

Survey of poliovirus antibodies during the final stage of polio eradication in Egypt

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Abstract

Background: Egypt provides ideal conditions for poliovirus (PV) transmission (high population density, high contact rates and low sanitation and hygiene in some areas). Despite excellent program performance, wild poliovirus type 1 (PV1) continue to circulate in 2004. To investigate potential causes for the persistence, we conducted a serological study.

Methods: Seroprevalence surveys were conducted in “polio-endemic” regions (Greater Cairo and Upper Egypt) and in one control region (Lower Egypt) in December 2004. Sera collected from infants aged 6–11 months were tested for antibodies to poliovirus by neutralization assay.

Results: A total of 973 subjects were tested. Seroprevalence to PV type 1 (PV1), PV type 2 (PV2) and PV type 3 (PV3) was 99, 99 and 91%, respectively. Significant variation in PV3 seroprevalence was found (range: 76–100%). Region, density, maternal education, socioeconomic status (SES), stunting and diarrhea were significant risk factors for lower seroprevalence in the univariate analysis.

Conclusions: Our study suggested that uniformly high immunity levels (>96%) were required to interrupt PV1 transmission in the last remaining reservoirs (last PV1 was isolated in mid-January 2005 in Egypt). It further suggests substantial regional differences in OPV immunogenicity, with rural areas and low SES achieving the lowest seroprevalence to PV3.

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1. Background

Polio has probably been endemic in Egypt since ancient times. An Egyptian stele dated between 1403 and 1365 BC featuring a young man on crutches with a withered leg and the foot in a typical equivarus position is thought to represent the oldest picture of a patient with poliomyelitis [1].

Polio control efforts were initiated in Egypt when OPV vaccination was made compulsory in 1968 [2]. These control efforts led to precipitous declines in poliomyelitis incidence, and to the elimination of indigenous wild PV2 in Egypt sometime in the 1970s [3]. Following type 2 elimination, endemic type 2 circulating vaccine-derived poliovirus (cVDPV) circulated in Egypt from 1983 to 1993 [3]. From the mid-1970s, Egypt started a national program for polio elimination in which OPV was used during campaigns in practically every year, although not of the standard expected for an eradication effort. Following adoption of the goal of global poliomyelitis

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eradication in 1988 by the World Health Assembly [4], the global eradication strategies were implemented in Egypt starting in 1989 [2,5]. Surveillance for poliovirus in Egypt relies on two complementing systems: (1) acute flaccid paralysis (AFP) that has met the performance indicators since 2000 [6,7] and (2) environmental surveillance that relies on weekly sewage sample collection from 31 sites located representatively in Upper and Lower Egypt, as well as in Greater Cairo [8]. These systems provided strong evidence that the indigenous wild poliovirus strains had been eliminated in Egypt.

Implementation of the eradication strategies led to further decreases in the incidence of poliomyelitis, and the elimination of wild PV3, last detected in December 2000 [6,7]. However, the elimination of wild poliovirus type 1 (PV1) remained elusive. All isolated wild polioviruses from Egypt appear to be indigenous to the country [8]. Starting from 2002, the program performance further accelerated with procurement of oral poliovirus vaccine (OPV) from WHO-pre-qualified manufacturers, house-to-house administration of OPV, house markings and the introduction of finger markings with indelible ink of vaccinated children, and other program enhancements [9,10].

By 2002, Egypt was the only polio-endemic country that had both a high-performing routine immunization program achieving >90% OPV3 coverage, and excellent execution of supplemental immunization activities (SIAs; national immunization days [NIDs], sub-national immunization days [SNIDs] and mop-up campaigns), with high reported coverage (>90%) [6,7,10,11]. The mass vaccination campaigns were conducted with increasing frequency and quality. For example, both in 2003 and 2004, five NID rounds were conducted. Despite these efforts, wild PV1 continued to circulate in Egypt until January of 2005.

Part of the proposed explanation for the continued circulation was the high population density, contact rates and low sanitation and suboptimal hygiene in some areas. The population density is $\sim 1500/\text{km}^2$ in the populated areas along the Nile Valley [12]. Serological studies in the pre-vaccine era reported that the average age of infection in Egypt was very low, suggesting a high force of poliovirus infection in the country [13].

