*Acta Microbiologica et Immunologica Hungarica, 62 (1), pp. 75–85 (2015)*
*DOI: 10.1556/AMicr.62.2015.1.6*

# COMPARATIVE STUDY OF ANTIBODY LEVELS DEVELOPED BY VACCINATION AGAINST POLIO VIRUS IN POPULATION AFTER VACCINE TYPE ALTERATION

ÁGNES FARKAS, NÓRA MAGYAR, KATALIN N. SZOMOR and MÁRIA TAKÁCS*

WHO National Poliovirus Reference Laboratory, Division of Virology,
National Center for Epidemiology, Budapest, Hungary

(Received: 6 January 2015; accepted: 30 January 2015)

During clinical trials, samples from Hungarian patients of different age groups were tested for antibodies against all 3 serotypes of poliovirus, a member of *Picornaviridae* family. During the virus neutralization serological test, blood samples were titrated using permanent virus concentration. Based on the cythopathic effect observed under a light microscope, the antibody level of the patient was assessed. The 100 people examined were classified into 5 groups based on age and type of original vaccine: I. Newborns, no vaccination given; II. Immunosuppressed patients; III. Born before 1986, received only OPV vaccine; IV. Born between 1992–2005, received a combination of OPV and IPV vaccines; V. Born after 2006, received only IPV vaccine. Results show that vaccination coverage meets all the criteria. None of the immunized persons was seronegative to all three polioviruses. Both IPV and OPV vaccines are effective against poliovirus. Blood samples from newborn babies with no immunization were also examined. Results show that most newborns have maternal antibodies in their blood. Results of group II show that immunosuppression does not have a negative influence on blood antibody levels against polioviruses. In spite of the low number of samples, our results show that seroconversion after immunization in the Hungarian population is adequate. For more accurate results about vaccination coverage in the population, further trials would be necessary.

**Keywords:** poliovirus, paralytic poliomyelitis, vaccine, antibody level

## Introduction

Paraliticus poliomyelitis caused by polio virus was one of the most feared contagious diseases in the 20th century, and in some endemic countries it still

* Corresponding author; E-mail: takmar@gmail.com

occurs. Poliovirus is a human enterovirus in *Picornaviridae* family [1]. The virion of polio is one of the smallest known virions with the size of 27–30 nm [2]. According to traditional taxonomy (valid until 2005), *genus poliovirus* was a separate group under its own name [3]. According to current taxonomy, all 3 serotypes (PV1-3) are members of the group enterovirus C (EV-C) [4].

The virion is very resistant against detergents. It can preserve its virulence for days under +4 °C, and for years under –20 °C [5]. Formaldehyde and chloro derivates are effective disinfectants against the virus. The virions are sensitive to heat, dehydration and UV radiation [6]. Only humans are susceptible to the virus. Infected humans are the source of infection, whether the clinical course is symptomatic or asymptomatic. Enteral spread is the most common way of infection, but nasal and throat secretions can also be contagious. Infected patients' stool can also be infectious, therefore the main transmission path of the virus is feco-oral. Due to its resistance, the virion can preserve its virulence and can spread via contaminated hands, food, kitchen tools, sewage, etc. The latency period is 7–14 days. Primer replication sites of the virus are the pharynx, the ileum of the intestines and the regional lymphatic system [5]. More than 90% of the cases are asymptomatic. In 4–8% of cases the virus enters the blood-stream, and causes viraemia with fever, which passes with no complications in a few days. In a small percentage of the cases, the virus can enter the central nervous system (CNS) and cause mild nervous symptoms (non-paralytic poliomyelitis). In 0.01–2.0% of all cases, the virus can replicate in the motoric neurons of the CNS. Depending on the number and the location of these neurons, different forms of paralitic poliomyelitis may develop [7].

In 1988, 125 endemic countries were registered and the estimated number of annual infections was 350,000. Due to polio eradication programmes, the number of endemic countries reduced to 3 (Afghanistan, Pakistan, Nigeria). Eradication in these countries has not been successful so far, and the virus is still circulating [8]. Virus transfer to non-endemic countries from endemic ones could be the cause of the 256 paralitic cases found in non-endemic regions in 2013. The 160 cases registered in endemic areas indicates that changes in vaccine coverage can lead to epidemics [9].

