

The Biologic Principles of Poliovirus Eradication

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The biologic principles for the global eradication of poliomyelitis are as follows: Poliovirus causes acute, nonpersistent infections, virus is transmitted by infectious humans or their waste, survival of virus in the environment is finite, humans are the only reservoir, and immunization with polio vaccine interrupts virus transmission. These principles appear to be sound. The potential for prolonged virus excretion by immunocompromised patients requires further definition, although there is no epidemiologic evidence of a threat to eradication. Survival of poliovirus in the environment is highly variable, but viral inactivation is usually complete within months. Higher primates may be infected with poliovirus, but they are unlikely reservoirs in nature. The only poliovirus reservoir remaining after eradication will be laboratory stocks. Serious attention must be given to reducing this potential source of infection. Polio eradication through immunization is evidenced by the documented absence of poliomyelitis in an increasing number of countries and the progressive disappearance of poliovirus genotypes.

The global strategy developed by the World Health Organization (WHO) for the eradication of poliovirus by the year 2000 is a product of nearly a century of biologic and epidemiologic observations and nearly 40 years of experience with prevention of poliomyelitis through immunization. Polio vaccine has been successfully used to eliminate indigenous poliomyelitis in multiple countries, including the entire Americas. Key to global eradication are the assumptions that poliovirus causes acute, nonpersistent infections, virus is transmitted only by infectious humans or their waste, survival of virus in the environment is finite, humans are the only reservoir, and immunization with polio vaccine interrupts virus transmission. Here we review the biologic basis for these assumptions.

Poliovirus Causes Acute, Nonpersistent Infections

Poliovirus types 1, 2, and 3 are members of the family Picornaviridae, genus *Enterovirus*, characterized by icosahedral symmetry, small size (22–30 nm in diameter), and the absence of an envelope. The viruses have four major polypeptides and a genome consisting of one strand of positive-sense infectious RNA.

All three types of poliovirus cause acute, short-term infections. Commonly, the ingested virus infects epithelial cells of the oropharynx, the tonsils, the lymph nodes of the neck, the small intestines, and Peyer's patches. Infection of susceptible cells progresses through cycles of viral replication, with virus release through cell destruction. The central nervous system may be infected through virus in circulating blood. Infection

may result in no or mild illness, aseptic meningitis, or paralytic poliomyelitis. Type 1 is the most paralytogenic and type 2 the least. Inapparent infections may exceed apparent infections by a margin of 100–1000 or more [1].

Poliovirus may be found in specimens from the oropharynx for 1–2 weeks and from the intestines for 1–2 months after infection. Excretion may be intermittent [2], but poliovirus is found in feces of virtually all poliomyelitis patients at the onset of paralysis. Thereafter, the rate of fecal excretion decreases by 10%–15% per week within the first month after onset of paralysis [3]. The virus is eliminated by normal immune defenses, usually within 1 or 2 months in immunocompetent persons.

There is no long-term carrier state of poliovirus in immunocompetent persons. Once fecal virus excretion has ceased, that person no longer is a source of infection. However, harboring of poliovirus may occur among immunocompromised persons. Vaccine virus was recovered from the cerebrospinal fluid of a patient 1 year after immunization [4] and continually from the stools of 2 patients 17 and 24 months, respectively, after immunization [5]. In a patient with agammaglobulinemia, replication of vaccine virus persisted for at least 684 days [6]. No information is available on wild virus excretion. Prolonged excretion of vaccine-like strains is described mostly in persons with B cell deficiencies [4, 7, 8] and rarely in persons with T cell deficiencies [9, 10]. B cell and B/T cell deficiencies predominate among immunocompromised persons with vaccine-associated paralytic poliomyelitis [11].

