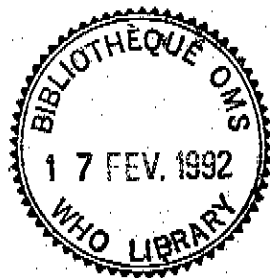


EUR/ICP/EPI.019 ✓

ENVIRONMENTAL
SURVEILLANCE OF WILD
POLIOVIRUS CIRCULATION
IN EUROPE



WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR EUROPE
COPENHAGEN

ABSTRACT

The last step in the eradication of poliomyelitis is the elimination of the wild poliovirus from circulation in both people and the environment; environmental surveillance is therefore crucial at this stage. WHO and the National Public Health Institute, Finland, held a joint meeting to launch collaborative studies to assess the most effective forms of environmental surveillance. The participants from Finland, France, Germany, the Netherlands, Sweden and the USSR agreed to take part in studies on the efficiency of sampling from sewage treatment plants, on various sampling methods, on the optimum sampling size, frequency and duration, and on viral detection and to report back to the Institute on a standard form. They also agreed that greater publicity about the importance of environmental surveillance would help raise the funds necessary to carry it out.

TARGET 5

REDUCING COMMUNICABLE DISEASE

Index terms

ENVIRONMENTAL MONITORING
POLIOVIRUS
COMMUNICABLE DISEASE CONTROL
DATA COLLECTION
EUR

This report is issued by the Regional Office for Europe in English, French, German and Russian. It may be reproduced, or translated into any other language, provided due acknowledgement is made.

38371

EUR/ICP/EPI 019
8000B
ORIGINAL: ENGLISH

**ENVIRONMENTAL
SURVEILLANCE OF WILD
POLIOVIRUS CIRCULATION
IN EUROPE**

Report on a Joint WHO/NPHI Meeting

**Helsinki, Finland
5-6 April 1991**

CONTENTS

	<i>Page</i>
Introduction	1
Aim of the meeting	1
Discussion	2
Participating countries	2
Sampling site	3
Sampling methods	3
Sample size	4
Sampling frequency	4
Duration of surveillance	4
Virus detection in environmental samples	5
Quality control programme	6
Exchange of information	6
Development of new methods for detection of wild polioviruses in environmental samples	6
Conclusions	7
Recommendations	8
Annex 1: Report protocol on the isolation of poliovirus from environmental samples	10
Annex 2: Participants	21

Introduction

One of the main objectives of the WHO poliomyelitis eradication programme is to rid countries of the poliovirus. The absence of circulating wild poliovirus in humans and the environment is one of the criteria used to declare countries free from poliomyelitis. In contrast to smallpox virus, poliovirus usually infects people without causing disease and is excreted by them for a considerable period of time. The virus may find its way into the environment via sewage, and may circulate and survive there for several months and may thus be the source of new infections. Environmental surveillance will, therefore, be crucial to the success of the eradication programme.

The use of modern techniques for detecting poliovirus in sewage makes it possible to detect wild poliovirus even if only one person in 10 000-50 000 is excreting the virus. Nevertheless, environmental surveillance of wild poliovirus will be particularly challenging in those areas where most of the population receives and excretes live vaccine viruses in large amounts, as they may easily mask the excretion of wild polioviruses. Poliovirus will not be eradicated without surveillance of the environment. In general, however, the importance of the environmental surveillance of wild poliovirus has been underestimated. Coordinated activities are needed to focus the attention of government bodies and funding agencies on the necessity for the environmental surveillance of wild poliovirus circulation.

Aim of the meeting

Screening sewage for poliovirus has been successfully used to monitor virus circulation during outbreaks and vaccination campaigns (with oral poliovirus vaccine (OPV)). Several methods for sample collection and treatment and for virus detection and characterization are in use in different countries. Therefore, a meeting was organized by the WHO Regional Office for Europe and the National Public Health Institute (KTL) of Finland, to develop a plan to identify and

establish practical, standard procedures for representative sampling of the environment and for the sensitive and specific detection of wild poliovirus in these samples, and to arrange collaborative studies for this purpose. The nine participants had good experience in the virological analysis of sewage (Annex 2) and agreed to take responsibility for various parts of this collaborative programme. They recognized that environmental surveillance of wild poliovirus will require cooperation among people of various scientific backgrounds including engineers, virologists, epidemiologists and statisticians.

