

Can post-eradication laboratory containment of wild polioviruses be achieved?

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Abstract The purpose of containment is to prevent reintroduction of wild polioviruses from laboratories into polio-free communities. In order to achieve global commitment to laboratory containment the rationale should be clear and compelling; the biosafety levels should be justified by the risks; and the objectives should be realistic. Absolute containment can never be assured. Questions of intentional or unintentional non-compliance can never be wholly eliminated. Effective laboratory containment is, however, a realistic goal. Prevention of virus transmission through contaminated laboratory materials is addressed by WHO standards for biosafety. The principal challenge is to prevent transmission through unrecognized infectious laboratory workers. Such transmission is possible only if the following conditions occur: infectious and potentially infectious materials carrying wild poliovirus are present in the laboratory concerned; a laboratory operation exposes a worker to poliovirus; a worker is susceptible to an infection that results in the shedding of poliovirus; and the community is susceptible to poliovirus infections. At present it is difficult to envisage the elimination of any of these conditions. However, the risks of the first three can be greatly reduced so as to create a formidable barrier against poliovirus transmission to the community. Final biosafety recommendations must await post-eradication immunization policies adopted by the international community.

Keywords Polioviruses; Poliomyelitis/transmission; Containment of biohazards; Laboratory infection; Recurrence/prevention and control; Disease susceptibility; Occupational exposure (*source: MeSH, NLM*).

Mots clés Poliovirus; Poliomyélite antérieure aiguë/transmission; Maîtrise risque biologique; Infection laboratoire; Récidive/prévention et contrôle; Sensibilité à maladie; Exposition professionnelle (*source: MeSH, INSERM*).

Palabras clave Poliovirus; Poliomiéлитis/transmisión; Contención de riesgos biológicos; Infección de laboratorio; Recurrencia/prevencción y control; Susceptibilidad a enfermedades; Exposición ocupacional (*fuentes: DeCS, BIREME*).

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Voir page 315 le résumé en français. En la página 315 figura un resumen en español.

Introduction

In May 1999 the World Health Assembly reaffirmed WHO's commitment to the eradication of poliomyelitis. All Member States were urged to move towards laboratory containment of wild poliovirus in order to prevent the reintroduction of polio from laboratories to polio-free communities (1). In December 1999, WHO distributed the Global Action Plan, linking containment steps to the global certification of eradication and subsequent decisions on post-eradication immunization strategies (2).

Over the past half-century, hundreds of laboratories have worked with various materials in which wild polioviruses were or might have been present. The logistics of identifying and disposing of or retaining such materials under appropriate biosafety conditions have prompted some to suggest that it might be easier to eradicate wild polioviruses in nature than to contain them in laboratories. The former can be verified; the latter cannot.

Absolute containment can never be assured. Questions of intentional or unintentional non-compliance could never be wholly eliminated. This and the remote possibility of the re-emergence of paralytic polio from unknown sources make it necessary to devise post-eradication strategies relating to contingencies, response plans, and vaccine stockpiles.

The prevention of inadvertent transmission to the community is a realistic goal. Such transmission can occur only if someone works under laboratory conditions that lead to the infection of himself, herself or others. We examine the opportunities for inadvertent transmission of poliovirus to the community and review the steps that are required in order to achieve biosafety levels appropriate to the risks of transmission.

Transmission of poliovirus from laboratory to community

In theory, viruses may be transmitted from laboratory to community through contaminated clothing, liquid or air

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effluents, or improper disposal of infectious materials. No evidence exists for poliovirus transmission by any of these routes, but the prevention of this possibility is dealt with in the WHO standards for laboratory design and biosafety practices (2). The principal challenge is to prevent transmission through unrecognized infectious laboratory workers. Such transmission can occur only if the four conditions described below are met.

Condition 1: infectious or potentially infectious materials are present in the laboratory

WHO defines wild poliovirus infectious materials as the following: stocks, isolates, cell cultures and products of laboratory research; clinical specimens from poliomyelitis patients; sewage or water samples in which wild poliovirus is present; and infected animals or specimens from such animals (2). Also considered wild for containment purposes are oral polio vaccine (OPV) derived polioviruses (VDPV) that have assumed the characteristics of wild virus in terms of neurovirulence and transmissibility (3).

Products of poliovirus replication, with virus concentrations of up to a billion infectious particles per ml, pose the highest risk. Less well recognized as laboratory risks are specimens that were collected for other purposes in which wild polioviruses may be present. Defined as potentially infectious materials, these include faeces, throat secretions, and environmental specimens collected for any purpose at a time and in an area where polio was known or suspected to be present and stored under conditions known to preserve the virus (2). The rationale for defining such materials as potentially infectious is based on the natural history of poliomyelitis. For every case of paralytic poliomyelitis, between 100 and 1000 additional poliovirus infections may go unrecognized.

