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# Enterovirus surveillance in the Netherlands 1996-1998: Indications for the absence of wild poliovirus circulation

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# Abstract

Vaccination, surveillance and certification are the cornerstones of the programme to reach the goal of global polio eradication by the year 2000. Certification of regions as polio free zones can only be obtained when intensive surveillance activities coupled to correct laboratory data have shown the absence of wild poliovirus circulation in all countries of the region during at least three years. Gold standard for the surveillance of poliomyelitis is AFP surveillance. A minimum rate of 1 case of non-poliovirus caused AFP in 100,000 children below 15 years of age is required for surveillance to be considered as sufficient, provided for each AFP case appropriate viral analysis of two qualified stool specimens has been performed in a WHO-accredited laboratory.

Non-polio enteroviruses have many characteristics in common with polioviruses, such as site of replication, disease manifestations and patterns of virus shedding and transmission, and can therefor be detected by the same diagnostic procedures in the same type of patients and materials. To be useful as proof for the absence of wild poliovirus circulation, a system of enterovirus surveillance needs to contain:

1) collective data on the number of all faecal specimens that were cultivated on poliovirus-sensitive cells obtained from children below the age of 15. 2) collective data on the number of enterovirus-positive isolates that were proven not to be wild polioviruses (by growth characteristics, typing, intratypic differentiation or genetic characterization), and 3) a quality control system guaranteeing the optimal use of all diagnostic procedures.

Application of enterovirus surveillance in the Netherlands has shown the proven absence of wild polioviruses in more than 15000 stool samples collected between 1996 and 1998. 980 samples were enterovirus positive, 8 isolates were Sabin-derived polioviruses, isolated from asymptomatic patients. In all cases the patients were recently vaccinated in OPV-using countries outside the Netherlands. 90% of the laboratories participating in the enterovirus surveillance activities passed the yearly proficiency test for isolation and typing of enteroviruses in stool samples. With these data the absence of wild poliovirus circulation in the Netherlands in the period of study was clearly demonstrated.

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# Samenvatting

Vaccinatie, surveillance and certificering vormen de hoekstenen van het WHO polio eradicatie programma dat wereldwijde eradicatie van de ziekte poliomyelitis en de verwekkers ervan, de wilde poliovirussen, in het jaar 2000 beoogt. Certificering van regio's als poliovrije zones kan enkel worden verkregen wanneer intensieve surveillance activiteiten, in combinatie met correct laboratoriumonderzoek de afwezigheid van wild poliovirus circulatie in alle landen van zo'n regio gedurende tenminste drie jaar hebben aangetoond. De gouden standaard voor de surveillance van poliomyelitis is surveillance van alle gevallen van acute slappe verlamming (AFP). Melding van tenminste één geval van niet door wild poliovirus veroorzaakte AFP per 100.000 kinderen onder 15 jaar is nodig als minimumeis voor adequate surveillance, mits gekoppeld aan juiste virologische analyse van twee correct afgenomen en vervoerde faecesmonsters in een door de WHO geaccrediteerd laboratorium. Voor Nederland zou optimale AFP surveillance laboratoriumanalyse van 60-70 faeces monsters per jaar vereisen.
Non-polio enterovirussen hebben een aantal eigenschappen met poliovirussen gemeen : identieke plaats van vermenigvuldiging in de mens, vergelijkbare klinische verschijnselen en patroon van virusuitscheiding en van virusoverdracht. Enterovirussen kunnen derhalve opgespoord worden met behulp van identieke laboratoriumtechnieken in identieke monsters van een gelijke groep patiënten. Wil een enterovirus surveillance system kunnen voldoen als systeem om de afwezigheid van wildtype poliovirus circulatie, dan zijn de volgende elementen onontbeerlijk:
1) representatieve gegevens over het totale aantal faeces monsters verkregen van personen onder de 15 jaar en gekweekt op cellen die geschikt zijn om poliovirus te vermeerderen.
2) representatieve gegevens over het totale aantal enterovirus-positieve isolaten, waarvoor (d.m.v. groeikarakteristieken, typering, intratypische differentiatie dan wel genetische karakterisering) uitgesloten werd, dat zij wild poliovirus bevatten en
3) een kwaliteitsbewakingssysteem dat optimaal virologische diagnostiek garandeert.
Enterovirus surveillance in Nederland over de jaren 1996-1998 heeft de afwezigheid van wild poliovirus aangetoond in meer dan 15.000 faeces monsters, verkregen uit kinderen beneden de leeftijd van 15 jaar. In 980 van deze monsters werd enterovirus aangetoond, 8 isolaten bleken OPV-afgeleide poliovirussen, geïsoleerd uit asymptomatische patienten. In al deze gevallen bleken de patienten recent met OPV gevaccineerd te zijn in het buitenland. Negentig procent van de in het enterovirus surveillance systeem deelnemende laboratoria behaalde een voldoende score van 80% of hoger in de jaarlijkse onder de auspiciën van de SKMM georganiseerde proficiency test voor isolatie en typering van enterovirussen uit faecesmonsters.
In meer dan 99% of de genoemde 15.000 faeces monsters, kon de aanwezigheid van wild poliovirus in het laboratorium worden uitgesloten. Optimalisatie van het systeem door inclusie van alle virologische laboratoria in Nederland omvat de jaarlijkse analyse van meer van 7500 faeces monsters per jaar, d.w.z ongeveer honderd maal meer monsters dan noodzakelijk voor optimale AFP surveillance.
Hoewel objectieve criteria als voor optimale enterovirus surveillance nog niet geformuleerd zijn, geven de data over het in Nederland opgezette systeem, zoals beschreven in dit rapport, sterke aanwijzingen voor de afwezigheid van wild poliovirus circulatie in Nederland. De data zullen door de WHO gebruikt worden om mathematische modellen te toetsen, waarmee de kracht van de verschillende surveillance systemen voor de surveillance van poliomyelitis worden vergeleken.

# Summary

Vaccination, surveillance and certification are the cornerstones of the program to reach the goal of global polio eradication by the year 2000. Certification of regions as polio free zones can only be obtained when intensive surveillance activities coupled to correct laboratory data have shown the absence of wild poliovirus circulation in all countries of the region during at least three years. Gold standard for the surveillance of poliomyelitis is AFP surveillance. A minimum rate of 1 case of non-poliovirus caused AFP in 100,000 children below 15 years of age is required for surveillance to be considered as sufficient, provided for each AFP case appropriate viral analysis of two qualified stool specimens has been performed in a WHO-accredited laboratory.

Non-polio enteroviruses have many characteristics in common with polioviruses, such as site of replication, disease manifestations and patterns of virus shedding and transmission, and can therefor be detected by the same diagnostic procedures in the same type of patients and materials. To be useful as proof for the absence of wild poliovirus circulation, a system of enterovirus surveillance needs to contain:

1) collective data on the number of all faecal specimens that were cultivated on poliovirus-sensitive cells obtained from children below the age of 15,

2) collective data on the number of enterovirus-positive isolates that were proven not to be wild polioviruses (by growth characteristics, typing, intratypic differentiation or genetic characterization), and

3) a quality control system guaranteeing the optimal use of all diagnostic procedures.