The potential for prolonged excretion of wild type poliovirus among persons infected with human immunodeficiency virus (HIV) is unknown. In one study of 198 consecutive adult patients admitted to the internal medicine ward in a Kinshasa hospital, only rotavirus, coronavirus, adenovirus, and small round structured viruses were detected [12]. Of these patients, 57% were HIV-positive and, among the HIV-positive patients,

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50% were classified as WHO AIDS stage IV. In this study, HIV-positive patients tended to shed virus more frequently, but there was no significant difference between HIV-positive and HIV-negative patients in the prevalence of enteric viruses in stool specimens. The short life expectancy and the predominant T cell deficiency of severely immunocompromised HIV-positive patients argues against the risk of prolonged poliovirus excretion. However, the frequency and duration of poliovirus excretion (wild and vaccine-like) among HIV-positive and other immunocompromised persons, especially children, should be further investigated. The findings from such studies have important implications for when and how to stop vaccination with oral polio vaccine (OPV) after eradication of wild poliovirus.

Virus Is Transmitted Only by Infectious Humans or Their Waste

Poliovirus is transmitted by infected humans directly or indirectly by droplets or aerosols from the oropharynx and by fecal contamination of hands, eating utensils, food, and water [1]. Epidemiologically, at least 80% of transmission appears to be person-to-person (fecal-oral or oral-oral), with the most effective source being a patient within 3 days before and after the first prodromal symptoms [13].

At the beginning of this century, it was widely believed that poliovirus was transmitted by insects. The mosquito had been shown to be the vector of yellow fever, and an insect vector was thought to explain the seasonal patterns of poliomyelitis as well. All biting insects—mosquitoes, lice, bedbugs, midges, and sand flies—were suspected and some were investigated without success [14].

Greatest attention was given to the biting stable fly because of an erroneous report of experimental transmission of polio between monkeys not in physical contact. Attention shifted to feces-eating flies in the early 1940s, with reports of virus isolations from several genera and species. Poliovirus was demonstrated to survive in the gut of active feces-eating flies for 2–3 weeks [15], in hibernating flies for 3 months [16], and in the cockroach for 51 days [17]. Poliovirus does not replicate in these insects.

The house fly has been proven to mechanically contaminate food during an epidemic [18]. Whether contamination of objects or food by flies plays any significant role in polio epidemics is questionable. Early attempts to control epidemics through fly abatement were remarkably unsuccessful [14, 19]. In any case, the role of flies as a potential mechanisms of extrahuman transmission of poliovirus becomes irrelevant in the absence of infected humans and their environmental waste.

Survival of Virus in the Environment Is Finite

Poliovirus is highly resistant to inactivation by common disinfectants such as alcohol and cresols (Lysol) but is readily

inactivated by dilute solutions of formaldehyde or free residual chlorine. In nature, chemical inactivation may be slowed or prevented by the presence of extraneous organic matter. Poliovirus remains viable in the laboratory at freezing temperatures for many years, in the cold for many months, and at room temperatures for days to weeks. In nature, rates of viral inactivation at these temperatures are greatly influenced by the immediate environment.

Soil. Contamination of soil may occur through human defecation near dwellings, crop fertilization with untreated or inadequately treated night soil or sewage, and recycled wastewater for irrigation. Conditions favoring survival of poliovirus in the soil are complex [20]. Poliovirus may survive in soil for weeks to months, often longer than in water [21]. Survival in soil is adversely affected by increased temperature, decreased moisture, low organic content, exposure to sunlight, and the presence of aerobic bacteria. Adsorption of poliovirus onto particulate solids in some soils provides resistance to the inactivating effects of temperature [22]. Aerobic microorganisms are a major independent factor in the decreased survival of poliovirus in soil [22]. In temperate climates, poliovirus inactivation under favorable soil conditions was found to be linear over time in both winter and summer months, with a decrease in virus survival of 1 log every 20 days in winter and every 1.5 days in summer [23].

Sewage. Studies >50 years ago demonstrated poliovirus in sewage in such abundance that it was suspected the virus multiplied in protozoa [24]. It is now known that the content of poliovirus in sewage reflects the hygienic level of the population and the prevalence of infection in the community [25]. In early landmark studies, no virus was recovered in nonepidemic periods, but virus could be found in sewage as early as 5 weeks before the first reported clinical case and up to 3 months after the last case [19, 26].