To better understand the reasons PV1 continued to circulate in the two final reservoirs (each with its own genetic lineage of poliovirus) of Greater Cairo and Upper Egypt, seroprevalence surveys were conducted in December 2004. Following the study, wild PV1 was only isolated twice in Upper Egypt in 2005, in Fayoum on 10 January 2005 and in Sohag on 13 January 2005.

2. Methods

The objectives of the study were to determine whether there were regional differences in OPV immunogenicity,

and if so, whether these differences could explain why some regions in Egypt remained PV1-endemic, and to determine if other factors affect seroprevalence, such as region, population density, socioeconomic status (SES), nutritional status and diarrhea. Immunity profiles of infants in areas with ongoing wild PV1 circulation in Upper Egypt (Assiut, Minia) and Greater Cairo were compared with areas in Lower Egypt (Beheira) without PV1 circulation (Fig. 1).

2.1. Sample size

PV3 was chosen as a marker of vaccine-induced seropositivity because there should be no natural immunity that would complicate interpretation, because PV3 circulation was interrupted 4 years earlier. Also, seropositivity for PV3 was expected from other OPV studies to be lower than for the other serotypes, providing a larger pool of seronegative children for analyzing risk factors. Sample size calculations were based on a projected 50% PV3 seroprevalence in the polio-endemic governorates and 70% in the control governorate, and an 80% probability of detecting a significant difference at the 5% level (two-tailed). The resulting sample size was 103 in each stratum, which was inflated to 120 to account for possible exclusions due to insufficient sera.

2.2. Eligibility criteria

Infants aged 6–11 months with consenting care-givers were eligible to participate, except those (a) born or residing outside of Egypt; (b) with serious acute illnesses requiring hospitalisation and (c) no written routine immunization records (clinic records, immunization cards, or birth certificates that included vaccination dates), with the exception that we included infants whose parents reported that the child had received no routine vaccinations at any clinic. We chose 6 months as the lower age limit to minimize the contribution of maternal antibody to seropositivity.

2.3. Site selection

Three governorates from Egypt's polio-endemic regions, Cairo, Assiut and Minia, were selected as polio-endemic study areas. Endemicity was defined as having at least one isolate from environmental (sewage) sampling positive for PV1 in 2004. Beheira governorate was chosen as the control site because it has had no PV1 detected from either environmental or AFP surveillance in the past 3 years. Enrollment was stratified by urban, semi-urban and rural population settings in Minia, Assiut and Beheira. Because Cairo is urban, enrollment was stratified by socioeconomic status by selecting three sites, each serving either a mainly low, medium or high SES population. A total of 37 HOs or RHUs were included in the study. For each stratum (urban, semi-urban, rural), the target number of children was distributed equally among the selected clinics.