Discussion

The presentations on poliovirus surveillance in sewage or surface waters in different countries showed that various procedures and methods are used. By whatever method, however, wild poliovirus has been detected in sewage or river water in recent years in nearly all the countries with participants at the meeting, even those that have been (nearly) free of poliovirus for several years, such as Sweden and the Netherlands.

Participating countries

The participants agreed that the environmental surveillance of wild poliovirus becomes necessary in stage A (or near stage A) countries, defined as effectively free of poliomyelitis. They proposed that the collaborative programme should include stage A countries using inactivated poliovirus vaccine (IPV) and OPV, as well as a country with well established circulation of wild polioviruses. The following countries are considered prime candidates for such a study: Finland, France, Germany, Netherlands, Spain, Sweden, Switzerland and USSR.

In the USSR, regions without recent cases of poliomyelitis (Estonia), with few recent cases (Moscow) and with quite a number of recent cases (Kirghizia) will be included.

Sampling site

Reports from different countries showed that environmental samples were collected from varying sampling sites. Consensus focused on sampling from sewage treatment plants, either at the inlet to the plant or from the activated sludge. The participants from Finland, France, Germany, Sweden and the USSR agreed to participate in a comparative study on the efficiency of viral recovery from samples taken at the inlet to sewage treatment plants or from activated sludge.

Sampling methods

Several sampling methods are in use and fall into two categories: continuous sampling and grab sampling.

Continuous sampling consists of suspending, for a certain period of time, gauze pads or macroporous glass beads packed in small gauze bags in sewage streams, at the inlet to sewage treatment plants. Viruses in the sewage are absorbed by the pads or beads and are eluted therefrom for inoculation onto susceptible cells.

Grab sampling consists of taking a single sample of a certain volume from the sewage system, either at the inlet to sewage treatment plants or from the activated sludge. After treatment to remove toxic or interfering factors, the sample is inoculated onto susceptible cells.

In some laboratories, treatment also involves concentrating the virus in the sample to improve its sensitivity. In view of the widely different methods used, the participants decided to start a collaborative study on sampling methods. This study will be combined with the study on the efficiency of viral recovery mentioned above and includes participation from laboratories in Finland, France, Germany, Sweden and the USSR. Besides their regular sampling procedures, these laboratories will also use macroporous glass beads packed in small bags for sampling from sewage. The macroporous beads will be distributed by Dr V. Kazantseva, of the Institute of Poliomyelitis and Viral Encephalitis of the Academy of Medical Sciences of the USSR, with the help of WHO. In addition, Dr Kazantseva will carry

out a study on the optimal sampling time when using these glass beads.

Sample size

The optimal sampling size and frequency depend on the sampling method, the nature of the sample, the use of concentration techniques and the virus detection method used. They have not yet been established, therefore. The sensitivity of environmental surveillance should be expressed in practical terms, by stating how many excretors in a certain population in a given period the test system used would detect. As to sampling volume, increasing the number of sewage plants sampled is preferable to increasing the volume sampled at any single sewage plant. Sampling size also relates to the proportion of the population monitored in the surveillance programme: a substantial percentage of the population in a country should be covered. Nevertheless, the exact size of the population to be covered, before a decision can be made about the absence of wild poliovirus circulation in a given area, is not yet clear and should be the subject of special studies in the future.

Sampling frequency

Although poliovirus excretion is known to show a certain seasonality, in temperate climates, environmental sampling should be carried out throughout the year. Given the limitations of the present, rather laborious and time-consuming methods for the environmental monitoring of poliovirus, sampling at sewage treatment plants should take place at least monthly. Later on, when better, more efficient virus detection methods are available, the frequency may be increased.

Duration of surveillance

Environmental surveillance will probably have to be continued for several years after the global disappearance of clinical cases of

poliomyelitis. The absence of the wild virus from the environment will have to be demonstrated over several years before a decision to stop vaccination can be made. How long this will take is not yet clear and should be the object of further discussions.

Virus detection in environmental samples

All the participants use tissue culture systems to detect viruses in environmental samples. Molecular methods, particularly the polymerase chain reaction, have not yet been developed sufficiently for application in a surveillance programme. Whichever method and cell system are used - the plaque method or the cytopathogenic effect method, using various cells (including BGM monkey cells, RD or HEp-2) in flasks, tubes or microlitre plates - the isolated strains must always be typed. In addition, poliovirus strains will have to be intratypically differentiated. For standardization purposes, Dr van Loon of the Laboratory of Virology, the National Institute of Public Health and Environmental Protection (RIVM), in the Netherlands, will arrange that BGM, RD and HEp-2 cells, as well as neutralizing sera for the typing of poliovirus and other enteroviruses, are distributed to all the participants in the studies. The Laboratory of Virology will also carry out the intratypic differentiation of poliovirus strains isolated by the Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes, Berlin, Germany. If the number of strains to be analysed is high, a request for intratypic differentiation will also be made to Dr R. Crainic at the Institut Pasteur, Paris, France.