Studies with enteroviruses as models have shown that sewage treatment may not always be effective. Sewage treatment as commonly practiced will substantially reduce virus concentrations, but some numbers may remain [27]. Increased temperature, ammonium concentrations, and pH are major factors in the natural inactivation of poliovirus in sewage or its byproducts [28]; the average time required for a 90% decrease in enterovirus titer in sewage sludge is 180 days at 2°C and 26 days at 23°C [29].

Surface water. Poliovirus contamination of surface waters can occur from discharge of untreated or inadequately treated sewage, overland runoff, and seepage from underground flows. Enteroviruses have been recovered many miles from untreated or improperly treated sewage effluents, in marine waters, near beaches, and in recreational waters that meet bacteriologic standards of cleanliness [30]. Suspended solids from contaminated marine water sediments may be five times more likely to contain polioviruses than water samples free of solids. Adsorption of polioviruses to these solids provides protection against thermoinactivation [31, 32]. Polioviruses appear to be more rapidly inactivated in marine than in fresh water [33].

Poliovirus from infected stool was reported to have survived in fresh water for 188 days at 4°C under laboratory conditions [34]. However, survival in nature is highly variable, depending on physical, chemical, and biologic factors in the environment [32]. The specific infectivity of poliovirus in river and creek waters in Ohio decreased between 98.4% and 99.97% in 4 days at 27°C, while there was little loss of infectivity in distilled water under the same conditions [35]. The presence of aerobic microorganisms appears to be the most important factor in poliovirus inactivation in natural fresh waters. Proteolytic bacterial enzymes presumably inactivate virus particles through cleavage of viral proteins and exposure of RNA to nuclease digestion [35].

Because of the use of different ambient temperatures, different virus concentrations, and different means for expressing destruction, reports in the literature are difficult to compare. In general, a 99% infectivity loss of poliovirus in various types of fresh surface waters was observed between 3 and 25 days (average, 11). In seawater from multiple sources, a 99% infectivity loss occurred between 1 and 12 days (average, 5) [32].

Some cases of poliomyelitis have been associated with drinking untreated water [19, 36]. Although there have been occasional reports of sporadic poliovirus isolations from municipal water supplies [37], no drinking water provided through appropriately controlled community systems has been implicated in outbreaks of poliomyelitis.

The frozen state. The exceptional stability of enteroviruses in the frozen state often has led to speculation of poliovirus overwintering in snow, frozen soil, or the ice of contaminated surface waters. Arguments against overwintering being a major factor in persistence of poliovirus are the absence of epidemiologic evidence and the qualitative differences between the frozen state in the laboratory and that in nature. Unlike the relatively stable conditions of the laboratory freezer, there are wide swings in temperature and humidity in cold climates. Repeated temperature changes, particularly freezing and thawing, and desiccation have a profound deleterious effect on virus survival.

Some have speculated that cadavers of persons buried in the tundra who died of poliomyelitis may be a future source of poliovirus if inadvertently uncovered or revealed by thawing or flooding conditions. Evidence does not favor such speculation. In 1951, an American expedition was unsuccessful in recovering influenza virus from the lungs of several persons exhumed from the Alaskan permafrost who had died during the 1918 influenza pandemic [38]. More recently, attempts in Siberia to recover smallpox virus from the bodies of persons who died of smallpox unearthed by a river changing course were also unsuccessful [39]. The recovery of poliovirus from cadavers buried for many years in the permafrost is unlikely. Natural biologic deterioration is slowed but not suspended under such conditions.

In summary, poliovirus in the environment is the direct result of recent poliovirus infections in the human community. The rate of poliovirus inactivation is dependent on numerous condi-

tions, but survival in the environment is finite. Interpolation of the available data indicates that poliovirus infectivity decreases by 90% in soil every 20 days in winter and every 1.5 days in summer, in sewage every 26 days at 23°C, in fresh water every 5.5 days at ambient temperatures, and in seawater every 2.5 days under the same conditions.