In Syria, no cases have been registered between 1999 and 2013. Vaccine coverage among 5-year-olds was 83% in 2009 and 2010. Children did not receive vaccines during the civil war, and vaccine coverage reduced to 53% by 2013. A new polio epidemic appeared in the country: in 2013 35 cases were known, in 2014 one infant developed paralitic poliomyelitis [10].

In 2014 (Until 17 December 2014), 333 new cases were registered globally. 19 happened in non-endemic and 314 in endemic regions [9].

The disease is incurable, only supportive therapy exists. Vaccination is the only option to achieve prevention. Two types of vaccines are used for immunization. One is the oral polio vaccine (OPV), which contains the virions alive. The other is inactivated polio vaccine (IPV). OPV (Sabin vaccine) is a trivalent vaccine containing living, attenuated strains from all 3 serotypes, and is received in drops. Two modes of seroconversion are known. First, vaccine induces immunoglobulin-G (IgG) antibodies to be synthesized by lymphocytes, so they can neutralize infective agents. Second, secretory IgA antibodies appear in intestinal mucosa and provides wider protection [11]. Inactivated polio vaccine (Salk vaccine), IPV is also trivalent and contains all 3 serotypes of the virus inactivated by formalin. IPV is received by injection and provides humoral prevention against polio, based only on IgG antibody synthesis [12].

According to World Health Organization's (WHO) recommendation, the Sabin vaccine should have been altered globally with inactivated Salk vaccine by 2010. Due to the mode of action of OPV, the living virus strains can replicate in the human body, and vaccine recipients can spread the virus by their stool. The only way to accomplish global polio eradication is to eliminate the circulation of all polio strains.

Current vaccination schedule in Hungary is regulated by the Act CLIV of 1997 and the Decree 18/1998 (VI. 3) of the Ministry for Welfare on the Epidemiological Protocols for Preventing Communicable Diseases and Outbreaks [13].

Oral polio vaccine was withdrawn in Hungary 1 April 2006. Since then, only IPV injection has been used for child immunization. According to the current vaccination schedule, infants receive trivalent IPV 5 times, at the ages of 2, 3, 4, 18 months and 6 years old [13].

## Materials and Methods

### *Study group classification and main objectives*

During the laboratory trial, blood samples from Hungarian persons of different ages were tested for antibodies developed after immunization, for all 3 serotypes of poliovirus with serological methods. The main goal was to achieve an insight of vaccination coverage amongst the Hungarian population and to compare the level of protection of two different types of vaccines.

All the persons tested were born in Hungary and were grouped based on age and the type of vaccine. OPV vaccine was introduced in 1959 in Hungary, and administered until 1992. In 2005, IPV vaccine was introduced. Between 1992 and 1 November 2005, children received IPV and OPV vaccines combined.

In 2006, OPV vaccine was discontinued. Since then, only IPV vaccine is used for immunization.

Immunosuppressed patients were also tested for antibody levels. The main question was, if immunosuppression have any negative effect on developed antibody levels. Considering the introduction and withdrawal dates of the vaccines, and the ages of the examined persons, 5 different study groups were classified:

I. Newborns, no vaccination given

II. Immunosuppressed patients

III. Born before 1986, received only OPV vaccine

IV. Born between 1992–2005, received a combination both of OPV and IPV vaccines

V. Born after 2006, received only IPV vaccine

Due to the altered dates of introduction of IPV in different geographical regions of Hungary in 1992 people born between 1986 and 1991 were not tested during this trial. Without knowing the exact history of immunization of the examined persons born within this period, it cannot be specified which group they belong to.

Based on differentiation, the main objectives were:

- Is there any difference between antibody levels or seroconversion developed by different types of vaccination?
- Is there any difference between the half-life of the antibodies developed by the two different types of vaccination?
- Does aging have any effect on the serum antibody level?
- Can protective amounts of maternal antibodies travel through the placenta providing protection for newborns until the first dose of vaccine given them at 2 months age?
- Does immunosuppression have any negative effect on antibodies?

*Virus neutralization serological test*

During the trial, blood samples were tested with virus neutralization serological test [14].

The experiment was performed under BSL2 circumstances in biosafety cabinet and 96 well plates were used for the probe. Before the neutralization test, the blood samples were inactivated in 56 °C for 30 minutes.

Twenty-five µl of RPMI medium (Rosewell Park Memorial Institute [RPMI], Sigma-Aldrich) was measured into each well. Twenty-five µl of the samples were added to the wells and diluted by twofold serial dilution. Each serum was tested in parallel.