Application of enterovirus surveillance in the Netherlands has shown the proven absence of wild polioviruses in more than 15,000 stool samples collected from children below 15 years of age between 1996 and 1998. In 980 of the faecal samples enterovirus was detected; 8 isolates were Sabin-derived polioviruses isolated from asymptomatic patients. In all cases the patients were recently vaccinated in OPV-using countries outside the Netherlands. 90% of the laboratories participating in the enterovirus surveillance activities passed the yearly proficiency test for isolation and typing of enteroviruses in stool samples.

In more than 99% of these 15.000 stool samples, the presence of wild poliovirus was excluded by laboratory analysis. Optimization of this system by inclusion of data from all virological laboratories in the Netherlands would imply the yearly analysis of more than 7,500 stool specimens, i.e. more than 100-fold the number of specimens to be analysed within the framework of optimal AFP surveillance.

Although objective quality indicators for optimal enterovirus surveillance have not yet been established by WHO, the present Dutch experience, as presented in this report, provides strong evidence for the absence of wild poliovirus circulation. WHO will use these data to evaluate mathematical models comparing the results of the various surveillance systems used to illustrate the absence of wild poliovirus circulation.

# 1. Introduction

## 1.1 Global polio eradication

In 1989 the World Health Assembly adopted a resolution to eradicate the disease poliomyelitis anterior acuta and its cause, the three wild polioviruses by the year 2000. The resolution had two objectives: 1) achievement of zero poliomyelitis cases caused by wild poliovirus, and 2) absence of wild poliovirus circulation in all clinical and environmental samples obtained throughout the world. The World Health Organization (WHO) was given the task to implement this resolution. (6)
Vaccination, surveillance and certification are the cornerstones of the programme to reach the goal of global polio eradication. (32,35) Vaccination strategies that have been successfully implemented in most parts of the world are maintenance of high levels of routine coverage with polio vaccine and delivery of polio vaccine in the manner most effective for interrupting wild poliovirus transmission, especially via regionally co-ordinated National Immunization Days (NIDs) (12). Effective surveillance activities to detect all cases of acute flaccid paralysis (AFP) in children below 15 years of age have identified regions with circulating wild poliovirus, to be targeted for intensified vaccination activities (house to house mopping up vaccination). Effective surveillance includes adequate virological analysis of two stool specimens from each AFP case, by standardized methods in WHO-accredited laboratories.(1,31) A complete network consisting of more than 100 laboratories at National and Regional levels and supported by 6 Specialized Reference Laboratories with specific tasks for quality control and optimization of laboratory methodology ensures that reliable virological data are available as the basis for action to implement eradication strategies.(11)
As a result of all activities the number of cases of poliomyelitis reported worldwide has been reduced to about 5000, with about 1500 wild poliovirus isolations. (33). Probable and known wild poliovirus circulation has been confined to three major reservoirs: sub-Saharan Africa (West and Central Africa), the Horn of Africa, and - Asia. In 1997/1998, a small reservoir of wild poliovirus circulation was identified in the South East of Turkey and the North of Iraq, which has lead to many additional immunization activities in that area, resulting in the absence of cases until date since October 1998.(3,34)

## 1.2 Certification of the absence of wild poliovirus circulation

Evidence for the absence of wild poliovirus circulation is collected by Regional Certification Commissions, that have to report to the Global Certification Commission. (25) As poliovirus circulation is not confined to single countries, individual countries are not certified as polio-free. Certification of regions can only be obtained when intensive surveillance activities coupled to correct laboratory data have shown the likely absence of wild poliovirus circulation in all countries of the region during at least three years. As the last case of polio in the America's was notified in 1991 and AFP surveillance has been kept at the required level since, the America's were officially declared free of wild poliovirus circulation in 1994. In the Western Pacific Region of WHO, no case has been reported since 1997. (34)
For the European Region of WHO (including the Russian Federation and all Newly Independent States), the goal is near: in 1998, isolation of only 26 wild polioviruses (24 x polio 1, 2 x polio 3) has been documented, all from the south-eastern part of Turkey. As

many of the 49 countries in European region of WHO have been free of polio for a long time, the European Certification Committee has started the certification process already in Western and Northern Europe. The data from four countries (Finland, Denmark, United Kingdom and the Netherlands) were reviewed in 1998, data from 15 other non-endemic countries in 1999. Certification documents have to contain a description of the vaccination programme in the country, the vaccination status of the population, an epidemiological review on poliomyelitis and wild poliovirus circulation in the country with special attention to the last ten years, and have to illustrate by that the ability of the country to detect imported cases of poliomyelitis and imported wild polioviruses.
Gold standard for the surveillance of poliomyelitis is AFP surveillance (1). A minimum rate of 1 case of non- poliovirus caused AFP in 100,000 children below 15 years of age is required for surveillance to be considered as sufficient, provided for each AFP case appropriate viral analysis of two adequate stool specimens (2 grams, obtained 24-48 hours apart within 2 weeks after onset, transported under cold conditions to the laboratory) has been performed in a WHO-accredited laboratory. In case AFP surveillance criteria are not met, additional evidence has to be given for the absence of wild poliovirus circulation. Countries have to prove that these additional data (f.i. from environmental or enterovirus surveillance) are at least equal to the data from adequate AFP surveillance.

## 1.3 Certification of the absence of wild poliovirus circulation in the Netherlands

Among the vaccine preventable diseases, poliomyelitis has always been given special attention in the Netherlands. A very high vaccination coverage of more the 97 % was reached and endemic wild poliovirus circulation was stopped by successful implementation of a routine vaccination programme since the late 50s using inactivated poliovirus vaccine (IPV). (23,26) However, the existence of a large socially isolated, but strongly organized community that refuses vaccination for religious reasons is a risk factor, that justifies the interest for poliomyelitis in the Netherlands. Two large outbreaks (a polio 1 epidemic in 1978 with 110 cases and a polio 3 outbreak in 1992/3 with 71 cases) and several small outbreaks in single villages in the early 70s have occurred within this community. All patients were not vaccinated against polio (19,23,24).
Active AFP surveillance by members of relevant medical professions (paediatricians, neurologists) with adequate analysis of stool samples has proven to be very difficult to organize in the Netherlands (4,20). The mean reported AFP-incidence rate of around 0.5 per 100,000 children below 15 years of age and adequate virological analysis of stools in only about 30% of the AFP cases are far below the requested WHO performance criteria of 1.0 per 100,000 children below 15 and 80% of cases with virological analysis of stool specimens.
A recent report evaluates the needs and possibilities for active surveillance for cases of poliomyelitis and for wild poliovirus circulation (29). On the basis of this information, the Netherlands certification committee has decided the documentation of the absence of wild poliovirus circulation in the Netherlands should contain data on four activities (8):
1) Obligatory and immediate notification of each suspected case of poliomyelitis to public health authorities.
2) Monthly retrospective notification by paediatricians of all cases of acute flaccid paralysis in children, preferably combined with laboratory analysis of stools.
3) Environmental surveillance by analysis of sewage water samples collected at secondary schools with a high percentage of non-vaccinated children.

