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Seroprevalence of Anti-polio Antibodies in Children From Polio High-risk Areas of Pakistan:

A Cross-Sectional Survey 2015–2016

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Abstract

Background: Pakistan is one of the 3 remaining wild poliovirus endemic countries. We collected sera from children to assess the prevalence of poliovirus antibodies in selected high-risk areas for poliovirus transmission.

Methods: Children in 2 age groups (6–11 and 36–48 months) were randomly selected between November 2015 and March 2016 in 6 areas of Pakistan (Sindh Province: Karachi and Kashmore; Khyber Pakhtunkhwa Province: Peshawar, Bannu and Nowshera; Punjab Province: Faisalabad). After obtaining informed consent, basic demographic and vaccination history data were collected, 1 peripheral venipuncture was obtained, and assays to detect poliovirus (PV)–neutralizing antibodies were performed.

Results: A total of 1301 children were enrolled and had peripheral blood drawn that analyzed. Study subjects were evenly distributed among survey sites and age groups. Anti-polio seroprevalence differed significantly among geographic areas ($P < 0.001$); in the 6–11 months group, it ranged between 89% and 98%, 58% and 95%, and 74% and 96% for PV serotypes 1, 2 and 3, respectively; in 36–48 months group, it ranged between 99% and 100%, 95% and 100%, and 92% and 100% for PV 1, 2, and 3, respectively. Having received inactivate poliovirus vaccine, malnourishment (stunting) and educational level of parents were found to be associated with presence of anti-polio antibodies.

Conclusion: The polio eradication program achieved overall high serologic protection; however, immunity gaps in young children in the high polio risk areas remain. These gaps enable sustained

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circulation of wild poliovirus type 1, and pose risk for emergence of vaccine-derived polioviruses. Focusing on the lowest socioeconomic strata of society, where malnutrition is most prevalent, could accelerate poliovirus eradication.

Keywords

polio; serosurvey; antibodies titer; eradication program

In 2016, despite intensive efforts of the Global Polio Eradication Initiative, Pakistan, together with Afghanistan and Nigeria, remained endemic for wild poliovirus serotype 1 (WPV1). Globally, the reported cases of poliomyelitis caused by WPVs have dropped from hundreds of thousands in 1988, when the global polio eradication program began, to 37 cases reported by the World Health Organization in 2016, of which 20 were detected in Pakistan.¹

Progress toward eradication in Pakistan is continuing: WPV1 transmission in most areas has ceased, and the main foci of transmission remained in the high-risk areas of the provinces of Khyber Pakhtunkhwa, Federally Administered Tribal Areas and parts of Sindh such as Gadap town of Karachi and the Kacha River area in the north of the province.¹

Oral poliovirus vaccine (OPV) has been used in routine immunization and in supplementary immunization (SIA) throughout the polio eradication initiative, and the dramatic decrease of poliomyelitis incidence is a result of massive OPV use.² The polio eradication program in Pakistan has implemented regular SIA, in many areas on monthly basis, for the past several years. Following the recommendations of the Polio Eradication and Endgame Strategic Plan 2013–2018, 1 dose of inactivated poliovirus vaccine (IPV) administered intramuscularly was added to the routine immunization schedule in Pakistan in 2015 and IPV campaigns in the high-risk areas have been conducted in 2014–2016.^{3,4}

As part of the Polio Endgame plan, a switch from trivalent OPV (tOPV) to bivalent OPV (bOPV) without type 2 poliovirus (PV) has been conducted in a globally synchronized manner in April 2016.⁵ Pakistan successfully completed the vaccine switch and, as of April 2016, bOPV vaccine has replaced tOPV vaccine for routine immunizations and SIAs.

In many countries, seroprevalence surveys of anti-polio antibodies have served as a tool for program performance evaluation as well as to assess population immunity in targeted age groups, and areas of high risk for PV transmission.^{6,7} A previous polio seroprevalence survey in Pakistan in 2011 demonstrated consistently high concentrations of antibody response to PV1 and more geographically varied response to PV2 and PV3. In the 2011 survey, children 6–11 months of age in 3 geographic locations of Pakistan (Lahore, Karachi and Peshawar) were selected to represent low socioeconomic at-risk populations. The average seroprevalence of PV1, PV2 and PV3 was 96.0%, 87.9% and 86.7%, respectively.⁷ The lowest seroprotection rate for all 3 serotypes was for Karachi with 90.2%, 73.8% and 78.8% for PV1, PV2 and PV3, respectively. Despite the observed high seroprevalence of anti-polio antibodies against PV1, WPV1 transmission continued uninterrupted.¹

In this report, we present results from a seroprevalence survey of anti-polio antibodies conducted between November 2015 and March 2016 in 6 high-risk areas for PV transmission and identify predictors for low seroprevalence.