The probability that a randomly collected specimen potentially harbours viable wild poliovirus hinges on whether the specimen was: collected from a person infected with poliovirus; obtained from a body site where poliovirus usually occurs, e.g. stools, throat secretions; or collected while the infected person was shedding poliovirus. The first of these conditions depends on the population infection rate; the second and third conditions depend on the reasons for collecting the specimen.

Faecal material may have poliovirus concentrations of 10 000 to 1 000 000 infectious particles/g. If the average duration of poliovirus shedding in stools is 21 days (4), the chance that a child infected at some time during the year is shedding virus at the time of sampling is approximately 6/100 (21/365). The wild poliovirus isolation rates from stools collected among healthy children mostly under 4 years of age were 8% in Cartagena, Colombia, in 1989 (5) and 19% in Mumbai, India, in 1994 (6).

Throat secretions present a lower risk than faecal materials. Wild polioviruses are found in throat secretions from a smaller proportion of infected persons, for shorter periods, and in lower concentrations than in stools (7, 8). The average duration of viral shedding in throat secretions is about a week, giving a probability of about 2/100 of collecting a sample while a child is shedding virus.

Environmental samples reflect the presence of wild polioviruses in the community. Infected persons may excrete millions of infectious poliovirus particles per gram of faeces daily. However, the viral content of sewage depends on many factors,

including the extent of population infection, the hygienic conditions of the community, the type of sewage disposal system, the location of the sewage collection site, and the dilution factor (9). Virus viability is reduced by increased temperature, ammonium concentrations, and pH; high bacterial growth; certain industrial toxic by-products and other factors. The poliovirus content of sewage collected from infected communities is often low, requiring sample concentration or enrichment if the virus is to be recovered successfully (10).

Laboratory products that are potentially infectious include mislabelled or contaminated cell cultures, untyped enterovirus-like isolates, and undifferentiated poliovirus isolates. Such unrecognized wild poliovirus materials are of particular concern because of possible high virus concentrations and further inadvertent propagation.

Materials not considered potentially infectious, even in areas of endemicity, include blood, serum, and cerebrospinal fluid collected from non-poliomyelitis patients. Although a brief viraemic phase appears to be a feature of poliovirus infection, viral blood levels are usually low and disappear after several days to a week, coinciding with the early onset of antibody production (8, 11). Poliovirus is rarely found in the blood of poliomyelitis patients at the time of clinical diagnosis. Moreover, the risks are smaller for sera collected from persons aged over 2 years, in whom the likely presence of vaccine-induced or naturally acquired serum antibodies inhibits viraemia. Poliovirus in blood collected on paper spots would be inactivated by the drying process (12).

Poliovirus is uncommon in cerebrospinal fluid, even in patients with acute poliomyelitis. In numerous studies conducted over a period of 13 years, cerebrospinal fluid was obtained from 543 poliomyelitis patients and poliovirus was isolated from 38 (7%) of the samples (13). The virus titres in the cerebrospinal fluid were low and laboratory contamination could not always be ruled out. Molecular methods have not increased the detection rate (14).

Clinical and environmental materials are not considered potentially infectious if they have been stored without refrigeration for three months or more, refrigerated for one year or more at 4–8 °C, heat-inactivated, treated with antipoliovirus disinfectants, or previously tested for the presence of enteroviruses with negative results (2). Lyophilized laboratory products are also regarded as not being potentially infectious, because the survival of poliovirus is poor during the drying process.

Condition 2: laboratory activities are performed that expose workers to poliovirus

Laboratory-associated infections are difficult to document in the absence of a formal reporting system. A review of laboratory infections that had occurred by 1976 identified 3921 involving various agents (15). Fewer than 20% were associated with known incidents. These included needle-sticks, spills, splashes or sprays, sharp objects, mouth pipetting, bites of animals or ectoparasites, and centrifuging. Exposure risks were greatest in facilities where laboratory workers handled comparatively large quantities and high concentrations of infectious agents (16).

Twelve cases of laboratory-associated poliomyelitis were reported between 1941 and 1976 (15, 17–22). The number of subclinical laboratory-associated poliovirus infections that occurred is unknown but is likely to have been many times higher.