4) Enterovirus surveillance: notification of results from laboratory analysis of stool specimens from patients with any clinical symptoms on poliovirus-sensitive cell lines.

Whenever a case of poliomyelitis is suspected, action is taken according to the Plan of Action, in which responsibilities and duties of all authorities and institutions involved are described (15). The same Plan of Action describes which steps are to be taken after isolation of a wild poliovirus from an asymptomatic patient or an environmental sample.
The National Institute for Public Health and the Environment (RIVM) in Bilthoven has been designated as WHO Collaborating Centre for Reference and Research on Poliomyelitis. The Virology Department of the Diagnostic Laboratory for Infectious Diseases and Screening (LIS-VIR) serves within the WHO Polio Laboratory Network on all three levels: as WHO-accredited National Laboratory, as Regional Reference Laboratory for Europe, serving 10 European countries (including Turkey, the last European reservoir for wild poliovirus circulation) and as Specialized Reference Laboratory, with a special task in preparation and distribution of standardized reagents and cell lines and in quality control of all laboratory activities. (11)

## 1.4 Use of data from enterovirus surveillance activities for the polio eradication initiative

The rationale for using enterovirus surveillance as tool to prove the absence of wild poliovirus circulation is that polioviruses belong to the genus enterovirus and therefore have many characteristics in common with non-polio enteroviruses, such as site of replication (gut, central nervous system), disease manifestations ("flu-like illness, aseptic meningitis) and patterns of virus shedding and transmission. Polioviruses and non-polio enteroviruses can both be detected by cell culture procedures from stool samples of the same type of patients. (13)
The present report describes methods for diagnosis of enterovirus infection in the Netherlands, and the quality control of the diagnostic laboratory services for enterovirus isolations. Notification systems for enterovirus surveillance will be evaluated on their suitability as tools for poliosurveillance. Recommendations will be given to improve the reporting systems for enterovirus surveillance in order to optimize the data that document the absence of wild poliovirus circulation in the Netherlands.

# 2. Enterovirus surveillance

## 2.1 Diagnosis of enterovirus infection in the Netherlands

Primary diagnosis of viral infections from patients with clinical symptoms is performed in regional microbiological laboratories, linked to major and academic hospitals or to provincial public health services. Nineteen of these laboratories, distributed all over the Netherlands, have facilities for cell culture and provide diagnostic services for respiratory and enteric infections by virus isolation. (Annexe 1). In the majority of these labs (15 out of 19) three types of cell lines are used for virus isolation: tertiary monkey kidney cells, human fibroblasts and human epithelial cells. All labs use at least two cell lines on which polioviruses can be propagated. (13)
In 12 regional laboratories, isolates with enterovirus-specific CPE are confirmed as enteroviruses by immunofluorescence with commercially available enterovirus-specific monoclonal antibodies or by home-made or commercial PCR tests. Fourteen labs use the RIVM enterovirus typing kit (PP,CP, AG-pools) for typing of enteroviruses (13), eight labs also use the WHO poliovirus typing sera. (31) Any poliovirus isolate is always immediately sent to the RIVM by special courier, for immediate confirmation and further characterization by intratypic differentiation (21,27,30) and, if necessary molecular analysis. (14,17)
Table 1 gives details on these polioviruses isolated between 1993 and 1998, on the patients from whom the polioviruses were obtained and on the most likely origin of the isolates. (17)
All but one polioviruses isolated after the end of the 1992/93 poliovirus type 3 epidemic were Sabin like viruses. The wild poliovirus type 3, isolated from a Dutch boy, 5 years of age and fully vaccinated, with gastro-enteritis after a recent visit to France, was a prototype wild virus, as used for vaccine production in France. Around this isolate, no further infections were found in direct family contacts nor in sewage samples taken near the home and the school of the patient. In three cases poliovirus isolation was the result of a laboratory contamination, linked to concurrent laboratory work on viruses of a proficiency test for isolation and typing of enteroviruses.
Up to 1998 non typed or non-typable enteroviruses were only sent to RIVM for further characterization in special cases with clinical, epidemiological or scientific interest.

*Table 1 Polioviruses isolated in the Netherlands, 1993*-1998*

| Year | Month | Virus | Age | Clinical Data | Epidemiology |
|---|---|---|---|---|---|
| 1993 | 10 | Polio 3 NSL | 5 y. | Gastroenteritis, *S. Paratyphi* positive. | Recent visit to France, virus=prototype wild virus |
| 1994 | 6 | Polio 3 SL | 10 m. | ?? | ?? |
| 1995 | 3 | Polio 2 SL | 32 y. | GB, polyneuropathy | Visit to the tropics |
| 1995 | 8 | Polio 2 SL | 2 m. | BMT | Recently vaccinated in Belgium |
| 1995 | 11 | Polio 2 SL | 3 m. | Diarrhea, vomiting | Recent return from stay in Venezuela |
| 1995 | 12 | Polio 3 SL | 5 m. | Healthy | Adopted child from Taiwan |
| 1996 | 7 | Polio 2 SL | 5 m. | Fever, diarrhea | German child on holidays, OPV vaccinated two weeks before |
| 1996 | 7 | Polio 2 SL | <1 m. | SIDS | ?? |
| 1997 | 1 | Polio 3 SL | 5 m. | Resp. disease, HIV+ | Visitor from South Africa, last vaccination two months before |
| 1998 | 1 | Polio 2 SL | 9m | Possible "heart disease" | Adopted from Taiwan |
| 1998 | 1 | Polio 1 SL | 10m | Diarrhea, vomiting | Recently vaccinated in USA |
| 1998 | 3 | Polio 3 SL | 4m | Healthy | Adopted from Colombia |
| 1998 | 6 | Polio 3 SL | 1.5y | diarrhea, flaccid muscle | IPV vaccinated, origin: Turkey, |
| 1998 | 6 | Polio 3 SL | 1y | enteritis, retardation | Origin: Morocco, recent visit to Morocco |
| 1994 | 6 | Polio 1 SL | 67 y. | Mammacarsinoma, fever | Laboratory contamination with virus from proficiency panel |
| 1998 | 2 | Polio 1 SL | 1w | neonate, sick | Laboratory contamination with virus from proficiency panel |
| 1998 | 3 | Polio 1 SL | 38y | respiratory disease | Laboratory contamination? recultivation original culture: P1 SL recultivation original material: negative |

*Polioviruses (wild or vaccine) isolated in 1993, but related to 1992/93 outbreak are not specified in this table.