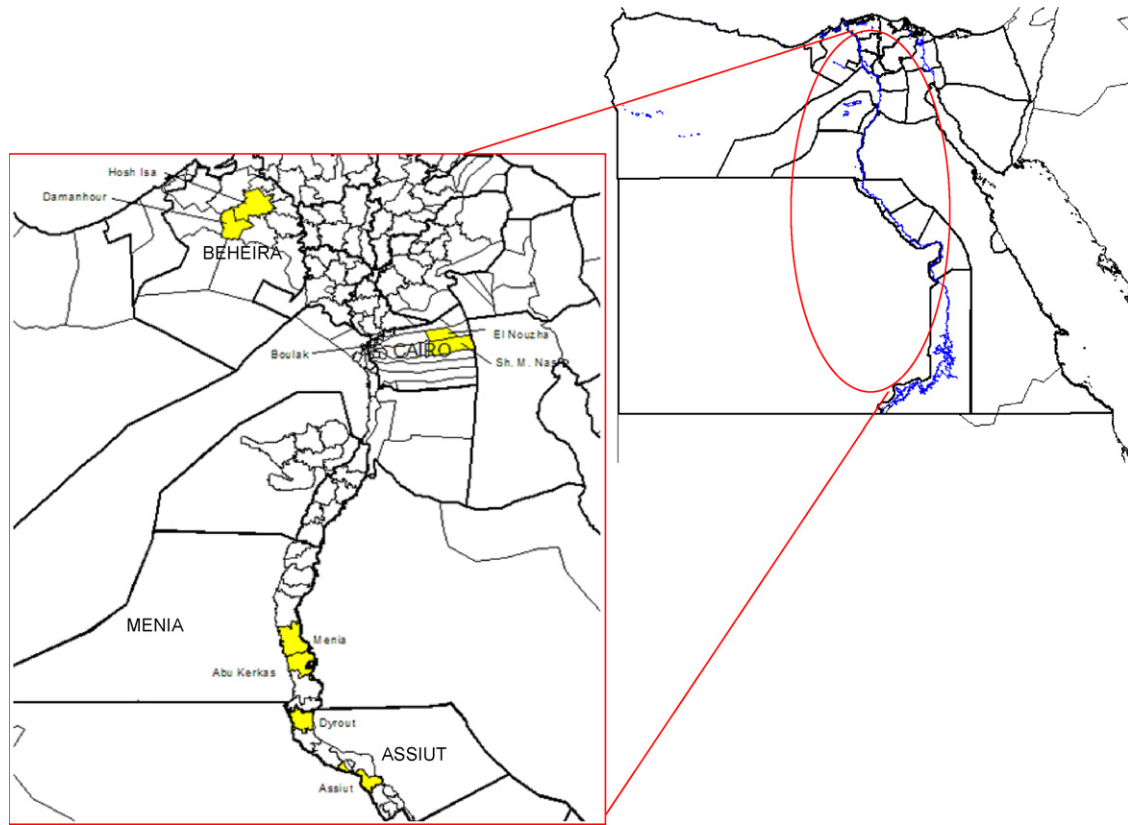


Fig. 1. Governorates and districts participating in poliovirus seroprevalence study, Egypt, 2004.

2.4. Enrollment and survey procedures

A study team was comprised of a physician and a nurse or pharmacist. Each of the 8 two-member study teams was responsible for enrolling approximately 120 infants. Infants were recruited when they presented for minor health care consultations (outpatient) or preventive care, including immunization. Eligible infants were recruited consecutively at each clinic site until the pre-determined sample size was achieved. After obtaining informed consent from parents or caretakers, a standardized questionnaire was administered. Vaccination histories for routine immunization were abstracted from immunization cards, birth certificates, or clinic records. Parent or care-giver recall for doses given at another clinic was acceptable if the parent or care-giver could specify the dose given. Supplemental vaccinations (vaccinations given during national or sub-national immunization days) were obtained through oral histories given by the parent or care-giver.

2.5. Blood collection and antibody testing procedures

One milliliter of blood was collected by heel stick using a Tenderfoot[®] 2.5 mm single-use lancet. Sera were stored at -20°C until transported refrigerated under reverse cold chain conditions to VACSERA Laboratory in Cairo for storage until shipment to the Centers for Disease Control and

Prevention (CDC) in Atlanta. Sera were tested in triplicate for levels of neutralizing antibody titers against poliovirus types 1, 2 and 3, respectively, using standard micro-neutralization techniques [14]. Seropositivity was defined as the presence of a neutralizing antibody titer of $\geq 1:8$.

The sera were tested in 16 runs and included multiple aliquots of an internal reference sera standard in each run. Sera were assigned randomly to each test run using a balanced block randomization scheme, where each run contained a constant proportion of samples from each site and proportion of positive control specimens. All sera from an individual child were tested in the same test run. Within each run the specimens were randomized to individual microtiter plates and position on the plates. This significantly reduces the potential for systematic error, and balances any variation between and within runs among the study sites equally.

