

# Seroprevalence of poliovirus antibodies among 7-month-old infants after 4 doses of oral polio vaccine in Sistan-v-Baluchestan, Islamic Republic of Iran

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## الانتشار المصلي للأجسام المضادة لفيروس شلل الأطفال بين الرضع في عمر سبعة أشهر بعد أربع جرعات من لقاح شلل الأطفال الفموي في سيستان وبلوشستان بجمهورية إيران الإسلامية

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**الخلاصة:** على الرغم من ارتفاع معدلات التغطية بلقاح شلل الأطفال في جمهورية إيران الإسلامية فإن معدلات الانقلاب السيرولوجي لدى الرضع قد تكون غير كافية. وقد قامت هذه الدراسة بقياس الانتشار المصلي للأجسام المضادة لفيروس شلل الأطفال من الأنماط المصلية 1 إلى 3 (فيروس شلل الأطفال 1 وفيروس شلل الأطفال 2 وفيروس شلل الأطفال 3) لدى الرضع في عمر سبعة أشهر من الذين تلقوا ما لا يقل عن أربع جرعات من لقاح شلل الأطفال الفموي الثلاثي التكافؤ استناداً إلى برنامج التمنيع الوطني الإيراني. فتم إجراء مسح سيرولوجي في عام 2010 في المناطق الريفية لشاباهار بمحافظة سيستان وبلوشستان. وباستخدام أخذ العينات العنقودي تم فحص 72 رضيعاً من الرضع المؤهلين للكشف عن وجود أجسام مضادة للأنماط السيرولوجية الثلاثة لفيروس شلل الأطفال وفقاً للدلائل الإرشادية لمنظمة الصحة العالمية. وقد اعتبرت عيارات الأجسام المضادة  $\geq 1:10$  إيجابية. وقد كانت معدلات إيجابية المصل للأجسام المضادة لفيروس شلل الأطفال 1 وفيروس شلل الأطفال 2 وفيروس شلل الأطفال 3 84.7% و95.8% و70.8% على التوالي. وكان لدى 63.9% فقط من المشاركين إيجابية مصلية للأجسام المضادة لجميع الأنماط السيرولوجية الثلاثة لفيروس شلل الأطفال. وإذا استثنينا فيروس شلل الأطفال 2 فإن الانتشار المصلي للأجسام المضادة للنمطين السيرولوجيين الآخرين لفيروس شلل الأطفال - لاسيما فيروس شلل الأطفال 3 - لم يكن مرضياً.

**ABSTRACT** Despite high coverage rates of polio vaccine in the Islamic Republic of Iran, the seroconversion rates of infants may be inadequate. This study measured seroprevalence of antibodies against poliovirus serotypes 1 to 3 (PV1, PV2 and PV3) in 7-month-old infants who had received at least 4 doses of trivalent oral polio vaccine. A serosurvey was conducted in 2010 in rural areas of Chabahar, Sistan-v-Baluchestan province. Using cluster sampling, 72 eligible infants were tested for antibody against the 3 poliovirus serotypes according to WHO guidelines. Antibody titres  $\geq 1:10$  were considered positive. The seropositive rates for antibody against PV1, PV2 and PV3 were 84.7%, 95.8% and 70.8% respectively. Only 63.9% of participants were seropositive for antibodies against all 3 poliovirus serotypes. Except for PV2, the seroprevalence of antibody against the other 2 poliovirus serotypes, especially PV3, was unsatisfactory.