Since 1998 all laboratories have been requested to send all non-typed and non typable enterovirus isolates from stool specimens to the National Polio Laboratory at RIVM, Bilthoven, where the presence of poliovirus in these isolates is excluded by virus typing, molecular methods, such as enterovirus- and poliovirus-specific PCR tests (16,36), and by cell culture on L20B cells, a murine cell line that expresses the human poliovirus receptor as a result of genetic modification, and thus gained the ability to propagate specifically polioviruses, and no other enteroviruses. (9,22) Table 2 contains the results of these analyses obtained in the years 1996-98.

*Table 2 Results of typing at RIVM of enteroviruses, isolated from faecal specimens in Regional Virological Laboratories*

| | 1996 | 1997 | 1998 | | 1996 | 1997 | 1998 |
|---|---|---|---|---|---|---|---|
| Polio 1 | | | 1(+2)* | ECHO11 | 1 | | 5 |
| Polio 2 | 2 | | 1 | ECHO16 | | 1 | |
| Polio 3 | | 1 | 3 | ECHO17 | | 1 | 1 |
| CB1 | 1 | | | ECHO18 | 3 | 9 | 1 |
| CB2 | | 1 | 2 | ECHO20 | 1 | | |
| CB3 | | 5 | 1 | ECHO21 | | | 1 |
| CB4 | | 7 | 3 | ECHO22 | 1 | | |
| CB5 | 3 | | | ECHO23 | | | |
| CA7 | 1 | | 1 | ECHO24 | 1 | | |
| CA9 | | 2 | | ECHO25 | | 2 | |
| CA10 | | | 1 | ECHO27 | | 1 | |
| CA13 | 1 | | | ECHO30 | | 3 | 1 |
| CA14 | 1 | | | Entero 71 | | 3 | 1 |
| CA16 | 1 | 1 | 4 | Negative | 5 | 2 | 6 |
| CA17 | | 1 | | Adeno | 3 | 2 | 1 |
| CA20 | | | 4 | NPEV | 2 | 2 | 49 |
| CA24 | 3 | 4 | 1 | NPV | 11 | 9 | 6 |
| ECHO3 | | | 7 | Pending | | | 1 |
| ECHO6 | | | | Herpes | | 1 | |
| ECHO7 | | 1 | 1 | | | | |
| ECHO9 | | | 1 | **Total** | **41** | **59** | **103** |

NPEV: growth on at least one cell line, but not on L20B, enterovirus PCR positive; NPV: growth on at least one cell line, but not on L20B, enterovirus PCR negative; *: 2 P1SL strains isolated as contaminations.

In several laboratories also serological tests for the diagnosis of enterovirus infection are performed: 6 laboratories analyse paired sera for a conversion or a significant rise in antibody titre against a specific enterovirus antigen (mainly CoxsackieB viruses, sometimes against the patient's own isolate) in a neutralization test, 7 laboratories do complement fixation tests using commercially available antigen preparations.
At RIVM, a test for detection of poliovirus-type specific IgM antibodies in serum and cerebrospinal fluid has been developed, that allows rapid diagnosis of recent poliovirus infection. (18) Experience during the 1992/3 type 3 polio epidemic has shown the potential of this test as an additional tool to prove or exclude poliovirus infection as cause of disease in cases of suspected poliomyelitis and other AFP patients in the Netherlands. (7,19) In the period 1996-1998, 25 sera and 7 CSF samples were tested for the presence of poliovirus type-specific IgM. All tests were negative. Indications for testing for polio-specific IgM are paralytic or neurologic symptoms, in combination with unknown vaccinations status. In many of the cases, a faecal sample was not available, or taken too late after onset of symptoms for successful isolation of poliovirus.

## 2.2 Quality control of enterovirus diagnostic procedures in regional virological laboratories in the Netherlands

Quality control of laboratories performing diagnostic microbiological services is co-ordinated by SKMM (Foundation for Quality Control in Medical Microbiology). Before 1998, no specific proficiency test for isolation and typing of polio and other enteroviruses was organized. A stool sample containing polioviruses was present in the 1994 and in the 1996 proficiency tests for methods for the diagnosis of viral gastro-enteritis (Table 3).
In both years the stool sample contained two enteroviruses. (1994: polio 1 + polio 3; in 1996 polio 1 + Coxsackie B5) Almost all of the participating labs were able to isolate enterovirus from both samples; only one lab reported one of the two samples as virus negative. Typing results were disappointing, as in only 28 of the 37 isolates (76%) the presence of poliovirus was reported. In the three laboratories, where type-specific poliovirus antisera were available, poliovirus typing was correct. (29)
In 1998 under the supervision of SKMM, a special proficiency test was organized by RIVM, specifically directed to the detection and characterization of polio and other enteroviruses in stool samples. A panel of four stool samples, containing 1 or 2 enteroviruses was sent to 19 laboratories. Results had to be reported within 6 weeks (Table 3).

*Table 3 Quality control of laboratory services: results from proficiency samples containing enteroviruses*

| Year | Virus | Number correct results | | | |
|---|---|---|---|---|---|
| | | isolation | poliovirus reported | poliovirus typing | enterovirus typing |
| 1994 | Polio 1 + Polio 2 | 17/18 | 15/17 | 3/15 | n.a* |
| 1996 | Polio 1 + CB 5 | 20/20 | 13/20 | 3/13 | 5/13 |
| 1998 | Polio 1 | 19/19 | 17/17 | 12/12 | n.a. |
| 1998 | Polio 2 | 17/19 | 15/17 | 7/8 | n.a. |
| 1998 | Polio 3 + ECHO 9 | 19/19 | 7/17 | 7/7 | 14/17 |
| 1998 | ECHO 30 | 17/19 | n.a. | n.a | 14/17 |

n.a.: not applicable

Four different labs reported one of the four samples as virus-negative: poliovirus type 2 and ECHO 30 were missed each in two labs, although all four labs used at least two cell lines that in principle should be able to propagate these viruses. Data on the growth characteristics of the enteroviruses present in the four samples, reported via a standardized questionnaire that accompanied the proficiency test, revealed large differences in the timing of the occurrence of a cytopathological effect (CPE) after inoculation and in virus yields of the various cell lines in use. A large variety of cells was used, of which the origin was not standardized. Quality control and standardization of cell lines in use at regional laboratories is strongly recommended.
Seventeen of the participating laboratories are able to do enterovirus typing using the RIVM enterovirus typing kit. (13) In 39 of the 51 samples containing poliovirus, the poliovirus was correctly typed. From two samples there was no isolate obtained. In the other ten cases, the poliovirus was not detected in samples that also contained another enterovirus. Non-optimal

use of the typing kit (3 cases) and performing typing on only one virus-positive cell line (7 cases) were reasons for not reporting the presence of poliovirus. In one laboratory an additional poliovirus was reported in one of the samples, as a result of cross contamination from another sample. Non polio-enterovirus typing was correct for 30 of the 34 viruses present in the samples analysed by the laboratories that performed typing of non-polio-enteroviruses. In two cases there was no isolate, in two other cases again there was non-optimal use of the typing kit as reason for not being able to type the isolate. In two samples a contamination with a non-polioenterovirus was reported.
Six laboratories reached a 100% score, six had 88%, five laboratories had a score of 75% and two labs scored 63%. The mean score was 86 %. WHO requires an 80% score in similar types of proficiency tests for the yearly accreditation of National Laboratories taking part in the WHO Laboratory Network supporting the polio eradication initiative (28). The results and recommendations of the 1998 proficiency test for isolation and typing of polio and other enteroviruses have been extensively evaluated with representatives of all participating laboratories. Staff of the laboratories with an insufficient result will receive extra training at the National Polio Laboratory at RIVM.

