Chapter 5

Surveillance – Technical Aspects
Protect - detect - protect

Polio eradication strategies can be summed up as “protect and detect” – protect children against polio by vaccinating them, and detect any poliovirus that continues to circulate. The big supplementary immunization efforts may have been the public face of the polio eradication initiative, but they were driven and supported by the equally important activity of detecting the wild poliovirus wherever it was. As the end drew nearer, the work gained in relative importance until the emphasis was reversed: the approach became “detect and protect”. Poliovirus could only circulate in areas where significant numbers of children were not protected by vaccination. Therefore, when the virus was detected in a community, it was an indication that the level of immunity needed to be raised by further vaccination.

The detection system for polio eradication was pioneered in the Americas and used throughout the world, but was developed into a more precise and powerful instrument in the Western Pacific Region. In order to appreciate that achievement adequately, it is necessary first to understand some of the technical aspects of the system. This chapter describes the science underpinning the system for detecting wild poliovirus. The following chapter describes the implementation and development of the system in the Region.

Disease surveillance: “information for action”

Keeping a close watch for cases of a given disease or condition is known as “surveillance” and is one of the most important activities of any public health system. Surveillance can detect outbreaks, monitor trends in the occurrence of a disease, or generate hypotheses about possible risk factors for developing a disease. Intrinsic to the concept of surveillance is the need for the information gathered to be disseminated to the people who contributed to its collection, as well as to those who can use it appropriately. There is no point collecting data if it is not used, and those who collect it are not likely to be very thorough if they do not see any outcome from their work.
**Surveillance for polio - difficulties**

There are several problems with polio – the disease caused by poliovirus and characterized by paralysis - as a candidate for surveillance in a programme intending to eradicate the disease by wiping out the virus which causes it.

Detecting polio is not a sensitive indicator for the presence of the poliovirus in the community. As fewer than 1% of susceptible people infected with poliovirus develop paralysis, it is possible for the virus to circulate "silently" for a considerable period of time before manifesting itself in a case of the disease.

Moreover, the clinical diagnosis of polio is not straightforward, as other diseases can produce very similar symptoms. Thus, a clinical diagnosis of polio (without laboratory evidence) is not a very specific indicator for the presence of the virus either. In 1991, at the beginning of the polio eradication activities in the Western Pacific Region, there was no agreed case definition for polio, so clinical diagnoses could be based on differing criteria. Laboratory tests, which can give a definitive answer, were not available for most cases of suspected polio at that time.
The solution: surveillance for acute flaccid paralysis

The solution was found in the use of an alternative surveillance indicator: acute flaccid paralysis (AFP). That type of paralysis – which comes on suddenly, and makes the affected limb floppy or flaccid (as opposed to the rigidity seen in spastic paralysis) – is seen in polio, but also in a number of other conditions. The advantage of using AFP as an indicator lay in the fact that it was more easily defined than polio, and could therefore be reported almost immediately upon detection, without waiting to confirm the finer details. Even more importantly, the level of reporting of AFP could be monitored to assess the performance of the system in practice.

Experience in the Americas had shown that, even when polio had been reduced to extremely low levels or eliminated, acute flaccid paralysis still occurred, caused by other diseases: there was a “background” rate of AFP beyond that caused by polio. Through observation in many countries, the background rate appeared to be 1:100 000 under the age of 15 years. In other words, at least one child under the age of 15 out of every one hundred thousand would develop the condition every year in the absence of the wild poliovirus. (Where polio was still common, the rate of acute flaccid paralysis could be much higher.) Therefore, the rate of one AFP case per 1 000 000 under 15 came to be used as a standard. The number of AFP cases expected in a country or area each year could be calculated from the number of children less than 15 years of age in the population. If fewer cases than expected were reported from an area, it could be an indication that the surveillance system was weak, and measures could be taken to strengthen it.