Sera were tested for levels of neutralizing antibodies for poliovirus types 1, 2 and 3 using a modified micro-neutralization assay [15]. After initially inactivating the sera to be tested at 56°C for 30 min, serial 2-fold dilutions of the sera (starting at 1:8 and ending at 1:1024) and 100 cell culture infectious doses (CCID_{50}) of poliovirus were added to the appropriate wells of 96-well microtiter plate. The three serotypes of poliovirus were used in the same run in different plates and all sera were tested in triplicate against the three serotypes in the same run and in the same relative plate position within a run. Each of the triplicate dilutions of a sin-

gle serum was located in contiguous positions on the same plate. A backtitration of the three viruses used in the assay was included in each run as a control. The sera-virus mixtures were then incubated at 36 °C for 3 h, followed by addition of 2×10^4 HEp-2(C) cells/0.1 ml to all the microtiter plate wells including a cell control plate used to monitor uninfected cell viability. After incubation at 36 °C for 5 days, each plate was stained with 0.05% crystal violet in 25% ethyl alcohol and then dried overnight. The optical density in each well was measured by an enzyme-linked immunosorbent assay (ELISA) plate reader at a wavelength of 590 nm. The final titer for each serum was estimated by the method of Spearman–Karber [16]. Serologic results were linked to questionnaire data through use of the unique specimen identification number.

2.6. Data management and analysis

Questionnaires were double-entered into an initial database using Epi info software [17]. Data analysis was done using SAS software (SAS Institute, Cary, North Carolina). Chi-square tests were used to determine differences in seroprevalence in univariate analysis. Logistic regression was used to calculate multivariate odds ratios. Logistic regression models were fit separately for Cairo and for the other three governorates. Total OPV doses were highly correlated with age ($r=0.81$), so age was dropped from the analysis under the assumption that any age effect was most likely to reflect an association between dose and seropositivity. Explanatory variables were grouped as demographic variables (region, population density [i.e., rural, semi-urban, urban status] or SES, and mother's education) and biological variables (diarrhea, stunting and number of OPV doses). The models were first fit including all two-way interactions within each variable group, then fit again omitting all non-significant ($p > 0.05$) interactions.

2.7. Definitions

A routine dose was counted if a date or vaccination session number was documented. Doses administered on the day children were enrolled in the study were excluded ($n = 352$). The number of SIA doses was calculated by determining the maximum number of SIA doses a child could receive based on their residence and age and subtracting the number of doses the care-giver reported the child missed. Entries with no response ($n = 11$) and a response of "unknown" ($n = 23$) to the missed doses question were categorized as having missed none of the SIAs. Total vaccination doses were the sum of routine and SIA doses. Doses were included regardless of how close they were in time to other doses.

Nutritional status was measured using height, weight and age. Height was measured using standard measuring boards; weight was measured using scales at each clinic. Height and weight were measured twice and the means of the measurements used in the analysis. Data were compared to the

standard distribution of a reference population using Epi Info software [16]. The reference population was the CDC's National Center for Health Statistics standard recommended for international use by UNICEF and WHO [17]. Categories of malnutrition were based on standard deviation (S.D.) units (z -scores) below the mean of the reference population. These categories were defined as normal (< 2 S.D.s), severe to moderate (2.0 – 2.9 S.D.) and extreme (≥ 3 S.D.). *Stunting* (low height-for-age) is a measure of chronic malnutrition; *wasting* (low weight-for-height) is a measure of acute malnutrition. Diarrhea was defined as > 2 loose or liquid stools per day, on at least 1 day during the preceding 2 weeks [18].

3. Results

3.1. Enrollment, exclusions and final study group

The study was conducted between 18 December 2004 and 1 January 2005. A total of 973 children were enrolled, completed the questionnaire and provided sufficient sera for laboratory testing; 6 were excluded because of lack of information on age resulting in a final sample size of 967 participants (Table 1).