Parenteral exposure was suspected in three of the twelve reports of occupation-associated poliomyelitis cases. Two were related to needle-sticks (18, 20) and one concerned a superficial hand or wrist wound (22). There is also evidence of the acquisition of poliomyelitis through the injection of inadequately inactivated experimental vaccines that were produced in the 1930s from infected monkey spinal cords (23). Incompletely inactivated poliovirus vaccine (IPV) resulted in a poliovirus type 1 outbreak in the USA in 1955; 10–25% of the vaccinated children became infected and there was considerable spread to household contacts and the community (24).

Oropharyngeal exposure poses the greatest risk in the laboratory, as it does in nature, where faecal-to-oral and oral-to-oral transmissions are the primary means of spread (12, 25, 26). The sources of infectious poliovirus in the laboratory may differ from those in nature, but the implications for laboratory workers are the same. Poliovirus introduced into the mouth is a potential source of infection, whether through mouth pipetting, sprays or splashes of infectious materials, contaminated hands, writing instruments, laboratory devices, or other means.

Aerosol exposure plays an uncertain role in nature. Polioviruses are present in the oropharynx during the early phase of infection but opportunities for expulsion of infectious aerosols may be limited. The throat is sore for only a day or two, if at all, and coughing and sneezing are uncommon. However, infectious virus aerosols at concentrations much higher than in nature may be generated by many common laboratory activities, including the pouring of fluids, the opening of culture containers, the expulsion of fluids from pipettes or syringes, homogenization, and centrifugation (16, 27).

Each of the twelve reported cases of occupation-associated poliomyelitis represents an opportunity for virus transmission to the community. Recent evidence suggests that silent transmission from the laboratory to the community can occur. A prototype wild virus was recovered from the 18-month-old child of a worker in an IPV facility (28). In a separate incident a common wild poliovirus laboratory reference strain was recovered from a child aged 3 years. The mechanism of transmission in the former incident and the source of infection in the latter are unclear.

Condition 3: workers are susceptible to infection resulting in poliovirus shedding

Both oral poliovirus vaccine (OPV) and IPV protect laboratory workers from poliomyelitis. Each stimulates comparable levels of serum IgG antibodies, thus preventing viraemia and infection of the nervous system (29, 30). Neither vaccine provides uniform protection against infection or reinfection but either may play an important role in reducing the risks of laboratory transmission.

OPV immunization more closely mimics the action of natural infection (31–33), offering the advantage that it more broadly stimulates nasal and duodenal secretory IgA antibodies (34). However, the differences between IPV and OPV are more qualitative than absolute (29, 30, 35). A high proportion of persons immunized with either vaccine and challenged with OPV demonstrate protection against infection of the oropharynx (36–38). Intestinal infections may occur in both groups, but fewer infections occur among OPV vaccinees (39–41). The duration of faecal virus excretion is shorter and the amount of virus excreted is smaller among infected vaccinees of either group than in non-immune groups.

Condition 4: community is susceptible to poliovirus infection

Mass OPV immunization prevents the spread of wild poliovirus. IPV immunization has less influence than OPV or naturally acquired immunity on the transmission of wild poliovirus within households or in areas of comparatively low socioeconomic status. On the other hand, IPV clearly provides herd immunity and limits the spread of wild poliovirus in areas of higher socioeconomic status (42, 43), possibly because oral–oral transmission plays a larger role in these settings than faecal–oral transmission. IPV is most effective in limiting oropharyngeal virus shedding, as it induces higher levels of neutralizing antibody in nasopharyngeal secretions (44). Whether high coverage with modern, more potent IPV can limit the spread of re-emerging polioviruses as effectively in developing countries as in developed countries has not been fully evaluated.

Discussion

The prevention of virus transmission on contaminated clothing, in liquid or air effluents, or through the improper disposal of infectious materials is the subject of well-established standards for laboratory design and biosafety practices (2). The implementation of these standards should be effective in preventing poliovirus transmission via the routes mentioned.

We have reviewed the risks of inadvertent poliovirus transmission from laboratory to community through unrecognized infectious laboratory workers, and have identified four major conditions that have to be met if this is to happen. At present the elimination of any of these conditions is unlikely to be achieved. However, the risks associated with three of the conditions could be greatly reduced and this could provide a formidable barrier to laboratory transmission. The fourth condition depends on the post-eradication immunization decisions of the international community.

Condition 1

The reduction of laboratory risks is already under way in many countries as a component of national/regional/global laboratory surveys — the first step towards containment. The purpose of the surveys is to alert laboratories to the anticipated eradication of poliomyelitis, to encourage disposal of all unneeded poliovirus materials, and to create national inventories of laboratories retaining such materials. In the Western Pacific and European Regions there has been a high degree of cooperation in cleaning up the laboratory environment. Laboratory products of poliovirus replication, presenting the highest risks, have been the easiest to identify, particularly in laboratories where the virus is familiar and all stocks present a high index of suspicion.