## 2.3 Weekly reports of laboratory confirmed viral infections

Regional virological laboratories report all positive findings for all viruses tested weekly to the RIVM Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, where the data are collected and tabulated, and reported back to all participating laboratories. Seventeen of the 19 laboratories that perform cell culture for enteroviruses have qualified for this reporting system, two smaller laboratories do not yet participate as they do not perform a required minimum of virological diagnostic methods.
Laboratories report only the number of patients with at least one positive virological or serological marker for virus infection diagnosed in the week of report. No data on the patient, the underlying illness nor the sample by analysis of which the diagnosis was made, are reported. All but only positive diagnoses of enterovirus infection, made by virus detection from stool, throatswab, CSF or any other body fluid (either confirmed by immuno-fluorescense, PCR or virus typing) or by serology (a significant rise in antibody titer in aneutralization or CFT, or a positive IgM test) are added up. In case, confirmation of enterovirus is done by virus typing, the type and number of typed viruses is reported, provided the typing was done in the week of report. The various serotypes of the typed enterovirus isolates can be reported in cases of epidemiological or scientific interest.

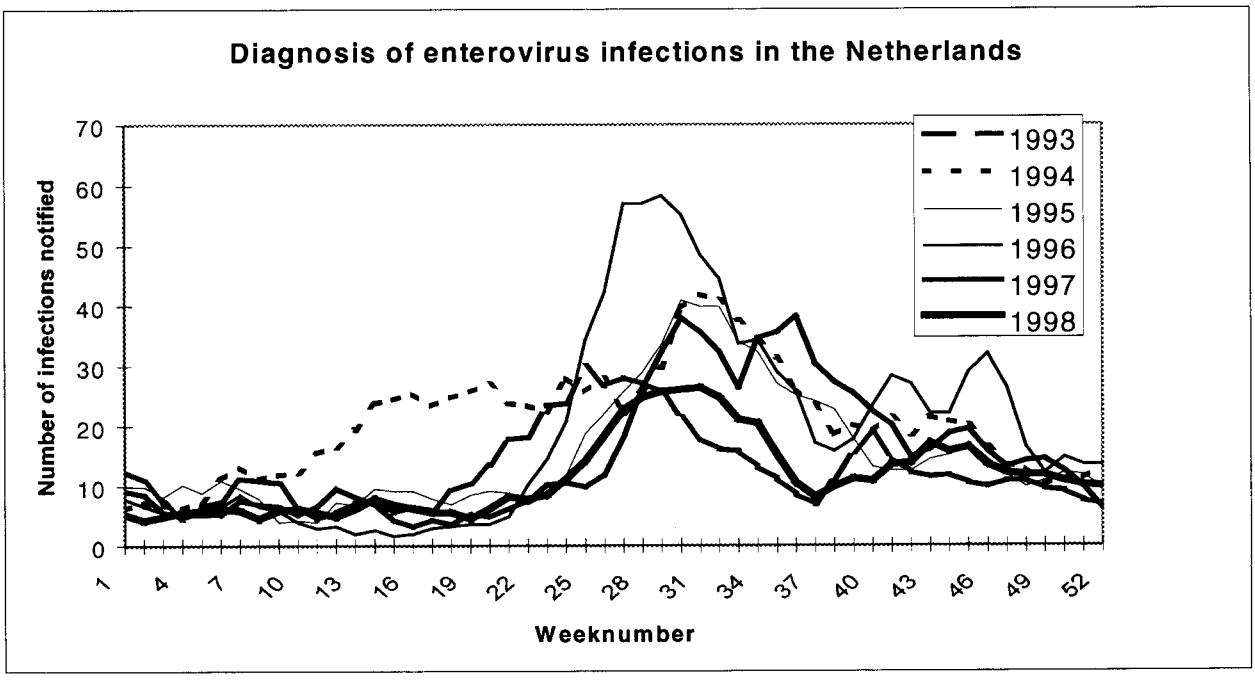


*Figure 1 gives the weekly distribution for the years 1993-1998 of the total number of notified enterovirus infections in the Netherlands. Enteroviruses occur in the Netherlands as a typical summer disease. The number of diagnosed infections usually starts to rise in May/June and reaches its peak value in July/August.*

Table 4 contains the total number of typed enterovirus isolates in these years, including the number of isolated polioviruses. From these data it can be concluded that for at least 35 % of the total number of enteroviruses isolated in the Netherlands between 1993-1998 the presence of wild poliovirus was excluded by typing of the isolate. However, only 4 of the 8 poliovirus isolates notified to the national public health services can be found in the weekly reporting system. The four other isolates were not typed in the week of reporting, and therefor were notified as non-typed enterovirus.

*Table 4 Annual numbers of enteroviruses typed in the Netherlands (according to Weekly Reports)*

| | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | Total |
|---|---|---|---|---|---|---|---|
| Polio 1 | | | | | | | 0 |
| Polio 2 | | | 1 | | | | 1 |
| Polio 3 | 3 | | | | | | 3 |
| CB1 | | | 1 | 61 | 6 | 5 | 73 |
| CB2 | | 1 | 36 | 2 | 4 | 21 | 64 |
| CB3 | | 1 | 5 | 10 | 6 | 11 | 33 |
| CB4 | | 3 | 13 | 8 | 12 | 3 | 39 |
| CB5 | | | 5 | 101 | 2 | 2 | 110 |
| CB6 | | | 6 | 2 | 9 | 4 | 21 |
| CBNT | | | | | | 9 | 9 |
| CA9 | | 5 | 16 | 2 | 15 | 2 | 40 |
| CA16 | | | 11 | 2 | 6 | 2 | 21 |
| ECHO 1 | | | | | 1 | 3 | 4 |
| ECHO2 | | | | | 2 | 7 | 9 |
| ECHO3 | | | | | | 5 | 5 |
| ECHO4 | | | | | | 2 | 2 |
| ECHO5 | | 9 | 1 | | | 1 | 11 |
| ECHO6 | | | 1 | | 18 | | 19 |
| ECHO7 | | | 1 | 33 | 4 | 8 | 46 |
| ECHO9 | | | 22 | 1 | 3 | 1 | 27 |
| ECHO11 | | | 19 | 3 | | 24 | 46 |
| ECHO12 | | | 1 | | | | 1 |
| ECHO14 | | | | 1 | | 1 | 2 |
| ECHO15 | | | | 1 | | | 1 |
| ECHO18 | | | 1 | 18 | 14 | | 33 |
| ECHO20 | | | 3 | 2 | 2 | 2 | 9 |
| ECHO21 | | | | 1 | | | 1 |
| ECHO22 | | 5 | 6 | 5 | 14 | 9 | 39 |
| ECHO25 | | 3 | | 1 | 25 | 3 | 32 |
| ECHO30 | | | 1 | 33 | 37 | 4 | 75 |
| Entero 71 | | | | | 3 | | 3 |
| EnteroNTa | 286 | 347 | 130 | 82 | 60 | 63 | 968 |
| EnteroNTb | 387 | 713 | 524 | 624 | 555 | 420 | 3223 |
| Total | 676 | 1087 | 804 | 993 | 798 | 612 | 4970 |
| Total typed | 289 | 374 | 280 | 369 | 243 | 192 | 1747 |
| % typed | 43% | 34% | 35% | 37% | 30% | 31% | 35% |