3.2. Characterization of groups by region

Because no population density areas were defined for Cairo, these data were analyzed separately. For non-Cairo areas, overall means of age, number of routine OPV doses, total number of OPV doses and maternal education differed by region (all $p < 0.01$) (Table 1). These variables also differed by population density within region. Within Minia and Beheira, age did not differ significantly by population density ($p > 0.07$ for all comparisons). In Assiut, children from the urban area were significantly younger than those from the semi-urban or rural areas. Overall, children in Beheira had significantly lower mean numbers of routine doses than those in the other two regions. In Minia, children from urban and rural areas had a lower mean number of routine doses than those from the semi-urban area ($p < 0.05$ for all comparisons). In Assiut and Beheira, children from the rural areas had significantly higher mean numbers of routine doses ($p < 0.01$ for all comparisons). The mean number of doses in semi-urban Minia was significantly higher ($p < 0.05$ for each comparison). The means did not differ between urban and rural areas. In Assiut and Beheira the mean number of doses in the urban area did not differ from the means in the semi-urban area ($p = 0.53$ in Assiut, $p = 0.43$ in Beheira). Means in both urban and semi-urban areas were significantly lower than the means in the rural areas ($p < 0.01$ for all comparisons). The mean number of total doses did not differ significantly by population density in Minia. In Assiut and Beheira, the mean number of total doses was significantly higher in the rural areas ($p < 0.05$ for all comparisons). The mean number of total doses in the urban and semi-urban areas

Table 1
Study sample, characteristics and poliovirus seropositivity in endemic (Minia, Assiut, Cairo) and non-endemic (Beheira) governorate study sites, Egypt, 2004

	Mean number						Percent					
	Sample size proposed	Sample size achieved	Mean Age (mo)	Routine OPV doses	Total OPV doses	Years maternal education	Moderate or severe stunting (extreme stunting) ^a	Moderate or severe wasting (extreme wasting) ^b	Diarrhea	P1 positive	P2 positive	P3 Positive
Minia			8.3	4.1	10.6	7.4	14 (4)	1	28	99	99	94
Urban (Minia City)	120	112	8.0	4.1	10.8	10.5	13 (9)	0 (0)	21	100	99	98
Semi-urban (Abu Korkas City)	120	122	8.5	4.3	10.9	6.8	20 (5)	1 (0)	33	99	99	96
Rural (Rural Abu Korkas)	120	125	8.3	4.1	10.5	4.6	10 (2)	2 (2)	30	98	98	89
Assiut			8.2	4.0	10.1	8.1	11 (2)	NA ^c	39	99	98	86
Urban (Assiut City)	120	119	7.9	3.8	9.8	10.8	6 (1)		40	100	100	97
Semi-urban (Dairut City)	120	116	8.4	3.9	10.1	10.8 ^d	20 (5)		34	97	97	76
Rural (Rural Dairut)	120	129	8.5	4.4	10.7	5.0	6 (5)		41	98	98	86
Cairo			7.6	3.5	7.4	8.4	10 (2)	4	46	98	100	92
High SES (El Nouzha)	40	35	7.9	3.6	7.6	11.0	3 (0)	3 (0)	37	100	100	97
Middle SES (El Galaa (Boulak))	40	30	7.8	3.6	7.6	8.4	17 (0)	0 (0)	47	100	100	100
Low SES (Hagganah (Nasr City-Shark))	40	52	7.6	3.5	7.3	6.7	12 (4)	8 (0)	51	96	100	85
Beheira			7.5	3.0	6.9	7.7	2 (0)	0 (0)	42	99	100	96
Urban (Damanhour City)	40	43	7.5	2.8	6.8	9.9	5 (0)	0 (0)	37	100	100	98
Semi-urban (Damanhour Rural)	40	44	7.4	2.7	6.5	9.2	2 (0)	0 (0)	49	100	100	93
Rural (Hosh Isa)	40	40	7.7	3.6	7.5	3.7	0 (0)	0 (0)	40	98	100	98
Overall	960	967								99	99	91

^a Stunting: moderate to severe = height-for-age 2–2.9 S.D. < standard mean; extreme ≥ 3 S.D. less than standard mean.

^b Wasting: moderate to severe = weight-for-height 2–2.9 S.D. < standard mean; extreme ≥ 3 S.D. less than standard mean.

^c Not calculated due to missing/unreliable weight data.

^d Missing = 71 (55%).

did not differ significantly ($p=0.17$ in Assiut, $p=0.46$ in Beheira). In Minia, mean years of maternal education was highest in the urban area, second highest in the semi-urban area and lowest in the rural area ($p < 0.01$ for all comparisons). Up to 20% of infants had moderate or severe stunting overall; stunting and wasting prevalence tended to be lower in Beheira ($p < 0.01$). Extreme stunting was rare. Wasting was uncommon and extreme wasting was almost non-existent. It was not possible to compare wasting in Assiut with the other areas because of an error in measuring the weight of the children. Diarrhea was common; up to 51% of parents reported that infants had diarrhea within the previous 2 weeks. The pro-

portions with diarrhea did not differ by population density areas within any of the three regions.