## Séroprévalence des anticorps du poliovirus chez des nourrissons âgés de sept mois après quatre doses du vaccin antipoliomyélitique oral dans la province du Sistan-Baloutchistan (République islamique d'Iran)

**RÉSUMÉ** En dépit de taux de couverture élevés par le vaccin antipoliomyélitique en République islamique d'Iran, les taux de séroconversion des nourrissons peuvent être inadéquats. La présente étude a mesuré la séroprévalence des anticorps dirigés contre les sérotypes 1 à 3 de poliovirus (PV1, PV2 et PV3) chez des nourrissons de sept mois qui avaient reçu au moins quatre doses du vaccin antipoliomyélitique oral trivalent. En 2010, une enquête sérologique a été menée dans des zones rurales de Chabahar, dans la province du Sistan-Baloutchistan. À l'aide d'un échantillonnage en grappes, 72 nourrissons éligibles ont fait l'objet de tests de dépistage des anticorps contre les trois sérotypes de poliovirus conformément aux lignes directrices de l'Organisation mondiale de la Santé. Des titres d'anticorps supérieurs ou égaux à 1:10 étaient considérés comme positifs. Les taux de séropositivité pour les anticorps dirigés contre les sérotypes 1, 2 et 3 de poliovirus étaient de 84,7 %, 95,8 % et 70,8 % respectivement. Seuls 63,9 % des nourrissons participants étaient séropositifs pour les anticorps dirigés contre les trois sérotypes de poliovirus. À l'exception du sérotype 2 du poliovirus, la séroprévalence des anticorps dirigés contre les deux autres sérotypes, en particulier le sérotype 3, n'était pas satisfaisante.

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## Introduction

The Islamic Republic of Iran joined the global campaign against polio in 1991 and the country was announced as polio-free in 2001. Since then the country has had no report of circulation of wild polioviruses throughout the country (1). Based on the Iranian national immunization schedule, by the 6th month of age, all infants should have received at least 4 doses of trivalent oral polio vaccine (OPV) (at birth and at 2, 4 and 6 months of age). All children should also receive another 2 doses at 18 months of age and before school entry (4–6 years of age). Added to these are the mopping-up activities of the national immunization days, which include all children aged less than 5 years and are implemented in January and February each year.

The main objective of these efforts is to establish and maintain an immunization coverage high enough to prevent recirculation of the wild polioviruses on probable re-introduction of the infection as has occurred several times within the last decade in the WHO Eastern Mediterranean Region and African Region and recently even in the Western Pacific Region (2). In fact the real protection level established by immunization activities depends on the proportion of the vaccinees who produce appropriate levels of anti-poliovirus antibodies in their blood.

Usually, however, there is a gap, small or large, between the immunization coverage rates and the seroconversion rates, i.e. not all those who receive vaccine always produce appropriate levels of antibody against the antigen. For example, in a study in the Islamic Republic of Iran on 20-month-old children who had received at least 5 doses of OPV, the seroconversion rates of poliovirus serotypes 1 to 3 (PV1, PV2 and PV3) were 94.1%, 96.7% and 78.3% respectively (3). Regardless of the quality of vaccination services it seems that the extent of this gap depends on the

virus serotype and also to some extent the other nutritional and demographic characteristics, such as a history of exclusive breastfeeding during the first 6 months of life and the socioeconomic status of the household (3,4). The presence of such a gap will jeopardize the confidence of the country in its high OPV immunization coverage (2,4–10).

Sistan-va-Baluchestan province, located in the south-east of the Islamic Republic of Iran, has common borders with 2 of the 3 countries that still having circulation of wild poliovirus. In 2010, reintroduction of the virus from Pakistan to China produced a polio outbreak 11 years after elimination of polio in the Western Pacific Region (2). The same may occur in other countries, especially those which have common borders with these countries. Sistan-va-Baluchestan province has common borders and strong cultural and economic ties with Afghanistan and Pakistan. The Iranian Ministry of Health and Medical Education (MOH) is therefore concerned about the occurrence of similar outbreaks in Islamic Republic of Iran. The present study was performed in Sistan-va-Baluchestan province to assess the seroconversion rate in newborn infants who had received 4 doses of OPV within the framework of the national immunization schedule.

