. Prog Med Virol. 1971;13:156–93.
331. Dowdle WR, Wolff C. Post-eradication poliovirus facility-associated community risks. Biologicals. 2006;34:127–32.
332. Angez M, Shaukat S, Alam MM, Sharif S, Khurshid A, Zaidi SSZ. Genetic relationships and epidemiological links between wild type 1 poliovirus isolates in Pakistan and Afghanistan. Virol J. 2012;9:51.
333. Nathanson N, Langmuir AD. The Cutter incident. Poliomyelitis following formaldehyde-inactivated poliovirus vaccination in the United States in the spring of 1955. II. Relationship of poliomyelitis to Cutter vaccine. Am J Hyg. 1963;78:29–60.
334. Offit PA. The Cutter incident: how America's first polio vaccine led to the growing vaccine crisis. New Haven: Yale University Press; 2005.
335. Deshpande JM, Nadkarni SS, Siddiqui ZA. Detection of MEF-1 laboratory reference strain of poliovirus type 2 in children with poliomyelitis in India in 2002 & 2003. Indian J Med Res. 2003;118:217–23.
336. World Health Organization. Update on actions taken following the isolation of MEF-1 reference poliovirus associated with acute flaccid paralysis cases in India in late 2002 and early 2003. Wkly Epidemiol Rec. 2003;78:284.
337. Nathanson N, Bodian D. Experimental poliomyelitis following intramuscular virus injection. III. The effect of passive antibody on paralysis and viremia. Bull Johns Hopkins Hosp. 1962;111:198–220.
338. Nathanson N. The pathogenesis of poliomyelitis: what we don't know. Adv Virus Res. 2008;71:1–50.
339. Nathanson N. David Bodian's contribution to the development of poliovirus vaccine. Am J Epidemiol. 2005;161:207–12.
340. Ren R, Constantini F, Gorgacz EJ, Lee JJ, Racaniello VR. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. Cell. 1990;63:353–62.
341. Koike S, Taya C, Kurata T, et al. Transgenic mice susceptible to poliovirus. Proc Natl Acad Sci U S A. 1991;88:951–5.
342. Crotty S, Hix L, Sigal LJ, Andino R. Poliovirus pathogenesis in a new poliovirus receptor transgenic mouse model: age-dependent paralysis and a mucosal route of infection. J Gen Virol. 2002;83:1707–20.
343. Ohka S, Igarashi H, Nagata N, et al. Establishment of a poliovirus oral infection system in human poliovirus receptor-expressing transgenic mice that are deficient in alpha/beta interferon receptor. J Virol. 2007;81:7902–12.
344. Ida-Hosonuma M, Iwasaki T, Yoshikawa T, et al. The alpha/beta interferon response controls tissue tropism and pathogenicity of poliovirus. J Virol. 2005;79:4460–9.
345. Gromeier M, Wimmer E. Mechanism of injury-provoked poliomyelitis. J Virol. 1998;72:5056–60.
346. Mueller S, Cao X, Welker R, Wimmer E. Interaction of the poliovirus receptor CD155 with the dynein light chain Tctex-1 and its implication for poliovirus pathogenesis. J Biol Chem. 2002;277:7897–904.
347. Gelfand HM, Fox JP, Leblanc DR, Elveback L. Studies on the development of natural immunity to poliomyelitis in Louisiana. V. Passive transfer of polioantibody from mother to fetus, and natural decline and disappearance of antibody in the infant. J Immunol. 1960;85:46–55.
348. Ogra PL, Fishaut M, Gallagher MR. Viral vaccination via the mucosal routes. Rev Infect Dis. 1980;2:352–69.
349. Ogra PL, Okayasu H, Czerkinsky C, Sutter RW. Mucosal immunity to poliovirus. Expert Rev Vaccines. 2011;10:1389–92.
350. Sabin AB. Experimental poliomyelitis by the tonsillopharyngeal route: with special reference to the influence of tonsillectomy on the development of bulbar poliomyelitis. J Am Med Assoc. 1938;111:605–10.
351. Paffenbarger Jr RS, Wilson VO. Previous tonsillectomy and current pregnancy as they affect risk of poliomyelitis attack. Ann NY Acad Sci. 1955;61:856–68.