In Cairo, age, number of routine OPV doses, number of total OPV doses, diarrhea, wasting and stunting did not differ by SES areas. Maternal education correlated with socioeconomic status; mean years of education was higher in the high SES area than in the other two ($p < 0.01$).

3.3. Seroprevalence

Overall, 99, 99 and 91% of study participants had antibodies to PV1, PV2 and PV3, respectively. To maximize the number of seronegative children available for analysis, PV3 seroprevalence was chosen as the primary marker of vaccine-induced seropositivity. PV3 seroprevalence varied substantially by area and site from 76% in semi-urban Assiut to 100% in medium-high-SES Cairo. Because more than half of the titers for PV1 and PV2 were higher than the highest dilution, median titers were not calculable for these serotypes. The overall median titer for PV3 was 724, ranging from 362 in semi-urban and rural Assiut to 1149 in high and middle SES Cairo.

3.4. Risk factors

In the univariate analysis, region ($p < 0.001$), high density ($p < 0.001$), SES ($p = 0.01$), high maternal educa-

Table 2
Poliovirus type 3 seropositivity by various factors, univariate analysis, Egypt, 2004

Factor	Category	N	P3 positive (%)	p-Value
Governorate	Minya	362	94	<0.001 ^a
	Assiut	366	87	
	Cairo	117	92	
	Beheira	127	96	
Age (months)	6–7	364	90	0.32 ^b
	8–9	378	92	
	10–11	225	92	
Total OPV doses	4–6	108	93	0.37 ^b
	7–9	375	90	
	10–12	396	90	
	≥13	88	98	
Gender	Female	486	90	0.21 ^a
	Male	480	92	
Density	Urban	417	97	<0.001 ^a
	Semi-Urban	298	88	
	Rural	298	89	
SES ^c	High	38	97	0.01 ^a
	Medium	30	100	
	Low	52	85	
Education (years) ^d	0	282	87	<0.001 ^b
	1–6	61	93	
	7–9	74	91	
	10–12	341	96	
	13+	138	100	
Stunting ^e	Yes	106	84	0.003 ^a
	No	896	92	
Wasting ^{f,g}	Yes	10	90	0.57 ^a
	No	593	94	
Diarrhea	Yes	346	88	0.02 ^a

^a Chi-square or Fisher's exact. Pairwise analysis: Assiut significantly different from each of the other governorates; urban different from semi-urban and rural. Lower SES different from upper and middle SES.

^b Test for trend.

^c Socioeconomic status, Cairo only.

^d 107 Missing.

^e Stunting – moderate to severe: height-for-age 2–2.9 S.D. < standard mean; extreme: ≥3 S.D. less than standard mean.

^f Excludes Dairut City site.

^g Wasting- moderate to severe: weight-for-height 2–2.9 S.D. < standard mean; extreme: ≥3 S.D. less than standard mean.

Table 3
Relation between factors and type 3 poliovirus seropositivity, multivariate prevalence ratios, Egypt 2004

Variable	Odds ratio	p-Value
Minia, Assiut and Beheira		
Governorate		0.02 ^a
Minia	0.32	
Assiut	0.19	
Beheira	Ref.	
Population density	1.16	0.62
Mother's education		<0.01
Density X mothers education		<0.01
Slope in urban area	1.33	<0.01
Slope in semi-urban area	1.08	0.19
Slope in rural area	1.04	0.35
Stunting	1.52	0.36
Diarrhea	0.69	0.63
Diarrhea X mothers education		<0.01
Slope in those with diarrhea	1.05	0.29
Slope in those without diarrhea	1.24	<0.01
Total OPV doses	1.24	0.02
Cairo		
SES	2.29	0.50
Mother's education	1.26	0.02
Stunting	6.73	0.08
Diarrhea	1.26	0.79
Total OPV doses	1.25	0.34