NTa: Not typed
NTb: Not typable

## 2.4 Quarterly Reports on enterovirus surveillance 1996-1998

Enterovirus surveillance by the weekly reporting system for virological infections has its limitations for use as system to prove absence of wild poliovirus circulation within the Netherlands. The notification system does not discriminate between virological and serological diagnoses and does not reflect the real number of typed enteroviruses isolated from stool samples. Furthermore the system gives no data on the total number of samples tested for the presence of enteroviruses, as only positive findings are reported. To overcome these drawbacks, a new notification system was developed in 1998, in which laboratories

report on a quarterly basis the total number of stool specimens inoculated on poliovirus-sensitive cells, the number of enterovirus-positive stools, and all typing results from these isolates. Data are to be reported separately for patients below and above 15 years. Data for 1996 and 1997 were collected retrospectively.
Thirteen laboratories agreed to provide complete data for the quarterly notification system. Table 5 shows that in the years 1996-98 more than 1000 enteroviruses have been isolated from almost 20,000 stool samples (5% virus-positive samples). The fraction enterovirus-positive stool samples is rather constant during the years of study for each of the participating laboratories, but varies from 1.08 to 9.19 % when compared to each other. This probably reflects differences in indications for virological investigation for various clinical symptoms and local preferences due to participation in scientific research programmes.
More than 95% of the enteroviruses are isolated from stool specimens from children below 15 ( Table 6), although more than 20% of the stool samples are from persons above 15 year of age (data from 12 laboratories). This results in a mean non-poliovirus isolation rate of 6.38 % for stools from all patients below 15 years. WHO requires a non-poliovirus isolation rate of 10% for stool samples from only AFP patients below 15 years of age, as one of the accreditation criteria for national laboratories of the WHO polio laboratory network. As in the Netherlands, adequate stool samples from only 30% of AFP patients are analyzed virologically (4,20), the two groups of patients overlap only partially.

*Table 5 Enterovirus isolations from faecal specimens by Regional Virological Laboratories (Quarterly Reports)*

| | Labnumber | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 3 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| **1996** | # samples tested | 464 | 1276 | 463 | 330 | 103 | 1596 | 468 | | n.d. |
| | # EV positive | 50 | 46 | 5 | 32 | 2 | 62 | 13 | | 141 |
| | % EV positive | 10.78 | 3.61 | 1.08 | 9.70 | 1.94 | 3.88 | 2.78 | | |
| **1997** | # samples tested | 596 | 1259 | | 309 | 116 | 1330 | 346 | n.d. | n.d. |
| | # EV positive | 49 | 64 | | 11 | 2 | 42 | 10 | 22 | 94 |
| | % EV positive | 8.22 | 5.08 | | 3.56 | 1.72 | 3.16 | 2.89 | | |
| **1998** | # samples tested | 148 | 939 | | 257 | 84 | 1410 | | | n.d. |
| | # EV positive | 12 | 12 | | 21 | 0 | 40 | | | 128 |
| | % EV positive | 8.11 | 1.28 | | 8.17 | 0.00 | 2.84 | | | |
| **Total** | # samples tested | 1208 | 3474 | 463 | 896 | 303 | 4336 | 814 | n.d. | n.d. |
| **96-'98** | # EV positive | 111 | 122 | 5 | 64 | 4 | 144 | 23 | 22 | 363 |
| | % EV positive | **9.19** | **3.51** | **1.08** | **7.14** | **1.32** | **3.32** | **2.83** | | |

| | Labnumber | 9 | 10 | 11 | 12 | 13 | 14 | **Total** | |
|---|---|---|---|---|---|---|---|---|---|
| **1996** | # samples tested | 923 | 315 | 21 | 585 | 562 | 704 | **7810** | |
| | # EV positive | 86 | 18 | 1 | 29 | 41 | 66 | **451** | |
| | % EV positive | 9.32 | 5.71 | 4.76 | 4.96 | 7.30 | 9.38 | 5.77 | |
| **1997** | # samples tested | 936 | 256 | 18 | 353 | 635 | 611 | **7055** | |
| | # EV positive | 7 | 18 | 3 | 18 | 43 | 60 | **406** | |
| | % EV positive | 8.23 | 7.03 | 16.67 | 5.10 | 6.77 | 9.82 | 5.75 | |
| **1998** | # samples tested | 689 | 127 | 25 | 405 | 591 | | **4868** | |
| | # EV positive | 21 | 1 | 1 | 30 | 33 | | **174** | |
| | % EV positive | 3.05 | 0.79 | 4.00 | 7.41 | 5.58 | | 3.57 | |
| **Total** | # samples tested | 2548 | 698 | 64 | 1343 | 1788 | 1315 | 19733 | |
| **96-'98** | # EV positive | 184 | 37 | 5 | 77 | 117 | 126 | 1031 | 385* |
| | % EV positive | **7.22** | **5.30** | **7.81** | **5.73** | **6.54** | **9.58** | **5.22** | |

* Number of isolates from laboratories giving no data (n.d.) on the total number of faecal specimens tested on poliovirus sensitivecells.

*Table 6 Enteroviruses isolated from stool specimens from children below 15 years of age*

| | 1996 | | | 1997 | | |
|---|---|---|---|---|---|---|
| | number samples tested | number EV positive | % EV positive | number samples tested | number EV positive | % EV positive |
| Total all patients | 7326 | 445 | 6.07 | 7037 | 403 | 5.73 |
| Total < 15 years | 5671 | 419 | 7.39 | 5649 | 394 | 6.97 |
| Total > 15 years | 1655 | 26 | 1.57 | 1388 | 9 | 0.65 |
| Total all patients | 100.00% | 100.00% | | 100.00% | 100.00% | |
| Total < 15 years | 77.41% | 94.16% | | 77.11% | 97.77% | |
| Total > 15 years | 22.59% | 5.84% | | 18.95% | 2.23% | |

| | 1998 | | | Total | '96-'98 | |
|---|---|---|---|---|---|---|
| | number samples tested | number EV positive | % EV positive | number samples tested | number EV positive | % EV positive |
| Total all patients | 4877 | 163 | 3.34 | 19240 | 1011 | 5.25 |
| Total < 15 years | 3848 | 155 | 4.03 | 15168 | 968 | 6.38 |
| Total > 15 years | 1029 | 8 | 0.78 | 4072 | 43 | 1.06 |
| Total all patients | 100.00% | 100.00% | | 100.00% | 100.00% | |
| Total < 15 years | 78.90% | 95.09% | | 78.84% | 95.75% | |
| Total > 15 years | 21.10% | 4.91% | | 21.16% | 4.25% | |