352. Ogra PL. Effect of tonsillectomy and adenoidectomy on nasopharyngeal antibody response to poliovirus. N Engl J Med. 1971;284:59–64.
353. Sutter RW, Prevots R. Vaccine-associated paralytic poliomyelitis among immunodeficient persons. Infect Med. 1994;11:426–38.
354. Khetsuriani N, Prevots DR, Quick L, et al. Persistence of vaccine-derived polioviruses among immunodeficient persons with vaccine-associated paralytic poliomyelitis. J Infect Dis. 2003;188:1845–52.
355. Dalakas MC. The post-polio syndrome as an evolved clinical entity. Definition and clinical description. Ann N Y Acad Sci. 1995;753:68–80.
356. Ramlow J, Alexander M, LaPorte R, Kaufmann C, Kuller L. Epidemiology of the post-polio syndrome. Am J Epidemiol. 1992;136:769–86.
357. Melchers W, de Visser M, Jongen P, et al. The postpolio syndrome: no evidence for poliovirus persistence. Ann Neurol. 1992;32:728–32.
358. Banerjee K, Hlady WG, Andrus JK, Sarkar S, Fitzsimmons J, Abeykoon P. Poliomyelitis surveillance: the model used in India for polio eradication. Bull World Health Organ. 2000;78:321–9.
359. Andrus JK, Strebel PM, de Quadros CA, Olive JM. Risk of vaccine-associated paralytic poliomyelitis in Latin America, 1989–91. Bull World Health Organ. 1995;73:33–40.
360. Brodie M, Park WH. Active immunization against poliomyelitis. Am J Public Health. 1936;26:119–25.
361. Kolmer JA. Vaccination against acute anterior poliomyelitis. Am J Public Health. 1936;26:126–35.
362. Leake JP. Poliomyelitis following vaccination against this disease. J Am Med Assoc. 1935;105:2152.

363. Morgan IM. Immunization of monkeys with formalin-inactivated poliomyelitis viruses. Am J Epidemiol. 1948;48:394–406.
364. Enders JF, Weller TH, Robbins FC. Alterations in pathogenicity for monkeys of Brunhilde strain of poliomyelitis virus following cultivation in human tissues. Fed Proc. 1952;11:467.
365. Ferguson M, Wood DJ, Minor PD. Antigenic structure of poliovirus in inactivated vaccines. J Gen Virol. 1993;74:685–90.
366. van Wezel AL, van Steenis G, van der Marel P, Osterhaus AD. Inactivated poliovirus vaccine: current production methods and new developments. Rev Infect Dis. 1984;6 Suppl 2:S335–40.
367. Theiler M, Smith HH. The effect of prolonged cultivation in vitro upon the pathogenicity of yellow fever virus. J Exp Med. 1937;65:767–86.
368. Theiler M. A mutant strain of the Lansing virus. In: Mechanisms of immunity in poliomyelitis. Baltimore: Department of Epidemiology, School of Public Health and Hygiene, the Johns Hopkins University; 1946. p. 29–36.
369. Li CP, Schaeffer M. Isolation of a non-neurotropic variant of type 1 poliomyelitis virus. Proc Soc Exp Biol Med. 1954;87:148–53.
370. Sabin AB, Boulger LR. History of Sabin attenuated poliovirus oral live vaccine strains. J Biol Stand. 1973;1:115–8.
371. Cabasso VJ, Jervis GA, Moyer AW, Roca-Garcia M, Orsi EV, Cox HR. Cumulative testing experience with consecutive lots of oral poliomyelitis vaccine. Live poliovirus vaccines First International Conference on Live Poliovirus Vaccines. Washington, D.C: Pan American Sanitary Bureau; 1959. p. 102–34.
372. Pan American Health Organization. Live Poliovirus Vaccines. Washington, D.C.: Special Publications of the Pan American Health Organization, Nos. 44 and 50; 1959, 1960.
373. Payne AMM. Field safety and efficacy of live attenuated poliovirus vaccines. Poliomyelitis papers and discussion presented at the fifth international poliomyelitis conference. Philadelphia: J.B. Lippincott; 1961. p. 257–61.