Humans Are the Only Reservoir

Critical to the eradication of poliovirus is the absence of an extrahuman reservoir, that is, no sustained transmission of the virus in any species independent of human transmission and no existence of the virus in any form from which it may re-emerge to affect humans at some future time.

Shellfish. Outbreaks of hepatitis A and nonbacterial gastroenteritis have been associated with raw or partially cooked oysters, clams, and mussels from polluted waters. Poliovirus has also been found in shellfish, but there are no reports of poliovirus infections from eating shellfish [40]. Poliovirus does not replicate in these organisms. However, filter-feeding shellfish can concentrate poliovirus in their tissues 10–900 times higher than the concentration in the surrounding polluted waters [40]. Polioviruses have been shown to survive 6–90 days in the oyster and theoretically may overwinter in dormant shellfish [27]. However, shellfish do not qualify as a reservoir. The virus is concentrated in shellfish only as long as the surrounding water remains polluted. An actively feeding oyster purges the concentrated virus once the source of contamination is removed.

Domestic and peridomestic animals. Throughout the early poliomyelitis literature, there are descriptions of simultaneous epidemics of paralysis in humans and animals. Epidemics among fowl were most frequently mentioned, but dogs, cats, and moles have also been suspected as possible reservoirs of infection. No poliovirus has been isolated from these animals despite repeated attempts [19, 26]. Mice, rats, muskrats, cats, chickens, cows, and hogs living in the vicinity of person with poliomyelitis also have been tested for poliovirus with negative results [41].

Sera from domestic cows, horses, chickens, dogs, goats, and sheep were found to neutralize poliovirus type 2, but with no evidence of infection with poliovirus [19]. Of particular concern was the nearly uniform finding in bovine colostrum of neutralizing activity against types 1 and 2. Epidemiologic studies had previously implicated contaminated milk in several poliomyelitis outbreaks [26]. The inability to experimentally infect antibody-negative calves and the pattern of natural antibody acquisition in cattle led to the speculation that the neutralizing properties of bovine colostrum and sera resulted from infections with an animal virus or viruses antigenically related to human poliovirus [42].

The origin of antibodies to poliovirus in domestic or peridomestic animals is unclear. Poliovirus has a highly restricted host range. Although some poliovirus strains have been adapted

to rodents through intracranial passages, fresh isolates from human sources are passaged readily only in certain primates or cultures of primate cells [43, 44]. Nonprimates and their cell cultures lack the human poliovirus receptors and are refractory to natural infection [45, 46]. There is no evidence that nonprimates are infected by poliovirus in nature or could serve as reservoirs.

Nonhuman primates. Experimental infections of nonhuman primates with poliovirus were described early in this century [14, 26]. For >40 years, until the advent of tissue culture, nonhuman primates were the laboratory animals of choice for studying poliovirus epidemiology and biology. Monkeys belonging to the *Cebus*, *Cercocebus*, *Cercopithecus*, *Erthrocebus*, *Macacus*, and *Papio* families were all found to be susceptible in varying degrees to poliovirus by intracerebral inoculation [26]. Lower primates, such as the spider monkey and the marmoset, were not. In the 1940s, the chimpanzee was recognized as being susceptible to infection by human strains via the oral route [47].

This experimental experience demonstrates a relative differential in the susceptibility of different primate species to poliovirus infection [48]. As a general rule, lower primates (rhesus and cynomolgus monkeys) are more susceptible to poliovirus infection through the unnatural intracranial route of inoculation than are the higher primates (chimpanzees). Conversely, through the natural oral/intestinal route, humans are more susceptible to poliovirus than are chimpanzees, and chimpanzees are more susceptible than are monkeys [49, 50]. Infections of monkeys by the oral route are often unpredictable and may be related to dietary and other conditions as well as the passage history of the challenge virus [51]. Many monkey species are markedly resistant to oral infection. Even in the cynomolgus monkey, considered to be one of the more susceptible to oral infection, poliovirus excretion is low and of short duration with very limited transmission [52].