METHODS

A community-based cross-sectional survey was conducted using 2-stage cluster sampling technique for data and sample collection. Areas assigned to 1 vaccination team (vaccination areas) were considered as primary sampling units (PSUs). In the first stage, 20 PSUs were randomly selected from each study area for enumeration. In the second stage, 10 children of each target age group (6–11 and 36–48 months) were selected systematically from each PSU using household listing. In case of refusal or if the house was found locked, the next household immediately on the right side was chosen. An eligible household was defined as a family sharing the same kitchen and having at least 1 child in the target age group. From each selected household, only 1 eligible child was randomly selected.

In order to identify high-risk areas for PV transmission, meetings with provincial health authorities and other stakeholders in the polio eradication program were organized in Sindh and Khyber Pakhtunkhwa. After the discussions with polio eradication program managers, the following 6 areas were identified and chosen to be included in the survey: (1) Sindh Province: Karachi, Gadap town Union Council (UC) No. 4; (2) Sindh Province: Kashmore, Kacha River Area; (3) Khyber Pakhtunkhwa Province: Bannu (UCs of Mumbati Barakzai, Domel and Ghoriwala); (4) Khyber Pakhtunkhwa Province: Nowshera (Jalozai, Rashakai and Akorha); (5) Khyber Pakhtunkhwa Province: Peshawar (Shaheen Town I and II) and (6) Punjab Province: UCs of Alama Iqbal Colony, Rasheedabad and Bhawana Road in Faisalabad. The last area was selected as an area of low risk to provide a reference and a comparator to the high-risk areas (Fig. 1). The polio eradication program in Pakistan defines polio high-risk areas as those with PV transmission, and/or restricted access for polio workers because of security issues.

Children 6–11 and 36–48 months of age with consenting caregivers were eligible to participate. Exclusion criteria included any disability and serious acute and/or chronic illnesses. This survey received approval from the Ethical Review Committees of the World Health Organization, Aga Khan University (AKU) and National Bioethics Committee of the Government of Pakistan.

We calculated a required sample size in each age group and each area assuming seroprevalence of 80%, error margin of $\pm 10\%$, $\alpha = 0.05$ and a power of at least 80%. The sample was further inflated by approximately 10% in order to account for potential nonresponse which resulted in the final sample size of 110 children in each age group and each study area.

Each survey team was comprised of district field supervisor, team leader, data collector, measurer of height and weight and phlebotomist. Survey teams collected data on key indicators related to immunization history, health-seeking patterns and nutritional status of children. Vaccination history for OPV received through routine immunization was assessed

from vaccination cards when available or by parental recall if cards were not available. OPV doses received through SIAs were estimated by the number of SIA rounds that were conducted in the study area during the life of each child.

The data collectors in the survey team collected the data from the caregivers; measured weight and height of children using the standard World Health Organization methodology.⁸ Moderate stunting was defined as weight for age Z score between -2 and -2.99 ; severe stunting as Z score: -3 ; moderate wasting as weight for height Z score between -2 and -2.99 ; severe wasting as Z score: -3 .⁸

The phlebotomists collected 2 mL of peripheral blood using standard venipuncture technique. On-the-spot hemoglobin tests were performed with HemoCue machine (HemoCue AB, Angelholm, Sweden) using 1 drop of collected blood, and results were recorded on questionnaire. Anemia was defined as hemoglobin value of <10 g/dL.⁹

After centrifugation, serum was separated, transferred into a labeled sterile cryovial, and immediately stored in a cold box with frozen ice packs and transported to the nearest laboratory collection point of AKU for further transportation to the Nutrition Research Laboratory of AKU in Karachi. At Nutrition Research Laboratory, 2 aliquots were prepared, 1 for backup and 1 for transport to the Centers for Disease Control and Prevention in Atlanta, United States, for neutralization assay.¹⁰ Seropositivity was defined as titers of PV neutralizing antibodies $>1:8$.^{6,10}