In Table 7 the typing results of the notified enterovirus isolates are shown. In each year three predominant serotypes were observed, each with more than 10% of the typed isolates. In 1996: CB1, CB5 and ECHO 22; in 1997: CB4, ECHO22 and ECHO30; in 1998: CB2, ECHO3 and ECHO 11. Overall 2 % of the isolates were notified as non typed, 15 % as non-typable enteroviruses. Most laboratories indicated to have sent these isolates to the National polio Laboratory in Bilthoven for further characterization or exclusion of poliovirus, but had not yet received the result, at the time of reporting. (See Table 3) Comparison of the typing results from the weekly reporting system and from the quarterly notification system ( Table 4 vs. Table 7) shows in general good correlation: the same serotypes are predominantly. In the quarterly reporting system, a higher percentage of isolates is typed (83% vs. 35%) and a larger diversity of serotypes is reported, as also the results of typing experiments performed at RIVM on isolates that were not or could not be typed at the regional virological laboratories are shown.

*Table 7 Typing results of enteroviruses from stool specimens ( Quarterly Reports)*

| | Actual numbers | | | | Percentages of total | | | |
|---|---|---|---|---|---|---|---|---|
| | 1996 | 1997 | 1998 | Total | 1996 | 1997 | 1998 | Total |
| Polio 1 | 0 | 1 | 1 | 2 | 0% | 0% | 0% | 0% |
| Polio 3 | 1 | 0 | 1 | 2 | 0% | 0% | 0% | 0% |
| Polio NT | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| CB1 | 90 | 0 | 2 | 92 | 22% | 0% | 1% | 10% |
| CB2 | 0 | 0 | 26 | 26 | 0% | 0% | 17% | 3% |
| CB3 | 5 | 10 | 11 | 26 | 1% | 3% | 7% | 3% |
| CB4 | 9 | 41 | 3 | 53 | 2% | 11% | 2% | 6% |
| CB5 | 87 | 2 | 0 | 89 | 21% | 0% | 0% | 10% |
| CB6 | 0 | 0 | 0 | 0 | 0% | 0% | 0% | 0% |
| CBNT | 19 | 4 | 8 | 31 | 5% | 1% | 5% | 3% |
| CA5 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| CA7 | 0 | 1 | 0 | 1 | 0% | 0% | 0% | 0% |
| CA9 | 4 | 20 | 1 | 25 | 0% | 6% | 0% | 3% |
| CA10 | 0 | 0 | 1 | 1 | 0% | 0% | 0% | 0% |
| CA13 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| CA14 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| CA16 | 2 | 0 | 1 | 3 | 0% | 0% | 0% | 0% |
| CA17 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| CA20 | 0 | 0 | 4 | 4 | 0% | 0% | 3% | 0% |
| CA24 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| ECHO1 | 2 | 2 | 0 | 4 | 0% | 0% | 0% | 0% |
| ECHO2 | 1 | 0 | 0 | 1 | 0% | 0% | 0% | 0% |
| ECHO3 | 0 | 5 | 21 | 26 | 0% | 1% | 13% | 3% |
| ECHO4 | 0 | 0 | 1 | 1 | 0% | 0% | 0% | 0% |
| ECHO5 | 0 | 1 | 0 | 1 | 0% | 0% | 0% | 0% |
| ECHO6 | 4 | 31 | 2 | 37 | 0% | 9% | 1% | 4% |
| ECHO7 | 31 | 5 | 8 | 44 | 8% | 1% | 5% | 5% |
| ECHO9 | 4 | 14 | 7 | 25 | 0% | 4% | 4% | 3% |
| ECHO11 | 7 | 4 | 22 | 33 | 2% | 1% | 14% | 4% |
| ECHO12 | 2 | 0 | 0 | 2 | 0% | 0% | 0% | 0% |
| ECHO13 | 2 | 0 | 0 | 2 | 0% | 0% | 0% | 0% |
| ECHO14 | 0 | 1 | 2 | 3 | 0% | 0% | 1% | 0% |
| ECHO17 | 0 | 0 | 1 | 1 | 0% | 0% | 0% | 0% |
| ECHO18 | 12 | 9 | 0 | 21 | 3% | 3% | 0% | 2% |
| ECHO20 | 4 | 0 | 2 | 6 | 0% | 0% | 1% | 0% |
| ECHO21 | 0 | 2 | 0 | 2 | 0% | 0% | 0% | 0% |
| ECHO22 | 47 | 35 | 7 | 89 | 11% | 10% | 4% | 10% |
| ECHO25 | 4 | 25 | 1 | 30 | 0% | 7% | 0% | 3% |
| ECHO27 | 0 | 8 | 0 | 8 | 0% | 2% | 0% | 0% |
| ECHO29 | 0 | 1 | 0 | 1 | 0% | 0% | 0% | 0% |
| ECHO30 | 14 | 45 | 3 | 62 | 3% | 13% | 2% | 7% |
| Entero 71 | 0 | 4 | 0 | 4 | 0% | 1% | 0% | 0% |
| EnteroNTa | 9 | 7 | 7 | 23 | 2% | 2% | 4% | 2% |
| EnteroNTb | 43 | 79 | 14 | 136 | 11% | 22% | 9% | 15% |
| Total | 409 | 357 | 157 | 923 | 100% | 100% | 100% | 100% |