Sorting the polio cases out from among AFP cases

Acute flaccid paralysis surveillance is very sensitive (although not very specific) as an indicator for polio: if all AFP cases are investigated, all paralytic polio cases will be among them (along with many others not due to polio). However, even a very highly-performing AFP surveillance system will still lack sensitivity for detecting wild poliovirus. That is difficult to avoid in view of the silent nature of most poliovirus infections, but makes it even more important that every polio case is detected. A single missed case of polio could mean overlooking poliovirus circulation among hundreds of people.
An adequate laboratory test to look for evidence of poliovirus is the most definitive way of determining whether a case of AFP is caused by polio. It is not always possible, however, to carry out such tests. Particularly early in the polio eradication activities, many AFP cases were not investigated in the laboratory. Any one of the following alternative criteria was, therefore, deemed sufficient to confirm the diagnosis of polio in a suspected case: residual paralysis at sixty days after onset; a link to another suspected or confirmed case; death; or lack of follow-up. Every case of suspected polio was expected to be classified as “confirmed - polio” or “discarded – non-polio” within ten weeks after onset of paralysis. In that way, countries – and the Regional Office – could keep track of the likely number of polio cases, even without laboratory results for all cases.

**The role of the laboratory: tracking the wild poliovirus**

The laboratory played an increasingly important role in the surveillance system as the polio eradication initiative progressed. With that increased contribution, the system could focus much more accurately on surveillance for the wild poliovirus.

**Options for detection of poliovirus**

Two kinds of laboratory test results can clinch a diagnosis of polio in a suspected case: finding and growing poliovirus in samples of faeces (stool) or throat secretions; or a four-fold or greater rise in the level of antibodies against poliovirus in the blood. The latter test is rarely carried out because of the difficulty of obtaining two blood samples at the right interval and because it cannot distinguish between antibodies produced in response to wild poliovirus and those produced by vaccination. Throat secretions are also not commonly tested: virus can be found in secretions from the throat in the first few days, but it is a much smaller amount of virus than that found in faeces, as well as being present for a shorter time.
The major laboratory test for polio is, therefore, to try to isolate poliovirus from stool samples. Patients infected with poliovirus continue to excrete the virus in their faeces for up to a month or longer, but the excretion can be intermittent, and the amount of virus excreted is higher in the early stages. Thus, the recommendation is that two stool samples should be taken at least 24 hours apart, within two weeks of the onset of paralysis. Taking two samples on different days lessens the chance of missing the poliovirus if the patient is indeed infected.

Isolating poliovirus from stool specimens

When a stool sample suspected of containing poliovirus is received in the laboratory, it is treated with chloroform to inactivate bacteria, fungi and other contaminants, and then spun in a centrifuge to separate solid material from the liquid which might contain the virus. The liquid portion is then inoculated onto sheets of special cells in tubes or flasks. The cells are of two specific types in which poliovirus can grow readily, and certain other viruses (particularly other viruses which, like poliovirus, grow in the gut – i.e. other enteroviruses) can also grow.

The cells are examined under a microscope every day for evidence of damage caused by virus growth. If no evidence of damage to either of the two types of cell is seen after fourteen days of examination, the sample can be deemed to be free of poliovirus and other enteroviruses.

One of the two cell types – known as L20B and derived from mouse cells genetically altered to contain human poliovirus receptors - is more selective for poliovirus, and shows a characteristic pattern of damage. If such damage is seen in L20B cells, it is almost certainly due to poliovirus.

The other cell type, derived from human cancer cells and known as RD, is susceptible to a wider range of enteroviruses. Damage to RD cells can be due to poliovirus or to other types of enterovirus. If a sample produces damage in RD cells without having caused damage to the L20B cells, it should be inoculated again onto L20B cells to re-test for poliovirus. A second negative L20B test confirms that the RD cell damage was due to non-polio enteroviruses. Detecting other enteroviruses in a certain proportion of samples is a sign that good specimen transport and laboratory techniques have been used. It is estimated that between 5% and 25% of children in any area have non-polio enteroviruses in their digestive systems. If the viruses have survived and have been detected by the laboratory, any poliovirus present would also have been likely to be detected.
Any sample, therefore, which produces the characteristic cell damage in L20B cells on either initial testing or re-testing, most likely contains poliovirus. To confirm the result, another test is undertaken, which also determines which of the three types of poliovirus is present in the sample.

**Identifying and typing poliovirus**

The primary aim of the laboratory with respect to poliovirus eradication is to positively identify polioviruses isolated from clinical specimens. That is done by testing the viruses isolated as described above, and those thought to be poliovirus with antibodies specific to poliovirus. The antibodies used in the test are obtained from animals exposed to each of the three different serotypes of poliovirus (types 1, 2 and 3).