Analysis was performed using STATA version 12 (Stata Statistical Software, College Station, TX). Frequencies and percentages were calculated for categorical variables. For seroprevalence, percentages with 95% confidence intervals (CIs) were reported. Medians with interquartile ranges and 95% CIs were reported for reciprocal antibody titers. A multivariable analysis using a logistic regression model to investigate the independent impact of each variables was done and expressed in unadjusted and adjusted odd ratio (AOR) with 95% CI; $P < 0.05$ was considered statistically significant. We tested bivariate analysis of factors associated with seropositivity to any PV with binary logistic regression and considered P value <0.25 in the bivariate analysis for inclusion in the multivariable logistic regression model. Covariates that were insignificant at the multivariable model were dropped consecutively from the model. The final model was selected on the basis of theoretical and statistical significance of predictors.

RESULTS

There were 1301 children enrolled in the survey (646 in 6–11 months age group, and 655 in 36–48 months age group); all of the enrolled children provided blood samples and their blood samples were analyzed.

There were differences in vaccination history, socioeconomic factors and nutritional status among children from different study areas. The proportion of caretakers who were literate ranged from 8% to 74%; the proportion of fully vaccinated children ranged from 6% to 86%; the prevalence of stunting ranged from 14% to 55%. Data on vitamin A supplementation in the previous 6 months showed that overall, 71% of children received

vitamin A supplementation with highest proportion (99%) in Peshawar and lowest (42%) in Bannu. In our sample, 39% and 24% of 6–11-month and 36–48-month-old children, respectively, had hemoglobin <10 g/dL (Table 1).

Seroprevalence to PV1 ranged between 89% and 99% in the 6–11 months old age group, with lowest prevalence found in Gadab and Bannu; it was nearly 100% in all areas in the 36–48 months old age group. Seroprevalence for PV2 ranged between 58% and 95% and 95% and 100% in the younger and older age groups, respectively. For PV3, it ranged between 74% and 96% and 92% and 100% in the younger and older age groups, respectively (Fig. 2).

We restricted risk factor analysis to the younger age group. In multivariate analysis, having received IPV (AOR: 4.43, 95% CI: 1.76–11.13) and no stunting (AOR: 1.90, 95% CI: 1.19–3.03) were found to be associated with higher seroprevalence of anti-polio antibodies, whereas being a resident of Gadap (AOR: 0.09, 95% CI: 0.04–0.24), Bannu (AOR: 0.08, 95% CI: 0.03–0.19) or Kashmore (AOR: 0.17, 95% CI: 0.07–0.41) was associated with a reduced odds of being seropositive (Table 2).

Distribution of reciprocal titers demonstrates the differences among study areas (Fig. 3). Median titer for PV1 was 724.1 (95% CI: 576–910.2) and 1152.1 (95% CI: 910.2–1448.2) for 6–11 and 36–48 months old, respectively; for PV2, it was 362 (95% CI: 181–455.1) and 576 (95% CI: 362–910.2); and for PV3, it was 227 (95% CI: 90.5–652.9) and 455 (95% CI: 181–838.2).

DISCUSSION

Our survey demonstrated that seroprevalence of anti-polio antibodies in young children varies among the selected study areas. In the highest risk areas of Gadap (where sustained circulation of WPV1 has been recorded for the past several years) and among internally displaced populations (IDPs) in Bannu, the PV1 seroprevalence among young children was <90%, but it was 97%–99% in the lower risk areas. Before the eradication of PV1 from India, which occurred in 2011,¹¹ the seroprevalence of PV1 antibodies among young infants from high-risk areas of Uttar Pradesh had consistently been above 97%.^{12–14} Socioeconomic conditions in India and Pakistan are considered to be similar; therefore, it is likely that the Pakistani eradication program has not yet reached seroprevalence concentrations needed for interruption of WPV1 transmission in its high-risk areas.