## Methods

### Study setting

Sistan-va-Baluchestan is one of the least developed parts of Islamic Republic of Iran. Chabahar district is a seaport in the coastal area of the south-east of the country, near the Pakistan border. About two-thirds of the Chabahar population live in rural areas (139 553 rural versus 77 128 urban) (11). The household economy in the rural areas is mostly based on subsistence agriculture, working on fishing boats and cross-border trading of fuel and other goods, and in the urban areas is mostly

based on small businesses, shop-keeping and working in public and private institutions.

### Sampling

#### Target population

The target population were 7-month-old infants (210- to 240-day-old infants, born from 25 April 2010 to 25 May 2010) living in the rural areas of Chabahar (i.e. people living outside the municipal territories of Chabahar). The criterion for entering the study was a recorded history of receiving 4 OPV doses scheduled at birth, 2, 4 and 6 months of age. Considering the birth date of the participants none of them had any opportunity for receiving vaccine during national immunization days or mopping-up activities.

#### Sample size

To calculate the sample size we used the following standard equation (12):

$$n = (z_{\alpha/2})^2(P)(1-P)/d^2$$

where  $n$  is the sample size;  $\alpha = 0.05$ ,  $z_{\alpha/2} = 1.98$  [for calculation of a confidence interval (CI) of 95%],  $P = 0.85$  (based on findings in other studies, we estimated the seroconversion rate to be about 85%) (13–16);  $1-P = 0.15$ ;  $d = 1/10$  of  $P = 0.085$  (12). The required sample size was 68.8.

Since we used the probability proportional to size cluster sampling method to compensate for clustering effects, the calculated  $n$  should be multiplied by the coefficient  $D$  for design effect (17):

$$D = 1 + (b-1)\rho$$

Where  $b$  = the average number of responses to the item per cluster (which was estimated to be at least 4);  $\rho$  is the rate of homogeneity (its value will be higher for those items whose value varies more between clusters). Since all the participants were from the rural areas of the same district, we considered  $\rho = 0.1$ ; thus the design effect was calculated to be 1.3, which for caution we rounded up to 1.5. More explanations could

be found in statistical references (17). Using  $n \times D =$  final sample size, then the sample required was 102, which we rounded up to 105.

### Sampling method

In both urban and rural areas of Chalabar there are complete lists of the households booked into each health centre. The cluster size was defined as at least 5 children eligible for the serological part (i.e. 21 clusters) and in each cluster location we continued interviews and blood samplings until completion of blood sampling of the 5 children. Using the results of the latest national census performed in 2006–07, cluster locations were defined for urban and rural parts of each district (11). A cumulative list of the study population was produced and a systematic sample was selected from a random start. By dividing the total population of the communities by the number of communities to be selected (i.e. 21 communities) the sampling interval was obtained. A random number between 1 and the result of the division was then chosen. This was fitted into position in the list to identify the first community in the sample. Then by adding the sampling interval to the initial random number the remaining communities were selected. We considered the size of each community to be 200 people.

### Data collection

From each selected community 5 households with infants in the required age range were randomly selected and the study questionnaire was filled during an interview with one of the parents (usually the mother) (3,17). If there were 2 eligible children in the same household, the interview was performed only for the younger one.

Research teams comprised a nurse well-trained in blood sampling of children, an interviewer fully adept in the local language and well-trained in using the study questionnaire, and a driver.

### Questionnaire

The questionnaire contained questions about important demographic information such as child's sex, age and birth date; child's full vaccination history based on the records of the vaccination log book of the local health house; child's feeding history in the first 6 months of life (exclusive breastfeeding versus other options such as feeding on powdered milk); parents' educational level and occupation; number of people in household and number of rooms (or living spaces); and household economic status (ownership of a car, mobile-phone and refrigerator).