Twenty-five µl virus-suspension was added to each well. The applied virus dilution was defined in advance as of $10^{-3}$. Vaccine-derived polio strains were used prescribed by WHO for the trial. Reference numbers were as follows: Sabin 1 NIBSC (National Institute for Biological Standard and Control [NIBSC]) Reference Number 01/528, Sabin 2 NIBSC Reference Number 01/530, Sabin 3 NIBSC Reference Number 01/532. Virus, serum and cell controls were added to each plates. Virus controls from all 3 serotypes of polio were added in the dilutions $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$ respectively.

Seropositive serum with known titre values was used as serum control.

After adding the virus, the plates were incubated for 2 hours in 37 °C, whilst antibodies and antigens were conjugated.

After incubation, 100 µl polio-susceptible L20B mouse-derived transgenic cells were added to each well.

During the 5–7 days incubation period at 37 °C, the virus destroyed the cells in those wells, where antibodies could not neutralize the virus.

The cythopathic effect was examined every day under the light microscope during the incubation period. Based on the cythopathic effect the antibody level of the patient was assessed.

## Results

Results of the study are summarized in order of the examination groups.

### *I. Newborns, no vaccination given*

Fifteen blood samples were tested in group I. Three infants had low antibody values (≤1:4 or ≤1:8). Most of the unvaccinated newborns had maternal antibodies in their blood. One newborn was found to be seronegative to all three serotypes of polio virus, but none of the examined infants had high (1:256 or 1:512) titre values. Relatively high antibody values against two serotypes were detected in case of three newborns. Against PV1 and PV3 the 1:4 was the most common titre value (27% and 47%), whilst against PV2 it was 1:256 (27%) (Fig. 1).

### *II. Immunosuppressed patients*

Fifteen blood samples were tested in group II. Antibodies were detected in the sera of immunosuppressed patients. This indicates immunosuppression has no negative influence on serum antibody levels. None of the patients was seron-

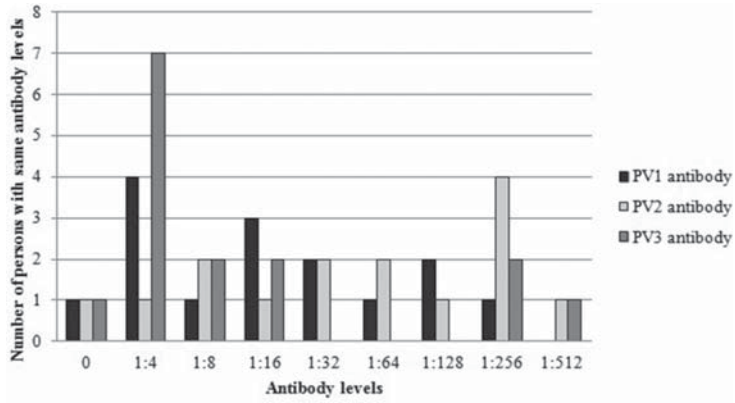


**Figure 1.** Distribution of antibody levels in group I. Horizontal axis shows antibody titre values, vertical axis shows the number of people who have the similar antibody level

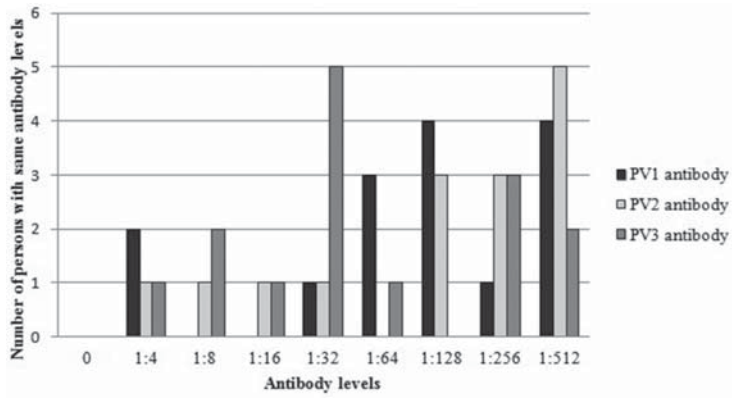


**Figure 2.** Distribution of antibody levels in group II

egative to all three serotypes. One person showed relatively low (≤1:4 or ≤1:8) titre values. Four patients had high antibody levels against all three serotypes. The most common titre values in case of PV1 serotype were 1:128 and 1:512 (27%, 27%). Against PV2, 1:512 was the most common value (33%). The lowest value observed against PV3 was the titre 1:32 (33%) (Fig. 2).