All of the children found to be seronegative for PV1 resided in areas where SIAs had regularly been conducted. There are 2 possible explanations for why some children remained seronegative: either they had been consistently missed during SIA rounds (as well as during routine immunization sessions) or despite having been vaccinated with multiple OPV doses, they did not seroconvert. The former points to failure of the program to reach the most vulnerable children, whereas the latter may be due to poor sanitation conditions and poor nutrition and is supported by previous findings of factors favoring nonseroconversion such as chronic malnutrition, chronic diarrhea or residing in areas with poor sanitation.^{15–19}

For both scenarios, high-coverage SIAs with OPV and supplemental IPV doses are the only solution to closing the remaining immunity gaps and securing PV eradication.²⁰

The number of vaccine doses received through routine immunizations also varied among surveyed areas. Almost onethird of children among IDP populations residing in Bannu had never received any vaccine, and in Kashmore, only 6% were fully immunized. Despite less frequent SIAs in Faisalabad, our reference area, the seroprevalence was slightly higher overall than in the other areas; this is likely due to a strong routine immunization program and better socioeconomic indicators.

Low seroprevalence for PV2 in the younger age group is a concern during the era when no type 2 containing live vaccine is used, and puts Pakistan at high risk of emergence of vaccine-derived PV type 2. The intense use of bOPV explains the difference in seroprevalence between PV2 and “PV1 and PV3”; however, in preparation for the tOPV to bOPV switch, Pakistan should have attempted to boost PV2 population immunity.

The National Nutrition Survey of Pakistan from 2011 estimated that 44% of children under 5 years of age are stunted.²¹ We demonstrated that stunting is associated with seronegativity. This may be important for the eradication program and suggests that focusing on the lowest socioeconomic strata of society, where malnutrition is most prevalent, may accelerate PV eradication. Our finding is in agreement with a previous study from Pakistan which assessed seroconversion in normally nourished and malnourished infants after administration of PV vaccines.¹⁶

The findings of our study are also consistent with the results of the seroprevalence survey conducted in Pakistan in 2011⁷; however, our study had some limitations. While data on routine vaccination history were available through vaccination cards or parental recall, we only estimated the total number of OPV doses that a child received based on the number of SIAs organized in the area during the life of the child. This is because there was no written documentation regarding the doses received during SIAs, and parental recall is unreliable when up to 12 SIAs may be conducted in 1 area annually. It was, therefore, impossible to correlate OPV dose history with seroprevalence or antibody titer. In addition, cross-sectional surveys usually do not provide the temporal relationship between the associated factors. Cases of WPV1 were detected in the study areas in 2015 and 2016; however, the WPV1 circulation is assumed to be of very low intensity, so we did not assume that seropositivity was caused by exposure to WPV1 but rather by exposure to PV vaccines.

The Pakistani polio eradication program has been able to achieve high seroprevalence of PV antibodies in most areas. However, pockets of under-immunized young children remain in high-risk areas and enable continued transmission of WPV1. In order to finally eradicate PV from Pakistan, it is imperative that the polio program reaches those children who are chronically missed by vaccination campaigns as well as those who live in low socioeconomic areas (e.g., IDPs) and may need more vaccine doses to seroconvert. At the same time, we found that children in lower risk areas have very high seroprevalence and therefore the eradication program may consider further strengthening the routine immunization in these locations rather than continuing to implement frequent SIAs.