The virus grown on cells, as described above, and thought to be poliovirus, is mixed with the test solution containing antibodies to all three types of poliovirus. If the unknown virus is indeed poliovirus, it will be inactivated by the antibodies. When the mixture of poliovirus and antibodies is then re-inoculated onto cells of the same type, it will no longer be able to cause cell damage. Thus, if no cell damage is observed in the test over a period of several days, it may be concluded that the original virus sample contained poliovirus of one or more serotypes.

By using different combinations of antibodies (e.g. to types 2 and 3, 1 and 3, and 1 and 2), the serotype of the unknown virus can be determined. A type 1 poliovirus will cause cell damage when inoculated with the first mixture (containing antibodies to type 2 and 3), but not with either of the other two combinations – since each contains antibodies which will neutralize it. Likewise, type 2 and 3 polioviruses will cause damage only when inoculated with mixtures which do not include their respective type-specific antibodies. By inoculating cells in multiple wells at the same time, with mixtures of the unknown virus sample and each possible combination of antibodies, it can be determined whether the sample contains a single, multiple or no poliovirus serotypes.

**Intratypic differentiation: distinguishing wild and vaccine-type poliovirus**

When a poliovirus of any serotype (type 1, 2 or 3) is found using the antibody tests described above, it is still not clear whether it is a wild (naturally-occurring) or a vaccine-type
poliovirus. Both wild and vaccine viruses occur in all three strains. The antibodies used in the tests cannot, therefore, distinguish wild from vaccine poliovirus.

That is a crucial distinction to make, as it determines the course of action to be taken. If wild poliovirus is found in the stool of a child with AFP, it indicates that the virus is circulating and could cause further cases of polio. The response must be rapid and thorough: immunization of all children in the vicinity of the case. If, however, the virus is found to be from oral polio vaccine (also referred to as Sabin-type or Sabin-like virus, named after the developer of the vaccine), it is very unlikely to cause further cases of polio. No response is normally needed: vaccine-associated paralytic polio is a rare, but recognized consequence of vaccination with OPV.

Distinguishing between wild and vaccine strains of poliovirus is known as “intratypic differentiation”, because it is carried out within serotypes (i.e. with the prior knowledge of the serotype involved). There are several tests which can make the initial distinction between wild and vaccine strains, without going to the extent of looking at the whole genetic sequence of the virus. They fall into two broad groups: antigenic and genetic (or molecular).
Antigenic methods make use of very specific antibodies to distinguish between wild and vaccine polioviruses. The vaccine virus (in any serotype) has a distinctive shape to its surface coat, which is part of the reason it is weak and ineffective in infecting human cells. The change was made deliberately when the vaccine was being developed from its wild poliovirus ancestors. Specific antibodies can be made, by rabbits exposed to vaccine virus, which target parts of (or antigens on) the surface coat of the vaccine virus. Because of the difference in surface coats between the vaccine and wild strains, even within the same serotype, the antibodies to vaccine virus will not bind to wild virus of the same serotype. Likewise, antibodies made by rabbits exposed to wild-type poliovirus will not bind to vaccine virus.

The identified, typed poliovirus is exposed to the two different kinds of antibody to determine whether it is of wild or vaccine origin. If an antibody made in response to a vaccine virus neutralizes the typed poliovirus, it indicates that the poliovirus is also a vaccine virus. Likewise, if an antibody made in response to a wild-type virus neutralizes the typed poliovirus, it indicates that it is a wild virus. There are various ways to determine whether an antibody has neutralized a virus – for example, by using enzymes, which cause visible reactions when they detect the presence of bound antibody-virus complexes. (That is where the name of the most widely used antigenic test comes from: ELISA, for enzyme-linked immunosorbent assay).

The other broad method of distinguishing between vaccine and wild strains of a particular poliovirus type is the genetic, or molecular method. Here, the genetic material of the virus (RNA or ribonucleic acid – analogous to the DNA in human cells), rather than its surface appearance to antibodies, is the basis for the test. Essentially, small pre-prepared fragments (“probes” or “primers”) of specific genetic material which exactly match sections of the poliovirus RNA – in either the vaccine or the recently-circulating strains of wild virus of the relevant type – are mixed with the identified, typed poliovirus. Whichever one finds an exact match will bind to the poliovirus RNA and cause some type of reaction which can be used to determine (through colour, size of product, etc) the identity (wild or vaccine) of the poliovirus.