*III. Born before 1986, received only OPV vaccine*

Twenty-one blood samples were tested in group III. None of the examined persons was seronegative to all three serotypes. One person had relatively low

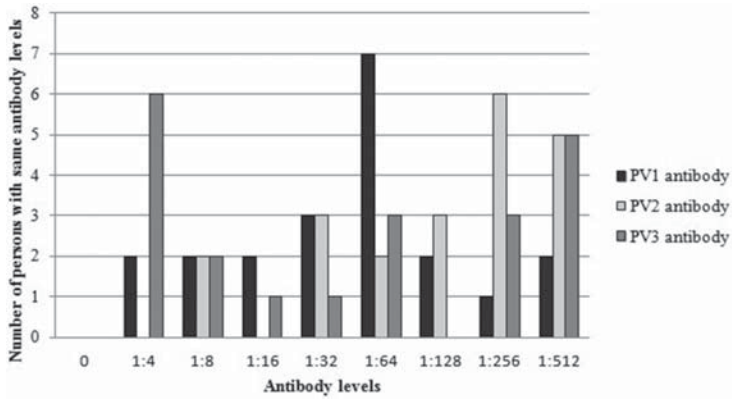


**Figure 3.** Distribution of antibody levels in group III

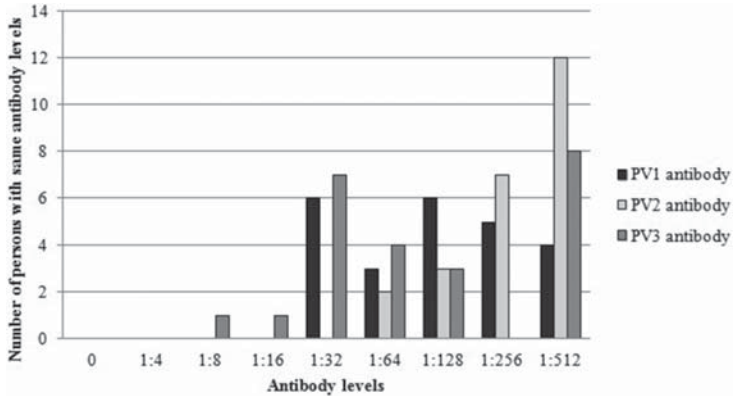


**Figure 4.** Distribution of antibody levels in group IV

antibody values (≤1:4 or ≤1:8). Three showed high titres. Four people exhibited high antibody values against two serotypes of polio. In case of PV1 serotype, the most common titre value was 1:64 (33%), and 1:256 against PV2 (29%). Two groups of extreme values were found against PV3: six persons had ≤1:4 titre value (29 %) and five showed 1:512 (24 %) (Fig. 3).

*IV. Born between 1992–2005, received a combination of OPV and IPV vaccines*

Twenty-four blood samples were tested in group IV. None of the examined persons proved to be seronegative against any serotype. High titre values were

detected in the group, the lowest antibody level was detected against PV3 with the titre 1:8. In case of PV1 serotype, the most common titre values were 1:32 (25%) and 1:128 (25%). Against PV2 and PV3 1:512 titre value was the most common (50% and 33%) (Fig. 4).

*V. Born after 2006, received only IPV vaccine*

Twenty-three blood samples were tested for antibodies against polio virus in group V. The highest titre values were seen in this group. Most infants (87%) of the group did not receive the complete programme of vaccination (5 doses) against polio, the most common titre value against PV1 and PV2 was 1:512 (39% and 65%). None of the infants was seronegative to all 3 serotypes. Eight showed high (1:256 or 1:512) titre values (35%) against all the serotypes. The most common antibody level against PV3 was 1:128 (39%) (Fig. 5).