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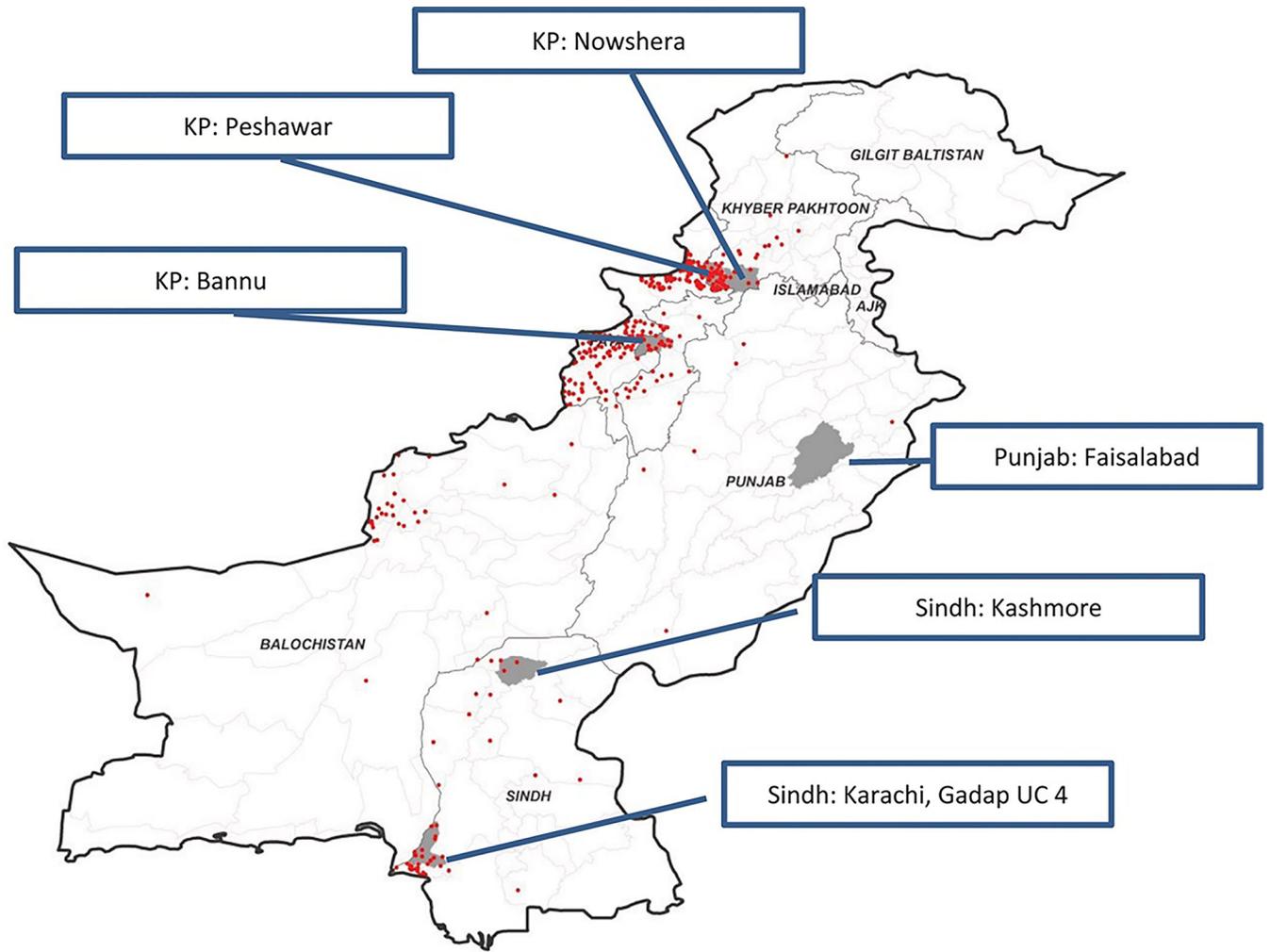


FIGURE 1. Study sites and location of detected cases of poliomyelitis in Pakistan: January 2014 to October 2016. Each dot represents 1 detected poliomyelitis case, and shaded areas represent districts where survey was conducted. KP indicates Khyber Pakhtunkhwa.