374. Racaniello VR, Ren R. Poliovirus biology and pathogenesis. Curr Top Microbiol Immunol. 1996;206:305–25.
375. Kitamura N, Semler BL, Rothberg PG, et al. Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature. 1981;291:547–53.
376. Racaniello VR, Baltimore D. Molecular cloning of poliovirus cDNA and determination of the complete nucleotide sequence of the viral genome. Proc Natl Acad Sci U S A. 1981;78:4887–91.
377. Racaniello VR, Baltimore D. Cloned poliovirus complementary DNA is infectious in mammalian cells. Science. 1981;214:916–9.
378. Omata T, Kohara M, Kuge S, et al. Genetic analysis of the attenuation phenotype of poliovirus type 1. J Virol. 1986;58:348–58.
379. Bouchard MJ, Lam DH, Racaniello VR. Determinants of attenuation and temperature sensitivity in the type 1 poliovirus Sabin strain. J Virol. 1995;69:4972–8.
380. Kawamura N, Kohara M, Abe S, et al. Determinants in the 5′ noncoding region of poliovirus Sabin 1 RNA that influence the attenuation phenotype. J Virol. 1989;63:1302–9.
381. Ren R, Moss EG, Racaniello VR. Identification of two determinants that attenuate vaccine-related type 2 poliovirus. J Virol. 1991;65:1377–82.
382. Macadam AJ, Pollard SR, Ferguson G, et al. Genetic basis of attenuation of the Sabin type 2 vaccine strain of poliovirus in primates. Virology. 1993;192:18–26.
383. Westrop GD, Wareham KA, Evans DM, et al. Genetic basis of attenuation of the Sabin type 3 oral poliovirus vaccine. J Virol. 1989;63:1338–44.
384. Tardy-Panit M, Blondel B, Martin A, Tekaia F, Horaud F, Delpeyroux F. A mutation in the RNA polymerase of poliovirus type 1 contributes to attenuation in mice. J Virol. 1993;67:4630–8.
385. Paul AV, Mugavero J, Yin J, et al. Studies on the attenuation phenotype of polio vaccines: poliovirus RNA polymerase derived from Sabin type 1 sequence is temperature sensitive in the uridylylation of VPg. Virology. 2000;272:72–84.
386. Rezapkin GV, Fan L, Asher DM, Fibi MR, Dragunsky EM, Chumakov KM. Mutations in Sabin 2 strain of poliovirus and stability of attenuated phenotype. Virology. 1999;258:152–60.
387. Stanway G, Hughes PJ, Mountford RC, et al. Comparison of the complete nucleotide sequences of the genomes of the neurovirulent poliovirus P3/Leon/37 and its attenuated Sabin vaccine derivative P3/Leon 12a1b. Proc Natl Acad Sci U S A. 1984;81:1539–43.
388. Macadam AJ, Arnold C, Howlett J, et al. Reversion of the attenuated and temperature-sensitive phenotypes of the Sabin type 3 strain of poliovirus in vaccinees. Virology. 1989;172:408–14.
389. Muzychenko AR, Lipskaya GY, Maslova SV, et al. Coupled mutations in the 5′-untranslated region of the Sabin poliovirus strains during *in vivo* passage: structural and functional implications. Virus Res. 1991;21:111–22.
390. Haller AA, Stewart SR, Semler BL. Attenuation stem-loop lesions in the 5′ noncoding region of poliovirus RNA: neuronal cell-specific translation defects. J Virol. 1996;70:1467–74.
391. Gromeier M, Bossert B, Arita M, Nomoto A, Wimmer E. Dual stem loops within the poliovirus internal ribosome entry site controls neurovirulence. J Virol. 1999;73:958–64.
392. Svitkin Y, Cammack N, Minor PD, Almond JW. Translation deficiency of the Sabin type 3 poliovirus genome: association with an attenuating mutation C472-U. Virology. 1990;175:103–9.
393. Minor PD, Dunn G. The effect of sequences in the 5′ non-coding region on the replication of polioviruses in the human gut. J Gen Virol. 1988;69:1091–6.