Antibodies to one or more poliovirus types have been reported in varying percentages from primate species, both free-living and in captivity. Monkeys with antibodies include *Macaca mulatta* (rhesus) [53, 54], *Cercopithecus aethiops* (green) [54, 55], *Macaca cynomolgus* [54], and *Erythrocebus patas* [54, 55], the four species most commonly used for laboratory research. In none of the studies of monkeys did the prevalence of antibodies to any poliovirus type exceed 6%. Other primates in which antibodies to one or more poliovirus types have been found include chimpanzees, orangutans [50, 54, 55], gorillas [56], and baboons [55]. Antibody prevalence was high only among chimpanzees (up to 49% for type 3) and varied widely with facility of origin. Serologic findings are difficult to interpret. It is unclear if these antibodies to poliovirus were acquired in nature or in captivity, whether the infecting viruses were vaccine or wild, or whether the infecting viruses were only antigenically related to human poliovirus and cross-reacted.

Illnesses and outbreaks of illnesses resembling poliomyelitis have been described in wild and captive primate species [57, 58], and several spontaneous outbreaks have been confirmed by

laboratory findings. A well-documented outbreak of poliovirus type 1 occurred in a primate center in 1964, affecting gorillas, orangutans, and chimpanzees [56]. Two gorillas and one orangutan developed paralytic poliomyelitis. The virus spread throughout the colony except among monkeys. None of the 150 monkeys became ill and only 3 of 25 yielded poliovirus. An outbreak of poliovirus type 1 was documented in Kenya in 1982 in a crowded, closed breeding colony of *Colobus abyssinicus* in which 3 monkeys became paralyzed [59].

In 1966, an epidemic of clinical poliomyelitis occurred among the chimpanzees in the Gombe National Park, Tanzania. The first cases occurred ~1 month after an outbreak of polio among the human population in a nearby district [58]. During the next 4 months, 10 chimpanzees from the study population were afflicted with paralytic disease. Laboratory confirmation of poliomyelitis was not attempted.

These reports suggest that the chimpanzee, and possibly other higher nonhuman primates, may acquire poliovirus in nature. However, these primates constitute an unlikely extrahuman poliovirus reservoir for the following reasons. First, poliovirus in primates, as in humans, causes acute, short-term infections, with virus being excreted for a finite period of time [47, 56]. There is no evidence of a persistent poliovirus carrier state. Second, nonhuman primates are less susceptible to experimental poliovirus infections than humans [49]. Third, epidemics of poliovirus among primate communities in the wild are likely to be rare events. Paralysis consistent with poliomyelitis was seen only once in the Gombe during >2 decades of observations. Further, the extent of the Gombe outbreak and the previous absence of lameness in the chimpanzee study population suggests that poliomyelitis had not occurred for a number of years before 1966.

Chimpanzee and gorilla populations occur at relatively low densities, living in groups of up to 30–150. Few chimpanzees remain in West and East Africa. Much larger populations occur in central Africa, primarily in the forests of Cameroon, Gabon, the Congo Republic, and Zaire (World Wildlife Fund, unpublished data). The chimpanzee population in Gabon, perhaps the largest in Africa, was estimated in 1981 as 64,000 [60]. The total chimpanzee population in Africa today is estimated to be <200,000. Populations are becoming smaller and increasingly fragmented because of poaching and progressive destruction of habitat for timber and farmland. Gorilla populations are even smaller in number (World Wildlife Fund, unpublished data). The western lowland gorilla is the most abundant, with an estimated population of ~40,000. The eastern lowland gorilla, found only in Zaire, numbers 3000–5000. Fewer than 650 mountain gorillas survive in Rwanda, Uganda, and Zaire. About 20,000 orangutans are thought to survive in Sumatra and Borneo. It is unlikely that these populations are sufficient to sustain poliovirus transmission in the absence of human infections [61, 62].

The laboratory. Once human infection ceases, the only known potential reservoir of poliovirus is in the laboratory.