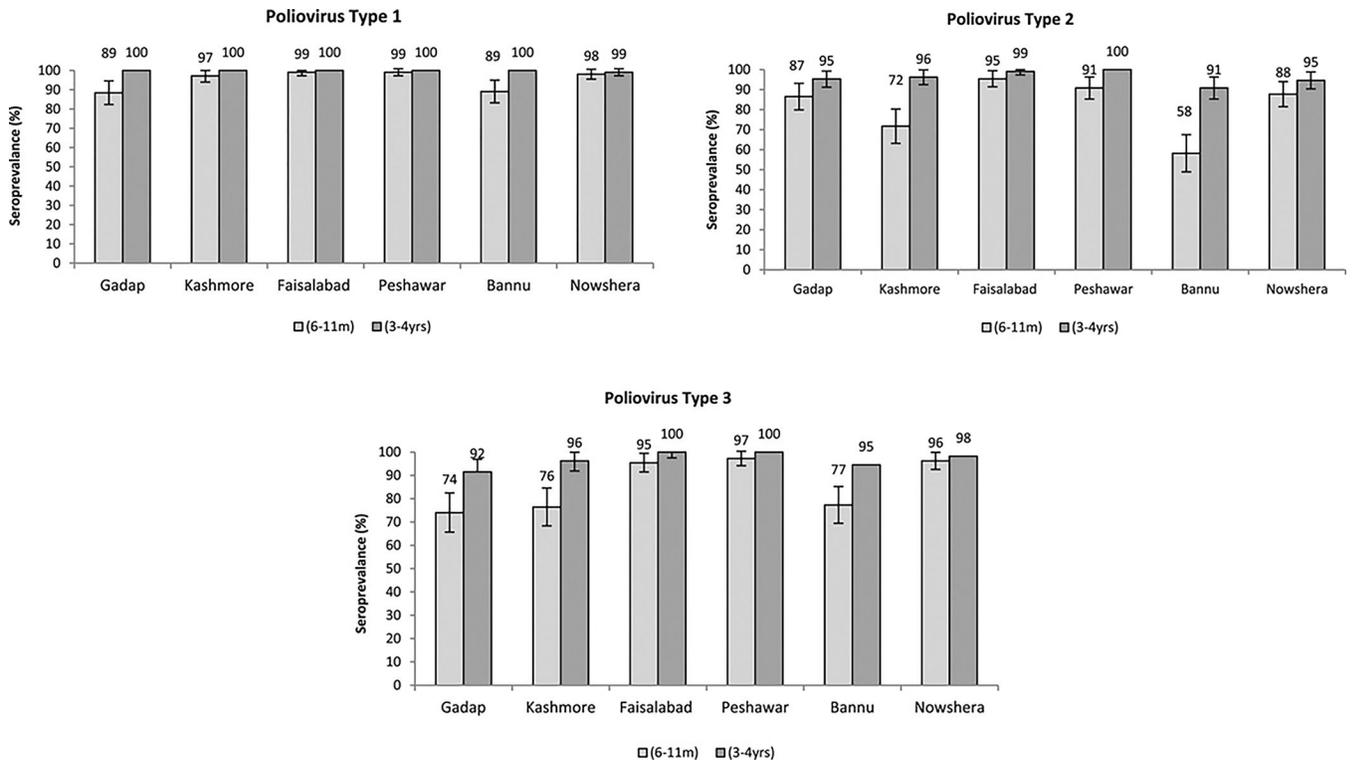


FIGURE 2. Seroprevalence value reported for each poliovirus serotype, age group and sampling site.