394. Guest S, Pilipenko E, Sharma K, Chumakov K, Roos RP. Molecular mechanisms of attenuation of the Sabin strain of poliovirus type 3. J Virol. 2004;78:11097–107.
395. Sutter RW, Cáceres VM, Más Lago P. The role of routine polio immunization in the post-certification era. Bull World Health Organ. 2004;82:31–9.
396. Terry L. The association of cases of poliomyelitis with the use of type 3 oral poliomyelitis vaccines. Washington, D.C: U.S. Department of Health, Education and Welfare; 1962.
397. WHO Consultative Group. The relation between acute persisting spinal paralysis and poliomyelitis vaccine –- results of a ten-year enquiry. Bull World Health Organ. 1982;60:231–42.
398. Esteves K. Safety of oral poliomyelitis vaccine: results of a WHO enquiry. Bull World Health Organ. 1988;66:739–46.
399. Joce R, Wood D, Brown D, Begg N. Paralytic poliomyelitis in England and Wales, 1985–91. Br Med J. 1992;305:79–82.
400. Kohler KA, Banerjee K, Hlady GW, Andrus JK, Sutter RW. Vaccine-associated paralytic poliomyelitis in India during 1999: decreased risk despite massive use of oral polio vaccine. Bull World Health Organ. 2002;80:210–6.
401. Centers for Disease Control and Prevention. Poliomyelitis prevention in the United States: Introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 1997;46(No. RR-3):1–25.
402. Georgescu MM, Balanant J, Macadam A, et al. Evolution of the Sabin type 1 poliovirus in humans: characterization of strains isolated from patients with vaccine-associated paralytic poliomyelitis. J Virol. 1997;71:7758–68.
403. Lipskaya GY, Muzychenko AR, Kutitova OK, et al. Frequent isolation of intertypic poliovirus recombinants with serotype 2 specificity from vaccine-associated polio cases. J Med Virol. 1991;35:290–6.
404. Cuervo NS, Guillot S, Romanenkova N, et al. Genomic features of intertypic recombinant Sabin poliovirus strains excreted by primary vaccinees. J Virol. 2001;75:5740–51.

405. Minor PD. Polio eradication, cessation of vaccination and re-emergence of disease. Nat Rev Microbiol. 2004;2:473–82.
406. Rosen FS, Cooper MD, Wedgwood RJP. The primary immunodeficiencies. N Engl J Med. 1995;333:431–40.
407. Hennessey KA, Lago H, Diomande F, et al. Poliovirus vaccine shedding among persons with HIV in Abidjan, Cote d'Ivoire. J Infect Dis. 2005;192:2124–8.
408. Melnick JL. Advantages and disadvantages of the killed and live poliomyelitis vaccines. Bull World Health Organ. 1978;56:21–38.
409. World Health Organization. Progress towards poliomyelitis eradication in India, 2002. Wkly Epidemiol Rec. 2003;78:66–71.
410. Grassly NC, Wenger J, Durrani S, et al. Protective efficacy of a monovalent oral type 1 poliovirus vaccine: a case-control study. Lancet. 2007;369:1356–62.
411. WHO Collaborative Study Group on Oral and Inactivated Poliovirus Vaccines. Combined immunization of infants with oral and inactivated poliovirus vaccines: results of a randomized trial in The Gambia, Oman, and Thailand. J Infect Dis. 1997;175 Suppl 1:S215–27.
412. World Health Organization. Global routine vaccination coverage, 2011. Wkly Epidemiol Rec. 2012;87:432–5.
413. Kim-Farley R, Bart K, Schonberger L, et al. The epidemiology of poliomyelitis in the United States: virtual elimination of disease caused by wild virus. Lancet. 1984;2:315–7.
414. Oker-Blom N, Penttinen K, Weckström P. Inactivated poliovirus vaccine in Finland. Rev Infect Dis. 1984;6 Suppl 2:S461–2.
415. Varughese PV, Carter AO, Acres SE, Furesz J. Eradication of indigenous poliomyelitis in Canada: impact of immunization strategies. Can J Public Health. 1989;80:363–8.