### Laboratory methods

Serum samples were screened for neutralizing antibody against PV1, PV2 and PV3 by micro-neutralization assay, which was performed by an in-house procedure according to WHO guidelines. Briefly, sera were inactivated at 56 °C for 30 minutes before the test, diluted 2-fold from 1:10 to 1:1280, and then incubated for 2 hours at 36 °C with an equivalent volume of 100 TCID<sub>50</sub> [50% tissue culture infectious dose] of PV1, PV2 or PV3. After the incubation period, 50 µL of L20B cell line ( $2 \times 10^4$  cells/0.1 mL) was added to all the microtitre plate wells (including control wells to monitor uninfected cell viability). Each test serum was investigated in triplicate. A back-titration of the 3 serotypes was included in each run as a control. After incubation for 5 days, the highest dilution of serum that prevented the development of virus induced cytopathogenic effects was recorded. A serum sample was considered positive if antibodies were present at a dilution  $1: \geq 10$  of the serum specimen. To calculate the geometric mean titre seronegative reports (titres  $< 1:10$ ) were considered as equal to 1:2 (3,18).

All required OPV vaccines in Islamic Republic of Iran are produced by Razi Vaccine and Serum Research Institute, Tehran, and all children who took part in this study had been

vaccinated by OPV produced by the Institute.

### Data analysis

After entering the data into a computerized database, the data were analysed using SPSS, version 15 and STATA, version 9. The data were analysed using descriptive tables, chi-squared test and Fisher exact test, and odds ratios (OR) were calculated using both univariate and multivariate (logistic regression) analysis methods. In calculating standard errors (SE) and 95% CI, the cluster sampling design was taken into account and adjusted for (17).

### Ethical considerations

The study protocol was reviewed and approved by the committee for medical research ethics of Zahedan University of Medical Sciences.

## Results

A total of 125 children were visited. Their mean age was 7.6 months (standard deviation 0.5 months). Table 1 shows some of the important demographic characteristics of the participants.

Table 2 shows the vaccination coverage of the study children; 92 of them (73.6%, 95% CI: 64.9–81.0%) had completed their vaccination schedule based on the national immunization programme. The multivariate analysis of the association between participants' vaccination status (complete versus incomplete 4 doses OPV schedule) and selected characteristics showed no statistically significant associations between any of the studied variables and vaccination status (Table 3).

Blood samples were taken from only 72 of the eligible children with complete vaccination; sampling was not successful in the remaining 20 eligible children due to problems in drawing blood. Table 4 shows the distribution of antibody titres for the 3 poliovirus serotypes separately in

**Table 1 Demographic characteristics and vaccination status of the study sample of 7-month-old children (*n* = 125)**

Variable	Incomplete vaccination schedule		Complete vaccination schedule		<i>P</i> -value <sup>b</sup>
	No.	%	No.	%	
<b>Sex</b>					0.498
Male	19	27.1	51	72.9	
Female	14	25.5	41	74.5	
<b>Father's education</b>					0.271
Illiterate	12	22.6	41	77.4	
Educated	21	29.2	51	70.8	
<b>Mother's education</b>					0.185
Illiterate	25	29.4	60	70.6	
Educated	8	20.0	32	80.0	
<b>No. of people in household</b>					0.433
1–5	18	25.4	53	74.6	
> 5	15	28.3	38	71.7	
<b>Household ownership of refrigerator</b>					0.128
Yes	6	17.6	28	82.4	
No	27	29.7	64	70.3	
<b>Household ownership of mobile phone</b>					0.404
Yes	23	27.7	60	72.3	
No	10	23.8	32	76.2	
<b>Household ownership of automobile</b>					0.497
Yes	27	26.0	77	74.0	
No	6	28.6	15	71.4	
<b>Method of feeding in first 6 months</b>					0.293
Exclusive breastfeeding	18	24.0	57	76.0	
Other feeding <sup>a</sup>	15	30.0	35	70.0	

<sup>a</sup>Including: breastfeeding by with feeding with powder milk; breastfeeding by with feeding with cow's milk; exclusive feeding with powder milk; exclusive feeding with cow's milk.

<sup>b</sup>1-sided Fisher exact test.

these children. The seropositive rates of PV1, PV2 and PV3 were 84.7% (95% CI: 76.3–93.1%), 95.8% (95% CI: 91.4–100.0%) and 70.8% (95% CI: 58.7–82.9%) respectively. There were 46 infants (63.9%; 95% CI: 49.2–78.6%) who were positive for all 3

poliovirus serotypes and 1 child (1.4%; 95% CI: 0.0–4.1%) who was positive for none of the serotypes.