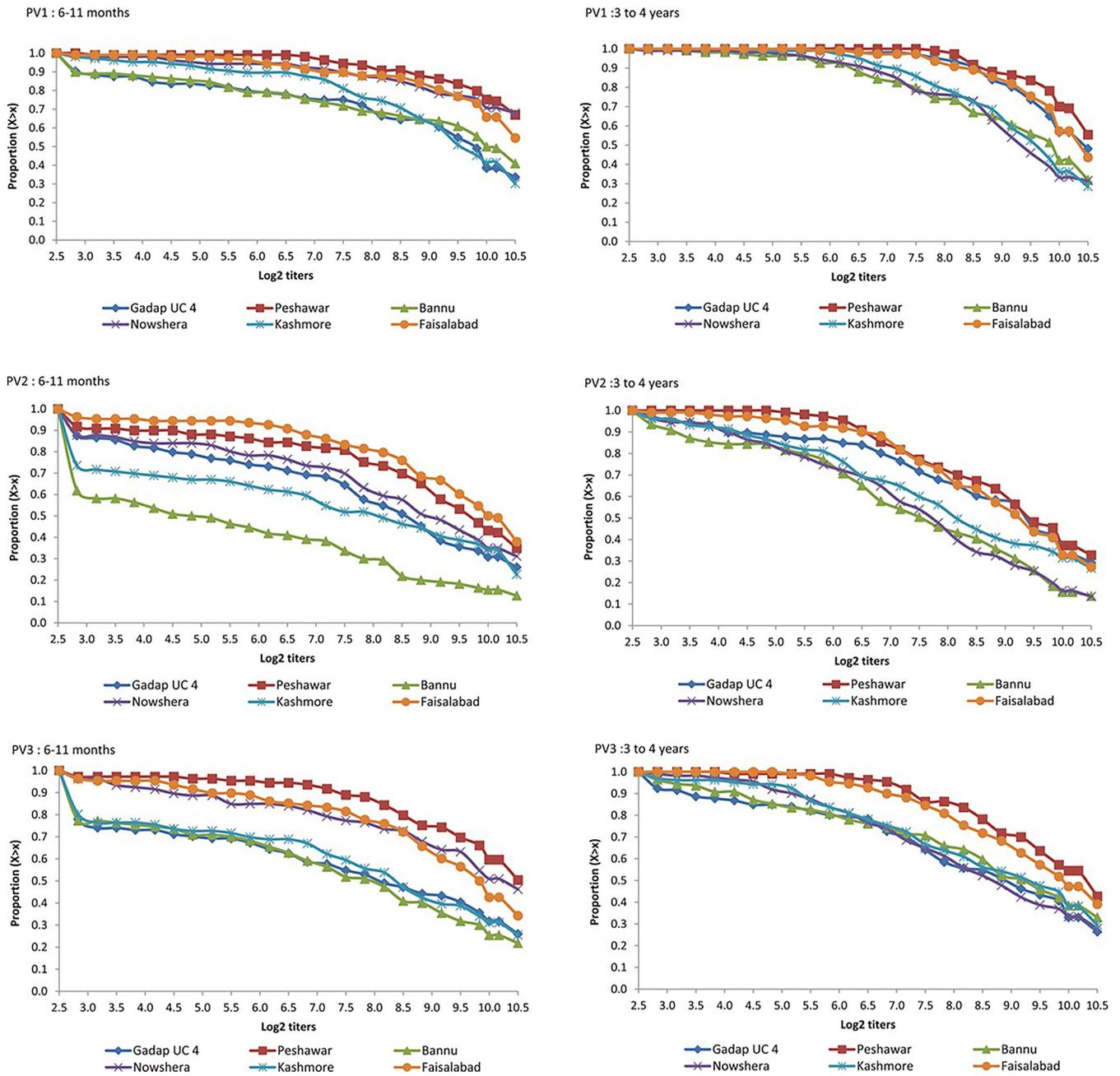


FIGURE 3.
Titer distribution.

TABLE 1.

Demographic and Other Attributes of the Study Population

	Overall	6–11 Months	36–48 Months	Karachi (Gadap)	Peshawar (High-risk UCs)	Bannu (IDP Population)	Nowshera (IDP Population)	Kashmore Kacha River Area	Faisalabad (Reference Area)
Gender									
Male	675/1301 (51.9)	311/646 (48.1)	364/655 (55.6)	110/212 (51.9)	122/219 (55.7)	115/220 (52.3)	122/218 (56)	100/211 (47.4)	106/221 (48)
Education level of respondent									
Literate	491/1301 (37.7)	237/646 (36.7)	254/655 (38.8)	44/212 (20.8)	112/219 (51.1)	73/220 (33.2)	82/218 (37.6)	16/211 (7.6)	164/221 (74.2)
History of diarrhea (last 2 weeks)	302/1301 (23.2)	196/646 (30.3)	106/655 (16.2)	60/212 (28.3)	28/219 (12.8)	32/220 (14.5)	42/218 (19.3)	75/211 (35.5)	65/221 (29.4)
History of routine vaccination									
Never received any vaccine	202/1301 (15.5)	107/646 (16.6)	95/655 (14.5)	29/212 (13.7)	8/219 (3.7)	67/220 (30.5)	8/218 (3.7)	88/211 (41.7)	2/221 (0.9)
Some routine immunization received	372/1301 (28.6)	197/646 (30.5)	175/655 (26.7)	138/212 (65.1)	35/219 (16)	24/220 (10.9)	36/218 (16.5)	110/211 (52.1)	29/221 (13.1)
Fully immunized	727/1301 (55.9)	342/646 (52.9)	385/655 (58.8)	45/212 (21.2)	176/219 (80.4)	129/220 (58.6)	174/218 (79.8)	13/211 (6.2)	190/221 (86)
Ever received IPV	235/1286 (18.3)	56/640 (8.8)	179/646 (27.7)	70/197 (35.5)	54/219 (24.7)	2/220 (0.9)	106/218 (48.6)	0/211 (0)	3/221 (1.4)
OPV dose history (RI + SIAs), median number of doses (IQR)									
6–11 months	10 (8–13)	—	—	12 (10–14)	13 (12–13)	10 (6–12)	12 (10–13)	9 (7–11)	7 (7–8)
3–4 years	38 (24–41)	—	—	39 (34–42)	40 (40–51)	34 (24–38)	44 (40–51)	33 (28–38)	14 (13–16)
Received vitamin A supplementation (during last 6 months)	919/1295 (71)	401/644 (62.3)	518/651 (79.6)	96/207 (46.4)	217/219 (99.1)	93/220 (42.3)	213/218 (97.7)	134/211 (63.5)	166/220 (75.5)
Nutritional status									
Stunting (HAZ <-2 SD)	402/1269 (31.7)	153/623 (24.6)	249/646 (38.5)	101/208 (48.6)	30/215 (14)	47/204 (23)	60/216 (27.8)	114/209 (54.5)	50/217 (23)
Wasting (WHZ <-2 SD)	188/1204 (15.6)	104/588 (17.7)	84/616 (13.6)	17/205 (8.3)	36/208 (17.3)	42/187 (22.5)	23/215 (10.7)	23/177 (13)	47/212 (22.2)
Child anemic status									
Severe and moderate deficiency (Hb <10 g/dL)	294/929 (31.6)	181/462 (39.2)	113/467 (24.2)	72/212 (34)	56/200 (28)	49/216 (22.7)	72/178 (40.4)	14/19* (73.7)	31/104 (29.8)