416. Emerson CI, Singer PA. Is there an ethical obligation to complete polio eradication? Lancet. 2010;375:1340–1.
417. Duintjer Tebbens RJ, Pallansch MA, Cochi SL, et al. Economic analysis of the global polio eradication initiative. Vaccine. 2011;29:334–43.
418. Gregg MB. Paralytic poliomyelitis can be eliminated. Rev Infect Dis. 1984;6 Suppl 2:S577–80.
419. Nomoto A, Arita I. Eradication of poliomyelitis. Nat Immunol. 2002;3:205–8.
420. Henderson RH. The expanded programme on immunization of the World Health Organization. Rev Infect Dis. 1984;6 Suppl 2:S475–9.
421. Castillo-Solórzano C, Marsigli C, Bravo-Alcántara P, et al. Elimination of rubella and congenital rubella syndrome in the Americas. J Infect Dis. 2011;204 Suppl 2:S571–8.
422. Reef SE, Strebel P, Dabbagh A, Gacic-Dobo M, Cochi S. Progress toward control of rubella and prevention of congenital rubella syndrome–worldwide, 2009. J Infect Dis. 2011;204 Suppl 1:S24–7.
423. World Health Organization. Progress in global control and regional elimination of measles, 2000–2011. Wkly Epidemiol Rec. 2013;88:29–36.
424. Sever J. Rotary International: a partner in polio eradication. Dev Biol (Basel). 2001;105:105–8.
425. World Health Organization. Progress towards poliomyelitis eradication, South-East Asia, 1998–1999. Wkly Epidemiol Rec. 2000;75:213–6.
426. Ku F-C, Dong D-X, Shi O-S, Niu J-T, Yang H-H. Poliomyelitis in China. J Infect Dis. 1982;146:552–7.
427. Yang B, Zhang J, Otten Jr MW, et al. Eradication of poliomyelitis: progress in the People's Republic of China. Pediatr Infect Dis J. 1995;14:308–14.
428. Fine PEM, Carneiro IAM. Transmissibility and persistence of oral polio vaccine viruses: implications for the global poliomyelitis eradication initiative. Am J Epidemiol. 1999;150:1001–21.
429. World Health Organization. Progress towards the global eradication of poliomyelitis, 2001. Wkly Epidemiol Rec. 2002;77:98–107.
430. World Health Organization. Expanded programme on immunization–certification of poliomyelitis eradication–the Americas, 1994. Wkly Epidemiol Rec. 1994;69:293–5.
431. World Health Organization. Certification of poliomyelitis eradication, European region, June 2002. Wkly Epidemiol Rec. 2002;77:221–3.
432. World Health Organization. Progress toward global poliomyelitis eradication, 2000. Wkly Epidemiol Rec. 2001;76:126–31.
433. World Health Organization. Progress towards polio eradication, Somalia and southern Sudan. Wkly Epidemiol Rec. 1999;74:246–52.
434. Tangermann RH, Hull HF, Jafari H, Nkowane B, Everts H, Aylward RB. Eradication of poliomyelitis in countries affected by conflict. Bull World Health Organ. 2000;78:330–8.
435. Hagiwara A, Yoneyama T, Yoshii K, et al. Genetic analysis of wild polioviruses towards the eradication of poliomyelitis from the Western Pacific Region. Jpn J Infect Dis. 1999;52:146–9.
436. World Health Organization. Progress towards the global eradication of poliomyelitis, 2002. Wkly Epidemiol Rec. 2003;78:138–44.
437. World Health Organization. Somalia is again polio-free. Wkly Epidemiol Rec. 2008;83:117–8.
438. World Health Organization. Progress towards eradicating poliomyelitis in India, January 2009–October 2010. Wkly Epidemiol Rec. 2010;85:497–503.
439. Chumakov K, Kew O. The poliovirus eradication initiative. In: Ehrenfeld E, Domingo E, Roos R, editors. The picornaviruses. Washington, D.C.: ASM Press; 2010. p. 449–59.
440. World Health Organization. Progress towards poliomyelitis eradication in Nigeria, January 2002 to March 2003. Wkly Epidemiol Rec. 2003;78:210–5.