Table 5 shows the contingency tables for the distribution of seropositive and -negative children for each poliovirus serotype, according to 3 important

variables: infant feeding, household crowding and father's education. There were no statistically significant associations or even noticeable differences in the distribution of these or other variables between seropositive and seronegative children.

**Table 2 Estimation of the vaccination coverage of trivalent oral polio vaccine (OPV) of the study sample of 7-month-old children**

No. of OPV doses received	No. of children	% of total (95% CI)	Cumulative %
4	92	73.6 (59.4–87.8)	73.6
3	23	18.4 (9.6–27.2)	92.0
2	6	4.8 (0.0–9.9)	96.8
1	4	3.2 (0.0–7.7)	100.0
Total	125	100	100.0

CI = confidence interval.



**Table 3 Multivariate analysis of the association between vaccination status (complete versus incomplete 4 doses oral polio vaccine vaccination schedule) and selected characteristics of the 7-month-old children ( $n = 125$ )**

Variable	OR (95% CI)	P-value
Exclusive breastfeeding	1.44 (0.63–3.28)	0.384
Sex	1.05 (0.46–2.41)	0.894
Father's education <sup>a</sup>	0.51 (0.21–1.27)	0.148
Mother's education <sup>a</sup>	2.30 (0.84–6.32)	0.104
Household car ownership	0.81 (0.28–2.39)	0.710
Household mobile phone ownership	1.28 (0.52–3.13)	0.470

<sup>a</sup>Illiterate = 0, capable of reading and writing = 1.  
OR = odds ratio; CI = confidence interval.

## Discussion

In interpreting these data it should be kept in mind that within the lifespan of these children no national immunization day or other kind of supplementary OPV vaccination had been performed (annual national immunization days are performed in January and February) and so what we observed was only the effect of the vaccines received based on the national immunization schedule. With regard to the sample size we could not reach the estimated number; however, we assume that it was reasonably large enough for estimating vaccination coverage. The fact that the sample did not include children of urban regions and that blood sampling was limited only to those children who had a complete vaccination profile are other limitations of the study design that decrease the external validity and generalizability of the findings. In multivariate analysis we could not find any statistically significant association between vaccination status and the characteristics

of the participants, which is presumably due to lack of heterogeneity in the sample and possibly the small sample size.

The calculated vaccination coverage for the study sample was 92.0% for at least 3 doses of OPV and 73.6% for 4 OPV doses. Although the rates of coverage of the 4th dose of OPV in most parts of the Islamic Republic of Iran exceed 90% (1), based on our findings Chabahar has not reached this target. It might be speculated that a considerable proportion of infants and toddlers are not vaccinated on time because of a lack of appreciation of the seriousness of poliomyelitis and this is an issue that deserves the attention of the health authorities. It should be kept in mind that country-specific averages can be deceptive. For instance in Tajikistan, during the 2008 polio outbreak, the rate of uptake of polio vaccine was reported to be 87%, only slightly below the WHO recommended minimum of 90%; however, the immunization rates were well below target levels in some regions of the country (5,6).

The acceptable level of seroprevalence of antibodies to PV2 among the infants (95.8%; 95% CI: 91.4–100.0%) indicates that the cold-chain supply of the vaccination programme was good. However, perhaps the most important finding of this study was the low seroprevalence of antibodies against PV3. Nevertheless, if we take into account the characteristics of the study population (which is an underdeveloped poor community), the findings might be interpreted as not unexpected, i.e. low immunogenicity of trivalent OPV, especially for PV3 and to a lesser extent for PV1, might not be considered as a new finding. These findings are comparable with those of another study in the same region on 20-month-old children (3). Also, findings similar to ours have been reported since almost the first days of introduction of OPV. For example, during a mass vaccination campaign carried out in 1958 with the serotype 1 CHAT strain (developed by Koprowski) in the former Belgian Congo it was noted that the seroconversion rate was lower compared with that observed in large field studies with the same vaccine in Poland (4,19). Poorer immunogenicity of OPV in lower-income settings has since been confirmed in numerous studies (4). Although rates of seroconversion following administration of trivalent OPV approach 100% in industrialized countries, only 73% (range 36–99%) and 70% (range 40–99%) of children in developing countries had detectable antibody to PV1 and PV3 respectively after 3 doses (7).