^a Overall p-value. Pairwise analysis: p-value Minia vs. Assiut=0.14, Minia vs. Beheira=0.06, Assiut vs. Beheira=0.01.

tion ($p < 0.0001$), no stunting ($p = 0.003$) and no diarrhea ($p = 0.02$) were all significantly associated with a higher PV3 seroprevalence (Table 2). In the multivariate analysis, total number of OPV doses ($p < 0.02$) and maternal education ($p < 0.01$) were significantly associated PV3 seroprevalence, although the relation between maternal education and seropositivity was limited to infants in urban and semi-urban areas. In Cairo, seroprevalence in the medium SES area was 100%, making estimates of the logistic parameters for SES unstable. For this reason, this area was omitted from the logistic regression models. By logistic regression, only maternal education ($p = 0.02$) was found to be associated with seropositivity in Cairo (Table 3). However, the range in mean OPV doses was narrow in Cairo (routine OPV range 3.5–3.6; and total range 7.3–7.6), limiting our ability to detect differences.

4. Discussion

Our study measured high seroprevalence of antibodies to PV1 and PV2, and slightly lower levels to PV3 among infants aged 6–11 months in Egypt. The PV1 levels likely represent both antibody induced by vaccine and natural infection [11]. Both PV2 and PV3 antibody are very likely due only to vaccine-induced immunity [3,6–8,10]. The PV2 and PV3 results confirm indirectly excellent program performance in implementing the polio eradication strategies and administering a high number of OPV doses to young infants. As predicted, PV2 were uniformly high and PV3 levels were variable and significantly lower than either PV1 or PV2 seroprevalence levels.

The levels of PV1 seroprevalence in Egypt (96–100%), together with the total number of OPV doses (range: 6.5–10.9) among the study participants aged 6–11 months, suggest that the eradication efforts nearly approached the programmatic and perhaps biological limits of eradication with OPV. In the polio-endemic areas, study infants had received a mean of 7.3–10.9 doses of OPV, indicating receipt of approximately one dose per month.

Our study demonstrates substantial regional variations in PV3 seroprevalence. Interestingly, similar variations in PV3 seroprevalence by region were reported from Oman [19]. Our study also demonstrated in the univariate analysis that low SES, low maternal education (perhaps another indication of SES), stunting and diarrhea were significantly associated with lower PV3 seroprevalence levels, consistent with other reports [20–25]. In the multivariate analysis, the number of OPV doses and maternal education appeared to best predict the PV3 seroprevalence. These data confirm that SES is an excellent predictor for OPV performance in developing countries [23,24].

Of special interest is the question of what seroprevalence levels may be needed to invoke herd immunity. In Egypt, the timing of the seroprevalence survey just before elimination of the last chains of PV1 transmission invited further examination. The range of PV1 seroprevalence in Egypt was

narrow, from 96 to 100%, suggesting a high threshold level for herd immunity in the study areas. To interrupt transmission, the immunity levels have to exceed the threshold levels for herd immunity for a sufficiently long period to ensure that all chains of transmission are eliminated. Ideally, one would measure resistance to challenge infection or mucosal immunity as a correlate for effective immunity. Data from a seroprevalence survey in the pre-vaccine era in Egypt confirm that the force of poliovirus infection is high [13], implying the need to achieve high levels of immunity to induce herd immunity.

The issue with estimating the threshold herd immunity level for PV3 is of interest. We observed a national seroprevalence level 91% with large variations by region. The programmatic experiences from the polio eradication initia-