441. World Health Organization. Progress towards poliomyelitis eradication in India, 2003. Wkly Epidemiol Rec. 2004;79:121–5.
442. World Health Organization. Progress towards global eradication of poliomyelitis, 2003 and January–April 2004. Wkly Epidemiol Rec. 2004;79:229–34.
443. World Health Organization. Progress towards poliomyelitis eradication in Nigeria, January 2003–March 2004. Wkly Epidemiol Rec. 2004;79:162–7.
444. World Health Organization. Progress towards interrupting wild poliovirus transmission, January 2006–May 2007. Wkly Epidemiol Rec. 2007;82:245–51.
445. World Health Organization. Progress towards interrupting of wild poliovirus transmission worldwide, January 2007–April 2008. Wkly Epidemiol Rec. 2008;83:170–6.
446. Morvan JM, Chezzi C, Gouandjika I, Reimerink JH, van der Avoort HG. The molecular epidemiology of type 1 poliovirus in Central African Republic. J Gen Virol. 1997;78:591–9.
447. Jenkins HE, Aylward RB, Gasasira A, et al. Implications of a circulating vaccine-derived poliovirus in Nigeria. N Engl J Med. 2010;362:2360–9.
448. Wassilak S, Pate MA, Wannemuehler K, et al. Outbreak of type 2 vaccine-derived poliovirus in Nigeria, 2005–2009: emergence and widespread circulation in an underimmunized population. J Infect Dis. 2011;203:898–909.
449. World Health Organization. Advisory Committee on Poliomyelitis Eradication: recommendations on the use of bivalent oral poliovirus vaccine types 1 and 3. Wkly Epidemiol Rec. 2009;84:289–90.
450. Cochi SL, Linkins RW. The final phase of polio eradication: new vaccines and complex choices. J Infect Dis. 2012;205:169–71.
451. O'Reilly KM, Durry E, ul Islam O, et al. The effect of mass immunisation campaigns and new oral poliovirus vaccines on the incidence of poliomyelitis in Pakistan and Afghanistan, 2001–11: a retrospective analysis. Lancet. 2012;380:491–8.
452. World Health Organization. 16th Informal Consultation of the Global Polio Laboratory Network, 22–23 September 2010 (http://www.polioeradication.org/Portals/0/Document/Resources/

GPLN_publications/GPLN_Meeting_recommendations_2010.pdf). Geneva. 2010.
453. MacLennan C, Dunn G, Huissoon AP, et al. Failure to clear persistent vaccine-derived neurovirulent poliovirus infection in an immunodeficient man. Lancet. 2004;363:1509–13.
454. Bellmunt A, May G, Zell R, Pring-Akerblom P, Verhagen W, Heim A. Evolution of poliovirus type I during 5.5 years of prolonged enteral replication in an immunodeficient patient. Virology. 1999;265:178–84.
455. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, July 2009–March 2011. MMWR Morb Mortal Wkly Rep. 2011;60:846–50.
456. Liang X, Zhang Y, Xu W, et al. An outbreak of poliomyelitis caused by type 1 vaccine-derived poliovirus in China. J Infect Dis. 2006;194:545–51.
457. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, January 2006–August 2007. MMWR Morb Mortal Wkly Rep. 2007;56:996–1001.
458. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, January 2008–June 2009. MMWR Morb Mortal Wkly Rep. 2009;58:1002–6.
459. Rakoto-Andrianarivelo M, Gumede N, Jegouic S, et al. Reemergence of recombinant vaccine-derived poliovirus outbreak in Madagascar. J Infect Dis. 2008;197:1427–35.
460. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, July 2012–December 2013. Morbid Mortal Wkly Rep. 2014;63:242–8.
461. Wringe A, Fine PEM, Sutter RW, Kew OM. Estimating the extent of vaccine-derived poliovirus infection. PLoS One. 2008;3:e3433.
462. Yoneyama T, Hagiwara A, Hara M, Shimojo H. Alteration in oligonucleotide fingerprint patterns of the viral genome in poliovirus type 2 isolated from paralytic patients. Infect Immun. 1982;37:46–53.