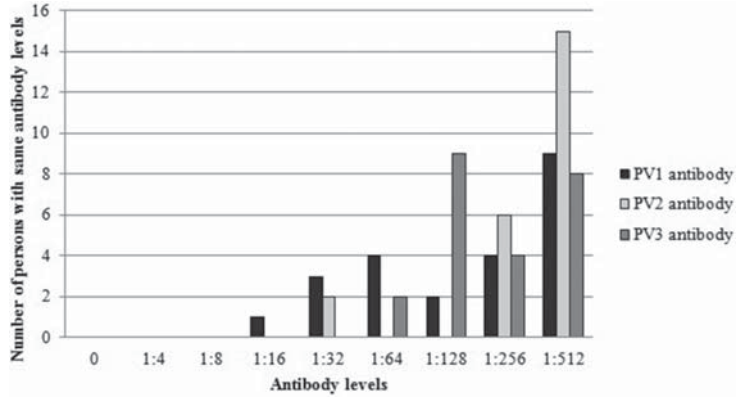


**Figure 5.** Distribution of antibody levels in group V

## Discussion

During the study, 100 blood samples were tested for antibodies against all three serotypes of polio virus. Figure 6 shows the distribution of the antibody levels amongst the tested groups. In conclusion, the vaccination coverage in Hungary exceeds the level required to prevent infections. Antibody titres are generally high, and none of the immunized group was seronegative to all three polioviruses.

Comparing the results of groups III, IV and V, it is proven that both IPV and OPV are effective against polio virus. Newly immunized infants in group V

showed the highest titre values. In group III antibody levels were lower with higher deviation value. As the level of neutralizing antibodies reduce in time, those newly immunized children who have higher titre values now, than earlier vaccinated adults, need to be monitored to complete the survey. Comparing the result of groups III and IV, it is evident that antibody levels are higher in group IV.

American scientists performed a similar clinical test amongst Guatemalan infants in 2007. The goal of the trial was to compare the efficacy of the IPV and OPV vaccines. Children were classified into three groups: IPV receivers only, OPV receivers only and those who received a combination of IPV and OPV vaccines. Results show that seropositivity against all 3 serotypes was 100% after three doses of IPV vaccination, while after OPV vaccination seropositivity was 99%, 100% and 97% against PV1, PV2 and PV3, respectively. IPV vaccinated infants showed higher titre values than OPV receivers, but had the same values as those who both IPV and OPV receivers [15].

It is important to highlight, that the highest antibody levels were experienced against PV2 serotype in all five groups (Fig. 6). The lowest levels were observed against PV3.

Highest deviation values are in groups I, II and III, the lowest are in groups IV and V. Examining the dilution rates in group V the average deviations were between 1 and 1.7. With the exception of group V, the highest deviation values were experienced against PV3 serotype.

An Italian study published in 2006 stated, newly immunized minors (1–17-year-old) showed much higher antibody titre values than formerly vaccinated elders (65–100-year-old). No seronegativity was experienced amongst the vaccinated population [16].

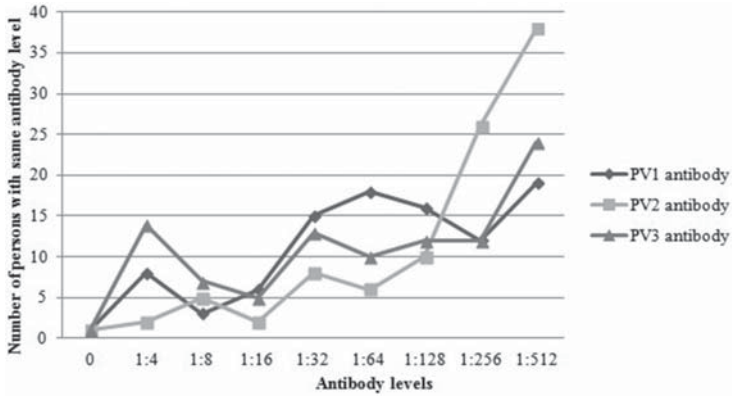


**Figure 6.** Vaccination coverage in Hungarian population:
Distribution of the antibody levels against 3 different virus serotypes among the 100 examined patients

Results of group I show, that maternal IgG antibodies can travel through the placenta and give the foetus a passive protection. Half-life of maternal antibodies in newborns' blood is 28 days. The protective period depends on the postnatal antibody level passed onto the newborn [5].

Blood samples from two newborn infants were collected on the day of birth. In the first newborn, the results against PV1, PV2 and PV3 serotypes were 1:4, 1:64 and ≤1:4. The second newborn's titre values were 1:64, 1:256 and 1:256.

Tested immunosuppressed patients show no deterioration in levels of protection. Patients in group II had average or high titre values, but results showed relatively high deviation.

The results cannot be considered as representative because of the low number in the sample groups. However, the study shows that seroconversion after vaccination in the Hungarian population is adequate. To achieve accurate results about vaccination coverage in the population, further trials would be necessary.

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