Frozen stocks of wild poliovirus are found worldwide. Research laboratories retain poliovirus stocks because of past or current studies on biologic and genetic properties and as models for studies on positive-strand viruses. Diagnostic laboratories retain wild poliovirus stocks and positive clinical specimens for their historical value and for reference purposes.

More recently, transgenic mice have been developed that express the human gene encoding cellular receptors for poliovirus [63, 64]. These binding sites are present in a wide range of tissues, but poliovirus replication occurred primarily in the brain, spinal cord, and skeletal muscle. Adult mice are refractory to infection by the oral route. Transgenic mice have been disseminated to laboratories worldwide as possible replacements for primates for poliovirus research and for assay of virus vaccines. Because the gene coding for the poliovirus receptor is dominant, concern has been expressed that these animals do not escape and create additional host ranges. Although the possibility of this happening is remote, laboratories should comply with the WHO recommendation on maintenance, containment, and transport of transgenic animals susceptible to pathogenic human viruses [65].

Before vaccine availability, poliovirus infections among laboratory workers were not uncommon [19, 26, 66–68]. It is not known whether others may have acquired asymptomatic poliovirus infections, placing at risk family members and the community. No additional laboratory-associated infections have been reported since the advent of poliovirus vaccine.

With adequate safeguards, wild poliovirus in the laboratory currently represents little or no risk to poliovirus eradication. Nevertheless, planning for the disposition and control of poliovirus laboratory stocks and infected laboratory animals is required before polio is eradicated and OPV discontinued.

Vaccination Interrupts Virus Transmission

Trivalent vaccine (OPV) successfully interrupts poliovirus transmission by producing both serologic and intestinal immunity. In industrialized countries, three doses of OPV result in high rates of seroconversion. In developing countries, lower seroconversion rates are reported, averaging 73%, 90%, and 70% for poliovirus types 1, 2, and 3, respectively [69]. Because excretion of wild poliovirus is prevented or is brief and low-titer in immunized persons, transmission dies out in highly immunized vaccinated populations [70]. WHO recommends that four doses of OPV be administered to all infants during routine immunizations [71]. In countries in which polio is endemic, extra doses of OPV are to be administered during national immunization days, which consist of double rounds of OPV administered annually to all children <5 years of age regardless of immunization status. In countries with focal transmission, OPV is administered as extra doses to all children <5 years of age in high-risk foci [72]. Simultaneous administration of OPV to all children in a specified geographic area interrupts wild poliovirus circulation by boosting population immunity

(particularly intestinal IgA levels) to the point that transmission cannot be sustained.

Evidence of the success of this immunization strategy over the past decade is the absence of poliomyelitis in increasing areas of the world and the disappearance of wild poliovirus genotypes over the past decade [73]. Distinct genotypes sharing $\geq 85\%$ nucleotide similarity within the VPI/2A interval on the poliovirus genome tend to cluster geographically. A genotype is eliminated through immunization or when another predominating genotype is introduced. Eight poliovirus genotypes are believed to have been eradicated through vaccination in the Americas, four in China, and two in the Philippines. Genotypic diversity decreases as eradication approaches. Currently, three type 1 genotypes account for the greatest number of polio cases, primarily on the Indian subcontinent, in central and southwest Asia, and in West and central Africa. Wild polioviruses isolated in areas in which polio is not endemic have been identified by molecular methods as recent imports from endemic reservoirs within other countries or regions [74].

Conclusions

The biologic principles of poliovirus eradication are sound, although the potential for prolonged virus excretion by immunocompromised patients requires further studies and better definition. The source of poliovirus transmission is infectious humans. Survival of poliovirus in the environment is influenced by many factors, but even under the most favorable conditions, the virus is inactivated within months. Higher primates may be naturally infected with poliovirus, although they are unlikely reservoirs in nature. The only remaining poliovirus reservoir after eradication will be laboratory stocks, and serious attention must be given to reducing this potential source of infection. Evidence of poliovirus eradication through immunization is the increasing number of countries with no poliomyelitis and the progressive disappearance of poliovirus genotypes.

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