463. Minor P. Characteristics of poliovirus strains from long-term excretors with primary immunodeficiencies. Dev Biol (Basel). 2001;105:75–80.
464. Martín J. Vaccine-derived poliovirus from long term excretors and the end game of polio eradication. Biologicals. 2006;34:117–22.
465. MacCallum FO. Hypogammglobulinaemia in the United Kingdom. VII. The role of humoral antibodies in protection against and recovery from bacterial and virus infections in hypogammaglobulinaemia. Medical Research Council Special Report Series. 1971;310:72–85.
466. Labadie K, Pelletier I, Saulnier A, Martin J, Colbere-Garapin F. Poliovirus mutants excreted by a chronically infected hypogammaglobulinemic patient establish persistent infections in human intestinal cells. Virology. 2004;318:66–78.
467. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses. MMWR Morb Mortal Wkly Rep. 2006;55:1093–7.
468. Misbah SA, Lawrence PA, Kurtz JB, Chapel HM. Prolonged faecal excretion of poliovirus in a nurse with common variable hypogammaglobulinaemia. Postgrad Med J. 1991;67:301–3.
469. Shahmahmoodi S, Mamishi S, Aghamohammadi A, et al. Vaccine-associated paralytic poliomyelitis in immunodeficient children, Iran, 1995-2008. Emerg Infect Dis. 2010;16:1133–6.
470. Hidalgo S, Garcia Erro M, Cisterna D, Freire MC. Paralytic poliomyelitis caused by a vaccine-derived polio virus in an antibody-deficient Argentinean child. Pediatr Infect Dis J. 2003;22:570–2.
471. Centers for Disease Control and Prevention. Laboratory surveillance for wild and vaccine-derived polioviruses, January 2003–June 2004. MMWR Morb Mortal Wkly Rep. 2004;53:990–3.
472. Tharmaphornpilas P. Vaccine-derived poliovirus, Thailand, 2003. Emerg Infect Dis. 2005;11:777–8.
473. Parvaneh N, Shahmahmoudi S, Tabatabai H, et al. Vaccine-associated paralytic poliomyelitis in a patient with MHC class II deficiency. J Clin Virol. 2007;39:145–8.
474. de Silva R, Gunasena S, Ratnayake D, et al. Prevalence of prolonged and chronic poliovirus excretion among persons with primary immune deficiency disorders in Sri Lanka. Vaccine. 2012;30:7561–5.
475. Gumede N, Muthambi V, Schoub BD. Immunodeficiency-associated vaccine-derived poliovirus type 3 in infant, South Africa, 2011. Emerg Infect Dis. 2012;18:992–4.
476. Yong PFK, Thaventhiran JED, Grimbacher B. "A rose is a rose is a rose," but CVID is Not CVID common variable immune deficiency (CVID), what do we know in 2011? Adv Immunol. 2011;111:47–107.
477. Oberste MS, Moore D, Anderson B, Pallansch MA, Pevear DC, Collett MS. In vitro antiviral activity of V-073 against polioviruses. Antimicrob Agents Chemother. 2009;53:4501–3.
478. Adu FD, Iber J, Bukbuk D, et al. Isolation of recombinant type 2 vaccine-derived poliovirus (VDPV) from a Nigerian child. Virus Res. 2007;127:17–25.
479. Centers for Disease Control and Prevention. Laboratory surveillance for wild and vaccine-derived polioviruses, January 2002–June 2003. MMWR Morb Mortal Wkly Rep. 2003;52:913–6.
480. Roivainen M, Blomqvist S, Al-Hello H, et al. Highly divergent neurovirulent vaccine-derived polioviruses of all three serotypes are recurrently detected in Finnish sewage. Euro Surv. 2010;15:pii/19566.
481. Smith J, Leke R, Adams A, Tangermann RH. Certification of polio eradication: process and lessons learned. Bull World Health Organ. 2004;82:24–30.
482. Breman JG, Arita I. The confirmation and maintenance of smallpox eradication. N Engl J Med. 1980;303:1263–73.
483. Chiba Y, Murakami H, Kobayashi M, et al. A case of poliomyelitis associated with infection of wild poliovirus in Qinghai Province, China, in October 1999. Jpn J Infect Dis. 2000;53:135–6.