In addition, several suggestions were made for improving the practicability and sensitivity of the methods for detecting and typing viruses in sewage on tissue culture systems.

One suggestion was the inoculation of cells with (unconcentrated) 5-ml sewage samples divided over the wells of microtitration plates, possibly also using neutralizing antiserum to coxsackie virus B. Low virus concentrations (< 10 PFU/ml) and the use of cells with restricted susceptibility to enteroviruses are prerequisites for such a scheme.

Another suggestion was the typing of isolated strains as poliovirus or non-poliovirus only, possibly using an enzyme-linked

immunosorbent assay (ELISA) to detect poliovirus antigen in the supernatant fluid of inoculated cells. Of course, the polioviruses must then be intratypically differentiated.

A final suggestion was the development of non-human cell lines that express the receptor for poliovirus.^a

Quality control programme

In this phase of development of the environmental surveillance methodology, implementation of an external quality control programme would be premature. Internal quality assessment, however, is already needed now.

Once the environmental surveillance methodology has been standardized and a regular programme begun, external quality control programmes will be fundamental to the success of environmental surveillance.

Exchange of information

Dr T. Hovi of the Enterovirus Laboratory, National Public Health Institute, in Finland, will coordinate the collaborative programme on environmental surveillance. Quarterly reports should be sent to him on standard report forms (see Annex 1).

Development of new methods for detection of wild polioviruses in environmental samples

The current methods for environmental surveillance based on cell cultures are laborious, time-consuming and expensive and they are

^a Kolke, S. et al. Transgenic mice susceptible to poliovirus. *Proceedings of the National Academy of Sciences*, 88: 951-955 (1991).

not good at detecting small amounts of wild poliovirus in samples with high levels of vaccine virus. Therefore, new methods are needed. Specific gene amplification, by polymerase chain reaction or other amplification methods, seem ideal for detecting wild poliovirus in environmental samples. The genetic variation of wild polioviruses, however, somewhat limits the application of specific gene amplification. The use of polymerase chain reaction primer panels, covering all wild virus genotypes recognized so far, would be one solution to this problem, but this would miss the unknown wild virus genotypes or the evolution of new genotypes.

An interesting solution may come from combining biological and molecular methods in the so-called antigen-capture polymerase chain reaction described for the detection of hepatitis virus A.^a This antigen-capture polymerase chain reaction may achieve selectivity for wild poliovirus if antibodies specific to wild virus are used, whereas it will achieve sensitivity if enterovirus-specific primers and probes are used. At the Centers for Disease Control in the United States, the National Public Health Institute in Finland, and RIVM in the Netherlands, work has started on developing this methodology for environmental surveillance.

Conclusions

No optimal and standard system for the environmental surveillance of wild poliovirus circulation is yet available. The participants agreed to start a series of collaborative studies to improve the practicability and sensitivity of current methods, especially to develop an optimal technique for the sampling of sewage.

In addition, new methods urgently need to be developed that allow easier detection of wild polioviruses in the presence of large

^a Jansen, R.W. et al. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. *Proceedings of the National Academy of Sciences*, 87: 2867-2871 (1990).

amounts of vaccine viruses. These methods will probably require a combination of biological and molecular methodology. As well as these technical improvements and developments, a strategy of environmental surveillance will have to be developed, requiring cooperation among different scientific fields such as engineering, epidemiology, virology and statistics. Such a strategy should also address the question of what action to take when wild poliovirus is isolated in countries that have been at stage A for many years.

Finally, many of the activities needed to develop an optimal strategy for the environmental surveillance of wild poliovirus cannot be carried out without additional funding. For this, the importance of the environmental surveillance of wild poliovirus circulation should be made better known. Only then will the additional resources become available.