In a review of 32 studies from lower-income countries 27% and 30% of

**Table 4 Distribution of antibody titres in 7-month-old children who had received 4 trivalent oral polio vaccine doses and for whom blood samples were obtained ( $n = 72$ )**

Poliovirus serotype	Minimum	Maximum	25th percentile	Median	75th percentile
PV1	< 1:10	1:1280	1:40	1:160	1:640
PV2	< 1:10	1:1280	1:40	1:640	1:1280
PV3	< 1:10	1:1280	< 1:10	1:80	1:1280

PV1 = poliovirus serotype 1; PV2 = poliovirus serotype 2; PV3 = poliovirus serotype 3.

children lacked detectable serum neutralizing antibodies to PV1 and PV3 respectively after 3 doses of trivalent OPV (7). In another study in a rural Mayan community, looking at factors affecting immunogenicity of the first 2 doses of OPV among unimmunized Mayan infants, sero-responses were 86% to Sabin serotype 1, 97% to Sabin type 2 and 61% to Sabin type 3 vaccines. Decreased OPV immunogenicity was primarily attributable to interference of Sabin type 3 by Sabin type 2 (16). In addition, a reduced immunogenicity of PV3 when compared to PV1 and PV2 has been shown for inactivated poliovirus vaccine (20) and, based on the above-mentioned evidence, presumably the same might be true for live attenuated OPV3.

The threat of infiltration of wild poliovirus into these areas and occurrence of outbreaks such the 2011 outbreak in China (2) or the 2008 outbreak in Tajikistan (21), is real and there should be measures to tackle this problem. Use of bivalent (containing serotypes 1 and 3 vaccine) and monovalent (containing only serotype 1 or 3) formulations might be the best solution for this. Bivalent OPV was first used in December 2009 in Afghanistan and was swiftly adopted more widely (4). In India it was first used in January 2010, and a year later, elimination of the remaining wild PV1 and PV3 was achieved, and in 2012 India was declared polio-free (4,22). Without doubt, the final decision is with health experts of the Iranian Ministry of Health and we hope that our findings will be useful in this regard.

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**Competing interests:** None declared.

Table 5 Distribution of seropositive and seronegative children ( $n = 72$ ) to the 3 poliovirus serotypes by infant feeding, household crowding and father's education

Poliovirus type and seropositivity	Infant feeding				Household crowding				Father's education			
	Exclusive breastfeeding		Other feeding		1-3 people/ room		> 3 people/ room		Father illiterate		Father literate	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>PV1</i>												
Seropositive	41	89.1	20	76.9	44	89.8	5	10.2	26	83.9	35	85.4
Seronegative	5	10.9	6	23.1	17	73.9	6	26.1	5	16.1	6	14.6
<i>PV2</i>												
Seropositive	44	95.7	25	96.2	48	98.0	21	91.3	30	96.8	39	95.1
Seronegative	2	4.3	1	3.8	1	2.0	2	8.7	1	3.2	2	4.9
<i>PV3</i>												
Seropositive	33	71.7	18	69.2	38	77.6	11	22.4	18	58.1	33	80.5
Seronegative	13	28.3	8	30.8	13	56.5	10	43.5	13	41.9	8	19.5

<sup>a</sup>2-sided Fisher exact test.

PV1 = poliovirus serotype 1; PV2 = poliovirus serotype 2; PV3 = poliovirus serotype 3.

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