484. World Health Organization. Wild poliovirus imported into Qinghai province, China. Wkly Epidemiol Rec. 2000;75:55–7.
485. Kojouharova M, Zuber PLF, Gyurova S, et al. Importation and circulation of poliovirus in Bulgaria in 2001. Bull World Health Organ. 2003;81:476–81.
486. World Health Organization. Certification of poliomyelitis eradication: Western Pacific Region. Wkly Epidemiol Rec. 2000;75:399–400.
487. World Health Organization. Global action plan for laboratory containment of wild polioviruses, 2nd ed. Geneva: World Health Organization; 2003. Report No.: WHO/V&B/03.11.
488. Dowdle WR, Wolff C, Sanders R, Lambert S, Best M. Will containment of wild poliovirus in laboratories and inactivated poliovirus vaccine production sites be effective for global certification? Bull World Health Organ. 2004;82:59–62.
489. Centers for Disease Control and Prevention. National laboratory inventory for global poliovirus containment—United States, November 2003. MMWR Morb Mortal Wkly Rep. 2004;53:457–9.
490. World Health Organization. Completion of national laboratory inventories for wild poliovirus containment—WHO Western Pacific Region, December 2008. Wkly Epidemiol Rec. 2009;84:373–82.
491. Anis E, Kopel E, Singer SR, et al. Insidious reintroduction of wild poliovirus into Israel, 2013. Eurosurveillance. 2013;18:1–5.
492. Resik S, Tejeda A, Más Lago P, et al. Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine administered intradermally by needle-free device in Cuba. J Infect Dis. 2010;201:1344–52.
493. Tano Y, Shimizu H, Martin J, Nishimura Y, Simizu B, Miyamura T. Antigenic characterization of a formalin-inactivated poliovirus vaccine derived from live-attenuated Sabin strains. Vaccine. 2007;25:7041–6.
494. Verdijk P, Rots NY, Bakker WA. Clinical development of a novel inactivated poliomyelitis vaccine based on attenuated Sabin poliovirus strains. Expert Rev Vaccines. 2011;10:635–44.

495. Liao G, Li R, Li C, et al. Safety and immunogenicity of inactivated poliovirus vaccine made from Sabin strains: a phase II, randomized, positive-controlled trial. J Infect Dis. 2012;205:237–43.
496. Burns CC, Shaw J, Campagnoli R, et al. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. J Virol. 2006;80:3259–72.
497. Macadam AJ, Ferguson G, Stone DM, et al. Rational design of genetically stable, live-attenuated poliovirus vaccines of all three serotypes: relevance to poliomyelitis eradication. J Virol. 2006;80:8653–63.
498. Mueller S, Papamichail D, Coleman JR, Skiena S, Wimmer E. Reduction of the rate of poliovirus protein synthesis through large-scale codon deoptimization causes attenuation of viral virulence by lowering specific infectivity. J Virol. 2006;80:9687–96.
499. Vignuzzi M, Wendt E, Andino R. Engineering attenuated virus vaccines by controlling replication fidelity. Nat Med. 2008;14:154–61.
500. Thompson KM, Pallansch MA, Duintjer Tebbens RJ, Wassilak SG, Kim JH, Cochi SL. Preeradication vaccine policy options for poliovirus infection and disease control. Risk Anal. 2013;33:516–43.
501. World Health Organization. Global Invasive Bacterial Vaccine Preventable Diseases (IB-VPD). Information and Surveillance Bulletin. 2013.
502. World Health Organization. Progress in introduction of pneumococcal conjugate vaccine worldwide, 2000–2012. Wkly Epidemiol Rec. 2013;88:173–80.
503. World Health Organization. Meningococcal disease in countries of the African meningitis belt, 2012—emerging needs and future perspectives. Wkly Epidemiol Rec. 2013;88:129–36.
504. MacCallum FO. Hypogammaglobulinaemia in the United Kingdom. VII. The role of humoral antibodies in the protection against and recovery from bacterial and virus infections in hypogammaglobulinaemia. Spec Rep Ser Med Res Counc (G B). 1971;310:72–85.