Laboratories in developing countries rarely, if ever, retain materials collected for diagnostic purposes; refrigerator and freezer space is severely limited or unavailable; and power failures are common. With few exceptions, the most extensive collections of potentially infectious materials are located in the research laboratories of a small number of developed and developing countries. The number of such laboratories and the extent of collections of faecal or throat specimens for bacterial, parasitic, viral or nutritional studies will not be known for several years, when the global inventory is completed as required by the Global Commission for the Certification of the Eradication of Polio (45).

Most of the laboratories with large collections of potentially infectious materials are in countries with established biosafety programmes. In some such laboratories, however, the workers' orientation may not be towards virology, and biosafety practices may be less formalized. Although the risks of laboratory transmission associated with most potentially infectious specimens are low, each collection should be carefully assessed. No laboratories are being asked to dispose of valuable specimen collections or research materials. However, if such materials are retained the laboratories in question should be listed in national inventories and should implement the biosafety practices and procedures appropriate to the level of risk at the time of global certification (46).

The passage of time is a major factor in cleaning up the laboratory environment and verifying containment, just as it is in certifying eradication. The process outlined by WHO provides three crucial opportunities for governments to ensure compliance: when the inventory of laboratories is established, i.e. now; when global certification takes place; and when decisions are taken on vaccine use after certification.

The purpose of national/regional/global inventories is to facilitate the passing of information to laboratories on advances in biosafety and on progress in eradication, and to notify them when to implement biosafety procedures appropriate to the materials stored and the activities performed. Furthermore, signed validations from responsible persons at the different levels, i.e. laboratory, institute and country, provide evidence of the status of each laboratory at each phase.

Condition 2

Laboratory-associated infections and deaths from Lassa and Marburg viruses, smallpox virus, and, later, human immunodeficiency virus, have increased awareness of the importance of laboratory safety over the past two decades, which has resulted in major improvements in facilities and equipment. Laboratory risks have been reduced but not eliminated. The highest risks of exposure to poliovirus are activities involving virus replication, regardless of the source materials. The lowest risks are non-replicative procedures that are satisfactory from the biosafety standpoint and are performed on potentially infectious clinical and environmental materials. WHO recommends Biosafety level 2 (BSL-2)/polio as the minimum pre-eradication biosafety standard for all laboratories working with such materials. This standard is consistent with the national and international standards for working with common viral pathogens (47). Its universal adoption would provide an extraordinary degree of protection against inadvertent poliovirus transmission, particularly in laboratories working with potentially infectious faecal and throat specimens.

After eradication, and before the world can be declared polio-free (2), all laboratories retaining poliovirus infectious materials must implement an appropriate containment standard, i.e. BSL-3/polio or its equivalent, for all wild polioviruses, including circulating VDPV. The more stringent BSL-3/polio requirements increase awareness of the laboratory risks, particularly when work is being done on products of virus replication, and ensure greater community protection. Voluntary attrition in the number of laboratories retaining wild and VDPVs is anticipated at each eradication phase as institutions and nations weigh the costs of containment against the value of retaining a virus of no diagnostic or public health value.

The WHO initial recommendations were developed for situations presenting the highest risks at the time of eradication (high containment) and after the cessation of immunization (maximum containment). However, not all laboratories present the same level of risk. For example, laboratories working with replicating polioviruses present a far greater risk than bacteriology or parasitology laboratories working with potentially infectious materials and using non-aerosol generating activities under appropriate physical conditions. This wide spectrum of potentially infectious materials and the equally wide spectrum of activities in non-enterovirus laboratories are under assessment by WHO to ensure that the recommended biosafety requirements are compatible with the risks.

Condition 3

IPV is expected to be produced and available for laboratory personnel for many years. IPV immunization prevents parenteral infection. Furthermore, high serum antibody titres following IPV immunization serve to enhance protection against oropharyngeal infection. If a laboratory infection occurs in an IPV-immunized person, the reduced duration and quantity of virus shedding lessens the probability of infecting others. Maximum effectiveness is achieved by ensuring that persons working in accordance with the BSL-2/polio standard or higher biosafety conditions are immunized with IPV or OPV in accord with national policy.