Recommendations

1. WHO headquarters and the Regional Office, together with the National Public Health Institute, Finland, should coordinate collaborative studies in several European countries on the optimal strategy for sampling sewage and analysing the samples.
2. WHO headquarters should promote collaborative studies on the use of polymerase chain reaction to detect wild polioviruses in sewage, especially by obtaining special primers for the detection of wild polioviruses in European countries and by combining the biological and molecular methods in the polymerase chain reaction technique.
3. The Regional Office should contact investigators in several other European countries who could be involved in collaborative studies on the environmental surveillance of polioviruses (such as Spain and Switzerland).
4. The Regional Office should stress the importance of environmental surveillance for the eradication of poliomyelitis to national

governments and intergovernmental organizations (such as the European Community), in the hope of obtaining the necessary funding to improve environmental surveillance and to launch special studies on the optimal strategy for the environmental surveillance of wild polioviruses.

5. WHO headquarters, together with the Regional Office for Europe and the Regional Office for the Americas, should organize the next meeting on the environmental surveillance of wild polioviruses at the beginning of 1992. The participants should be the experts involved in the WHO collaborative studies on the environmental surveillance of wild polioviruses being carried out in Europe and the United States and in the studies on the use of polymerase chain reaction to detect wild polioviruses in environmental samples.

ANNEX 1

**Report protocol on the isolation of poliovirus
from environmental samples**

To be sent quarterly to:

**Dr Tapani Hovi
Head, Enterovirus Laboratory
National Public Health Institute (KTL)
Mannerheimintie 166
00300 Helsinki, Finland**

Date of the report:

Reporting laboratory:

.....

.....

.....

Name of the reporting investigator:

.....

.....

Tel.:

Fax:

A. Sampling site information**A.1. Wastewater treatment plant(s)****Plant 1**

Name and location of the plant:

.....

.....

.....

Amount of wastewater processed daily: m^3

Number of persons served by the plant:

Amount of water allocated per capita: litres

Volume of the activated sludge basin: m^3

Detention time of the wastewater: hours

Average age of the sludge: hours/weeks

Plant 2

Name and location of the plant:

.....

.....

.....

Amount of wastewater processed daily: m³
Number of persons served by the plant:
Amount of water allocated per capita: litres
Volume of the activated sludge basin: m³
Detention time of the wastewater: hours
Average age of the sludge: hours/weeks

Plant 3

Name and location of the plant:
.....
.....
.....
.....

Amount of wastewater processed daily: m³
Number of persons served by the plant:
Amount of water allocated per capita: litres
Volume of the activated sludge basin: m³
Detention time of the wastewater: hours
Average age of the sludge: hours/weeks

A.2. Surface water(s) (rivers and coastal waters)

River(s). Describe name of the river(s) and location of the sampling site. If available, give other relevant information about the hygienic status, e.g. *Escherichia coli* or coliform concentration.

River 1:

.....

.....

.....

.....

River 2:

.....

.....

.....

.....

River 3:

.....

.....

.....

.....

Coastal water(s). Describe location and give other relevant information as described under rivers.

Coastal water 1:
.....
.....
.....
.....

Coastal water 2:
.....
.....
.....
.....

Coastal water 3:
.....
.....
.....
.....

A.3. Drinking-water and other

Drinking-water:.....
.....
.....
.....

Other:
.....
.....
.....

A.4. Remarks, if any:

.....
.....
.....
.....

*Annex 2***PARTICIPANTS****Temporary Advisers**

Professor Margareta Böttiger

Epidemiological Department, National Bacteriological Laboratory,
Stockholm, Sweden

Dr Sylvie Dubrou

Ingénieur Hygiéniste, Laboratoire d'Hygiène de la Ville de Paris, France

Ms Leena Hiisvirta

Environmental Health Engineer, National Agency for Welfare and
Health, Helsinki, Finland

Dr Tapani Hovi

Head, Enterovirus Laboratory, National Public Health Institute (KTL),
Helsinki, Finland

Dr Valentina Kazantseva

Institute of Poliomyelitis and Viral Encephalitis of the Academy of
Medical Sciences of the USSR, Moscow, USSR

Dr Anton M. van Loon

Head, Laboratory of Virology, National Institute of Public Health and
Environmental Protection (RIVM), Bilthoven, Netherlands

Professor Juan López-Pila

Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheits-
amtes, Berlin, Germany

Ms Tuija Pöyry

Enterovirus Laboratory, National Public Health Institute (KTL), Helsinki,
Finland

Ms Mirja Stenvik

Enterovirus Laboratory, National Public Health Institute (KTL), Helsinki,
Finland

World Health Organization

Regional Office for Europe

Dr George Oblapenko

Medical Officer for Eradication of Poliomyelitis

Ms Liz Shrapnel

Secretary, Eradication of Poliomyelitis programme

Headquarters

Dr Yuri Ghendon

Medical Officer for Communicable Diseases and Immunology