# 3. Conclusions and recommendations

## 3.1 Conclusions

The existence of a large group of socially strongly organized persons that refuse vaccination for religious reasons is a constant public health risk, that justifies permanent surveillance for the spread of vaccine-preventable infectious diseases, such as poliomyelitis in the Netherlands. As mandatory vaccination is not a viable option within the Dutch public health system, global polio-eradication is the only way to prevent poliovirus epidemics in the Netherlands.
The gold standard for certification of the absence of circulation of wild polioviruses is active AFP surveillance in children below 15 years of age coupled to adequate virological analysis of two stool specimens, taken 24-48 hours apart within two weeks after onset of symptoms. Based on data of the Statistics Netherlands the age group in question comprises about 3 million children. Optimal AFP surveillance would imply therefor analysis of 60-70 stool specimens per year. ( 4,20) Within the Dutch public health system, active AFP surveillance has been very difficult to organize and to sustain at the required WHO standards. For these reasons the Dutch Committee for the Certification of Polioeradication has formulated additional surveillance activities, i.e. nation-wide enterovirus surveillance and environmental surveillance of sewage water around secondary schools with a high percentage of non-vaccinated children. (8)
The relevant data for adequate enterovirus surveillance as a tool to prove absence of wild poliovirus circulation could only be obtained via a newly developed notification system. Regional virological laboratories, that perform cell culture for the diagnosis of respiratory and gastrointestinal virus infections were asked to report the results of all inoculations of stool samples from children below 15 years of age, on cell types on which polioviruses can be cultivated. The quality of the data is assured by a quality control system, that at least once a year measures the proficiency of the participating laboratories. Proficiency tests, containing samples with or without polio- and other enteroviruses are sent to all virological laboratories performing cell culture and enterovirus typing tests. All proficiency tests are accompanied by a questionnaire, from which additional information is collected on use and quality of reagents and cells, as well as on procedures followed. On the basis of these data, new panels are developed to reveal possible weaknesses in the performance of the participating laboratories. The fact that 3 of the 17 poliovirus isolations reported in the Netherlands between 1993 and 1998 originate from proficiency panel testing is worrying, and illustrates the need for critical review of laboratory procedures and sample routings in the laboratory.
The present report describes the analyses of data from 14 regional virological laboratories for the years 1996-1998. From 19,733 stool samples tested in cell culture on poliovirus-sensitive cells 1031 enteroviruses have been isolated, five of which were polioviruses.(Table 7). All poliovirus isolates were characterized as Sabin-derived viruses, originating from recent vaccinations in countries using OPV. Provided that the quality of the data generated is sufficient, these data imply that in none of the almost 20,000 stool specimens wild poliovirus could be detected. Even more impressive data could be produced provided all 19 regional virological laboratories would participate. Based on the data from the weekly reports, and using an enterovirus isolation rate of 5 %, the actual numbers would increase by more than 50%.

Corrected for the age group (15168 stool samples came from children below 15 years of age) this outnumbers by far the data that would be provided by optimal active AFP surveillance, by which just about 210 stool samples would have to be tested only during the same period of three years. Performance indicators for enterovirus surveillance have however not yet been formulated. Indicators may include the completeness of the data, the regional and seasonal representativity of the data, the quality of the procedures and tests performed, and the enteroviruses isolation rate. It is clear that AFP surveillance targets a group of patients with a severe clinical symptom that is characteristic for but not exclusively linked to poliovirus infection. On the other hand, enterovirus surveillance targets a much larger group of patients with less severe symptoms that are less specific for wild poliovirus infection. However these symptoms may occur as much as ten times more often in poliovirus infected persons. Comparison of enterovirus surveillance data with AFP surveillance data can therefore only be done after precise modelling. Only then the sensitivity of both systems will be known and estimation of their relative contribution to the prove of the likeliness of the absence of wild poliovirus circulation can be made.

## 3.2 Recommendations

1) The use of the quarterly notification system for results of inoculations of stool specimens on poliovirus-sensitive cells should be continued as the system provides relevant data to document the absence of wild poliovirus circulation in the Netherlands.
2) The quarterly reporting system for enterovirus surveillance in the Netherlands should be optimized by increasing the number of laboratories participating, and improvement of the performance of the laboratories in the yearly proficiency test for quality control of procedures and tests for enterovirus isolation and typing. Participation of all laboratories serving part of the risk-area, with concentrations of unvaccinated persons is necessary for optimal use of enterovirus surveillance as a tool to prove absence of wild poliovirus circulation in the Netherlands.
3) Performance indicators should be developed by WHO, to validate enterovirus surveillance as an additional tool to prove absence of wild poliovirus circulation. The data presented in this report could form part of the basis for the formulation of such criteria. Comparison with data from other countries (Belgium, England, Finland, USA), with different notification systems and vaccination policies (OPV vs. IPV) will also provide relevant information.(2,5,10)

# Annex 1

Regional Virological Laboratories in the Netherlands performing cell cultivation for enteroviruses.

a) Eemland Ziekenhuis Amersfoort.
b) Academisch Medisch Centrum, Amsterdam.
c) Dijkzigt Ziekenhuis, Erasmus Universiteit, Rotterdam.
d) Radboud Ziekenhuis, Nijmegen
e) Streeklaboratorium voor de Volksgezondheid, Tilburg.
f) Streeklaboratorium St. Maartensgasthuis, Venlo.
g) Streeklaboratorium voor Pathologie en Microbiologie, Enschede
h) Streeklaboratorium voor de Volksgezondheid, GG& GD, Amsterdam
i) Diagnostisch Centrum SSDZ, Delft.
j) Streeklaboratorium voor de Volksgezondheid, Veldhoven
k) Stichting Streeklaboratorium Zeeland, Terneuzen.
l) Virologisch Laboratorium Zuiderziekenhuis, Rotterdam.
m) Academisch Ziekenhuis Leiden.
n) Streeklaboratorium voor de Volksgezondheid, Groningen
o) Diakonessenhuis, Utrecht
p) Academisch Ziekenhuis Utrecht, Utrecht.
q) Streeklaboratorium voor de Volksgezondheid, Leeuwarden.
r) Academisch Ziekenhuis Maastricht, Maastricht.
s) Slotervaart Ziekenhuis, Amsterdam.

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# Abbreviations

| | |
|---|---|
| AFP: | Acute Flaccid Paralysis |
| CB: | Coxsackie B virus |
| CFT: | Complement Fixation test |
| CPE: | Cyto-Pathological Effect |
| CSF: | Cerebrospinal fluid |
| IPV: | Inactivated Poliovirus Vaccine |
| LIS: | Diagnostic Laboratory for Infectious Diseases and Screening |
| NID: | National Immunisation Days |
| OPV: | Oral Poliovirus Vaccine |
| PCR: | Polymerase Chain Reaction |
| RIVM: | Rijksinstituut voor Volksgezondheid en Milieu ( National Institute for Public Health and the Environment) |
| SKMM: | Stichting Kwaliteitscontrole in Medische Microbiologie ( Foundation for the Quality Control in Medical Microbiology) |
| WHO: | World Health Organization |

# Appendix 1 Mailing list

| | |
|---|---|
| 1 | Hoofdinspecteur voor de Preventieve en Curatieve Gezondheidszorg |
| 2 | Dr. H.J. Schneider, Directeur-generaal van de Volksgezondheid |
| 3 | J.K. van Wijngaarden, arts, Inspecteur Infectieziekten van de Inspectie Gezondheidszorg |
| 4 | Prof.dr. J.J. Sixma, Voorzitter van de Gezondheidsraad |
| 5-8 | Nationale Certificeringscommissie Polio Eradicatie |
| 9-11 | WHO-EPI, Genève |
| 12-14 | WHO-EURO, Kopenhagen |
| 15 | Landelijke Coördinatiestructuur Infectieziektenbestrijding, Den Haag |
| 16-25 | Regionale Virologische Laboratoria |
| 26 | SKMM, Leeuwarden |
| 27-31 | Europese Certificatie Commissie Polio Eradicatie, Denemarken, Kopenhagen |
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| 33 | prof.dr. R.A. Coutinho, GG&GD, Amsterdam |
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