Both the antigenic and the genetic methods of intratypic differentiation give reasonably accurate and reliable results, although the genetic methods are generally more sensitive when it comes to confirming a relationship to the vaccine virus. Neither, however, is 100% accurate. Each is based on specific characteristics of parts of the poliovirus or of its genetic material, rather than on the whole entity. The most definitive way to determine
the identity of a poliovirus is to directly examine larger parts of its genetic material in a process known as sequencing.

**Genomic sequencing: the gold standard**

Genomic sequencing is the process of determining the exact makeup of the nucleic acid (RNA or DNA) in an organism. The entire strand of nucleic acid in a cell or organism is known as its genome: it contains all the genes for that entity. Nucleic acid is made up of nucleotides (or bases) which code for amino acids, the building blocks of proteins for cells. Thus, genomic sequencing is the process of determining (“mapping”) the order of nucleotides in a given strand of nucleic acid.

Poliovirus is an RNA virus, which means that its nucleic acid is in a “ribo” form, as compared to the “deoxyribo” form that is DNA. Thus, RNA is the blueprint for the poliovirus, and determines all its properties, just as the DNA in human cells codes for the myriad of characteristics that make each individual unique. The complete genome of the poliovirus consists of approximately 7500 nucleotides. Most genomic sequencing, however, focuses only on a small portion.

The most commonly sequenced part of the poliovirus genome is that which codes for an important part of the surface coat of the virus. Known as the VP1 section, it is about 900 nucleotides in length, thus representing about 8% of the virus genome. That is a reasonable sample of the whole, enough to provide the information that is usually required. In some circumstances, other parts of the genome are also sequenced.

**Relationships between polioviruses**

Every wild poliovirus has a different sequence of nucleotides, even within the small sample that is usually sequenced. The characteristic appearance is sometimes called the “oligonucleotide fingerprint” of the virus. Just as every human being has a unique fingerprint, so every wild poliovirus has a unique nucleotide sequence by which it can be identified and distinguished from other viruses. Vaccine polioviruses, by contrast, start out with very similar nucleotide sequences or fingerprints, since they have been developed from the same original strains and do not evolve during the processes of virus production.
In its natural environment, the human digestive tract, the poliovirus is one of the most rapidly evolving organisms on earth. All polioviruses – both wild and vaccine-type – evolve as they replicate in the human gut, so that their nucleotide sequences are constantly changing. Viruses recently descended from a common ancestor are very similar in nucleotide sequence and can be considered to be “related”: a genotype or family is a group of viruses which are at least 85% similar in their nucleotide sequences in the VP1 section. The relationships can be mapped on a dendogram, a kind of family tree. Genomic sequencing can thus accurately determine the relationships between polioviruses, in a way that no other test can.

Vaccine viruses, being weaker and less transmissible, do not persist for as long as wild viruses, and so do not have a chance to accumulate as many changes. Even by passing through one body (that of the vaccinated child), however, a vaccine virus may develop one or a small number of changes to its genome in the sequenced area. It is thus no longer a vaccine virus, exactly, but rather vaccine-like or vaccine-derived. In exceptionally rare circumstances (only two confirmed episodes worldwide in the twenty years for which it is possible to examine the question), notably in the presence of low population immunity rates, the mutated virus can regain enough activity to be transmitted further, and even cause paralysis in a number of people. That is known as circulating vaccine-derived poliovirus.

Wild polioviruses, persisting as they do for much longer, are able to mutate much more than vaccine viruses – although the evolutionary pressure on them to increase their fitness is nowhere near as great, and they do not seem to mutate into more virulent strains. The rate of poliovirus mutation appears to be relatively constant through time, whether viruses are replicating in one person or being transmitted among many. It is thus possible, by plotting the date of detection of the particular virus against the number of mutations in the genome (as compared to a common ancestor), to determine when a particular branch of the virus family diverged from the trunk.

Genomic sequencing is thus an extremely powerful tool for characterizing and tracking wild polioviruses. Its application in the polio eradication initiative, in conjunction with the other weapons in the armoury of the surveillance system, is described in the following chapter.