Condition 4

Post-certification immunization options are still under discussion at the global level. Further studies are in progress on VDPVs and on prolonged excretion of vaccine-derived virus in individuals (48). Key questions of projected community susceptibility relate to the OPV cessation strategy and the extent and duration of IPV use. The latter provides an additional margin of safety to BSL-3/polio practices. In countries where all polio immunization has ceased, susceptibility to polioviruses can be expected to increase rapidly, and more stringent levels of laboratory containment become paramount. Whatever the eventual timetable or strategy for stopping OPV immunization, decisions will have to be taken on the basis of evidence that wild and VDPVs are retained under appropriate biosafety conditions.

Conclusion

The effective containment of wild and OPV-derived poliovirus is achievable if the rationale behind it is clear and compelling, the biosafety levels are justified by the risks, and the objectives are realistic. Much remains to be learnt about establishing biosafety procedures appropriate to the risks presented by the great variety of infectious and potentially infectious materials in a wide range of settings. The first step is the global laboratory survey and inventory, now under way. The final recommendations on containment will have to be consistent with the decisions of the international community on immunization following eradication. It is vital to keep the dialogue open between the international bodies and national authorities, on the one hand, and the institutions, laboratories and scientists concerned, on the other, in order to attain the goal of effective global containment of polioviruses. ■

Conflicts of interest: none declared.

Résumé

Le confinement des poliovirus sauvages en laboratoire après l'éradication est-il réalisable ?

Le but du confinement en laboratoire est d'empêcher la réintroduction du poliovirus sauvage dans des communautés indemnes de poliomyélite à partir des laboratoires. Pour obtenir un engagement au niveau mondial en faveur du confinement en laboratoire, les justifications doivent en être claires et irréfutables, les niveaux de sécurité biologique en rapport avec les risques et les objectifs réalistes. Le confinement absolu ne peut jamais être assuré. Le non-respect délibéré ou accidentel des mesures de confinement ne peut jamais être totalement exclu. Un confinement efficace en laboratoire est toutefois un objectif réaliste. La prévention de la transmission du virus par des matériels contaminés détenus par les laboratoires est prévue par les normes de sécurité biologique établies par l'OMS. La principale difficulté est d'éviter la transmission par des membres du personnel de laboratoire qui seraient infectieux sans le savoir.

Une telle transmission n'est possible que si les conditions suivantes sont réunies : des matériels infectieux et potentiellement infectieux contenant du poliovirus sauvage sont présents dans le laboratoire concerné, une manipulation de laboratoire expose un membre du personnel au poliovirus, un membre du personnel est sensible à une infection qui entraîne l'excrétion du poliovirus, et la communauté est sensible à l'infection par le poliovirus. Il est actuellement difficile d'envisager l'élimination de l'une quelconque de ces conditions. Cependant, les risques associés aux trois premières peuvent être fortement réduits de façon à constituer une barrière quasi infranchissable contre la propagation du poliovirus dans la communauté. Pour établir les recommandations définitives en matière de sécurité biologique, il faudra attendre l'adoption par la communauté internationale des politiques vaccinales applicables à la période post-éradication.

Resumen

¿Es posible el confinamiento en laboratorio de los poliovirus naturales después de la erradicación?

El objetivo del confinamiento es prevenir la reintroducción de los poliovirus naturales albergados en laboratorios en comunidades libres de poliomiélitis. A fin de conseguir el compromiso mundial necesario para asegurar el confinamiento en el laboratorio, las razones que se aduzcan deben ser claras e imperiosas; los niveles de bioseguridad deben estar justificados por los riesgos; y los objetivos deben ser realistas. Garantizar el confinamiento absoluto es imposible. No es posible tampoco eliminar los interrogantes acerca de los casos de incumplimiento, deliberado o no. Pese a todo, el confinamiento eficaz en laboratorio es un objetivo realista. La prevención de la transmisión del virus a través de material de laboratorio contaminado es una cuestión abordada en las normas de la OMS sobre bioseguridad. El principal reto consiste en prevenir la transmisión mediada por personal de laboratorio infeccioso no

identificado. Esa transmisión sólo es posible cuando concurren las siguientes circunstancias: presencia de material infeccioso o potencialmente infeccioso portador del poliovirus natural en el laboratorio en cuestión; procedimiento de laboratorio que expone al poliovirus a un trabajador; trabajador vulnerable a una infección que supone la liberación del poliovirus; y comunidad vulnerable a las infecciones por poliovirus. Por el momento es difícil pensar en eliminar cualquiera de esas contingencias. Sin embargo, el riesgo de ocurrencia de las tres primeras puede reducirse sensiblemente, lo que representa ya una formidable barrera de protección de la comunidad contra la transmisión del poliovirus. Habrá que esperar a que la comunidad internacional adopte políticas de inmunización posterradicación para disponer de recomendaciones definitivas en materia de bioseguridad.

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