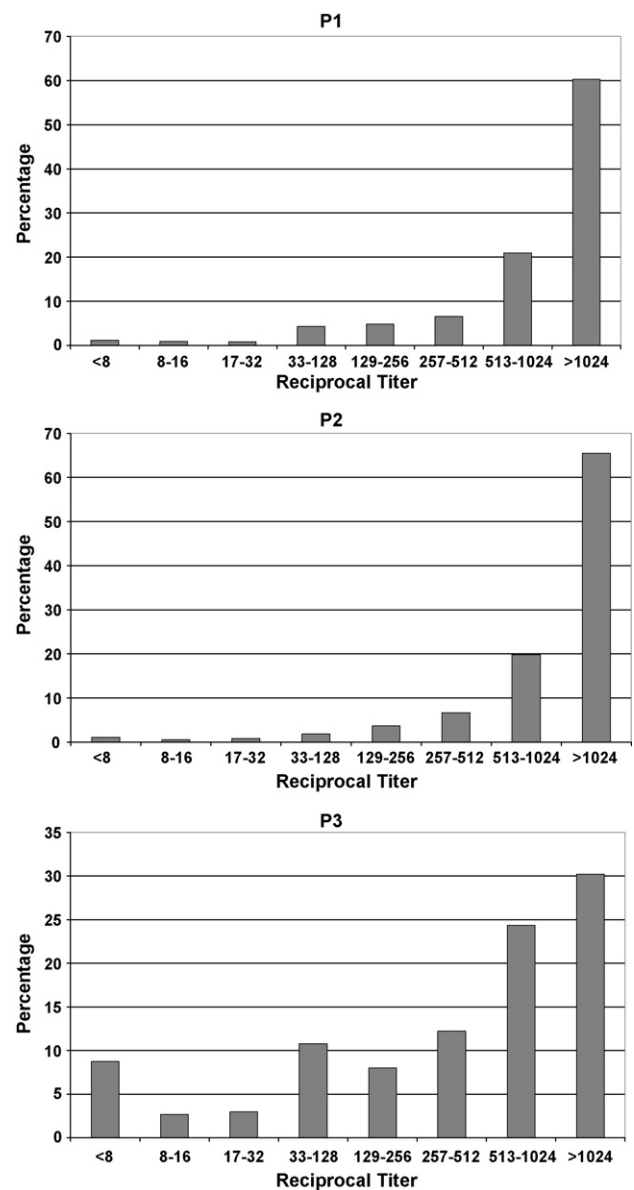


Fig. 2. Percentage of infants with antibodies to poliovirus serotypes 1, 2 and 3 (P1, P2, P3), by reciprocal titers, Egypt, 2004.

tive suggest that PV3 circulation is usually local, with no PV3 importations detected globally during 2003–2005. In contrast, 22 PV1 importations were detected globally during the same period [26]. Furthermore, PV3 circulation is usually eliminated before PV1 [11]. While these lines of evidence suggest that the force-of-infection for PV3 infection may be lower than for PV1, they contrast with data from the seroprevalence surveys conducted in the pre-vaccine era that suggest little difference between the serotypes in average age of infection [11,13].

The survey is subject to limitations. Infants in this study may not be representative of all infants of the same age in the areas studied. Infants receiving care at clinics may have higher immunization coverage and seropositivity than children not receiving care at clinics. However, financial and distance barriers to accessing health care are not significant in Egypt, and the distance barriers are not significant. Because selection of sites within governorates was not random, but rather chosen by availability of clinic resources to do the study and patient visit volume, the clinic sites may not be representative of all clinics. This limitation is somewhat mitigated by the fact that numerous health offices or rural health units ($n = 37$) were included in the study.

The laboratory cut-off for positivity was a reciprocal titer of eight. It is possible that the very small proportion of infants with very low positive titers may have been due to maternal antibodies (Fig. 2). Since we did not do a follow-up survey, we cannot be certain that all low positive samples were “truly” positive.

Our study confirmed that the polio eradication program in Egypt provided a high number of doses of OPV to very young infants, resulting in high seroprevalence. Eventually, the threshold for herd immunity was surpassed, and Egypt became polio-free in early 2005. Our study highlights the programmatic challenges of eradicating polio in one of the most difficult-to-eradicate countries, and provides a measure of the limited biological capabilities of OPV in developing countries. For the remaining endemic countries in similar circumstances to Egypt, the development, licensure and use in 2005 of more immunogenic vaccines (monovalent type 1 oral poliovirus vaccine [mOPV1] and monovalent type 3 OPV [mOPV3]), together with further improvements in program quality, could provide the necessary “immunity” booster to make global eradication a reality.

Conflict of interest

The authors do not have a commercial or other interest which might pose a conflict of interest.

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