* Only 19 children had Hb concentration measured in Kashmir.

IQR indicates interquartile range; HAZ, height for age Z scores; RI, routine immunization; WAZ, weight for height Z scores.

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Univariate and Multivariate Analysis for Risk Factors Associated With Seroprevalence, Restricted to Younger Age Group (6–11 Months)

TABLE 2.

	Any Type Poliovirus		Unadjusted Odds Ratio for Seropositivity	P	Adjusted Odds Ratio for Seropositivity*	P
	Seronegative	Seropositive				
Sex of child						
Male	78 (25.2)	232 (74.8)	1.0 (0.7–1.43)	0.985	—	—
Female	84 (25.2)	249 (74.8)	Reference	—	—	—
Site						
Gadap UC	4 (35 (33.7)	69 (66.4)	0.14 (0.06–0.33)	<0.0001	0.09 (0.04–0.24)	<0.0001
Peshawar	14 (12.8)	95 (87.2)	0.47 (0.18–1.22)	0.119	0.50 (0.19–1.3)	0.155
Bannu	52 (47.3)	58 (52.7)	0.08 (0.03–0.18)	<0.0001	0.08 (0.03–0.19)	<0.0001
Nowshera	15 (14.2)	91 (85.9)	0.42 (0.16–1.08)	0.071	0.44 (0.17–1.14)	0.091
Kashmore	39 (36.8)	67 (63.2)	0.12 (0.05–0.28)	<0.0001	0.17 (0.07–0.41)	<0.0001
Faisalabad	7 (6.5)	101 (93.5)	Reference	—	Reference	—
Child ever received IPV						
Yes	8 (14.3)	48 (85.7)	2.13 (0.98–4.6)	0.055	4.43 (1.76–11.13)	0.002
No	152 (26.2)	429 (73.8)	Reference	—	Reference	—
Total OPV doses (RI + SIA8)						
4	157 (25)	472 (75)	1.67 (0.55–5.06)	0.364	—	—
<4	5 (35.7)	9 (64.3)	Reference	—	—	—
Education level of respondent						
Literate	126 (30.9)	282 (69.1)	2.47 (1.64–3.73)	<0.0001	—	—
Illiterate	36 (15.3)	199 (84.7)	Reference	—	—	—
Nutritional status						
Normal	95 (20.3)	372 (79.7)	2.39 (1.61–3.56)	<0.0001	1.90 (1.19–3.03)	0.007
Stunting (HAZ <–2 SD)	58 (37.9)	95 (62.1)	Reference	—	Reference	—
Normal	113 (23.5)	368 (76.5)	0.98 (0.59–1.61)	0.928	—	—
Wasting (WHZ <–2 SD)	24 (23.1)	80 (76.9)	Reference	—	—	—
Child anemic status						
Normal(10 g/dL)	78 (27.9)	202 (72.1)	0.77 (0.5–1.19)	0.238	—	—
Severe and moderate deficiency(<10 g/dL)	41 (22.9)	138 (77.1)	Reference	—	—	—

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P 0.25 included in multivariable model.
*

HAZ indicates height for age Z scores; RI, routine immunization; WAZ, weight for height Z scores.