

## Immunity to polio, measles and rubella in women of child-bearing age and estimated congenital rubella syndrome incidence, Cambodia, 2012

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### SUMMARY

Significant gaps in immunity to polio, measles, and rubella may exist in adults in Cambodia and threaten vaccine-preventable disease (VPD) elimination and control goals, despite high childhood vaccination coverage. We conducted a nationwide serological survey during November–December 2012 of 2154 women aged 15–39 years to assess immunity to polio, measles, and rubella and to estimate congenital rubella syndrome (CRS) incidence. Measles and rubella antibodies were detected by IgG ELISA and polio antibodies by microneutralization testing. Age-structured catalytic models were fitted to rubella serological data to predict CRS cases. Overall, 29·8% of women lacked immunity to at least one poliovirus (PV); seroprevalence to PV1, PV2 and PV3 was 85·9%, 93·4% and 83·3%, respectively. Rubella and measles antibody seroprevalence was 73·3% and 95·9%, respectively. In the 15–19 years age group, 48·2% [95% confidence interval (CI) 42·4–54·1] were susceptible to either PV1 or PV3, and 40·3% (95% CI 33·0–47·5) to rubella virus. Based on rubella antibody seroprevalence, we estimate that >600 infants are born with CRS in Cambodia annually. Significant numbers of Cambodian women are still susceptible to polio and rubella, especially those aged 15–19 years, emphasizing the need to include adults in VPD surveillance and a potential role for vaccination strategies targeted at adults.

**Key words:** Congenital (intrauterine) infection, epidemiology, measles (rubeola), polio, rubella.

### INTRODUCTION

Over the past 15–20 years, vaccination coverage in Cambodian children has increased markedly, leading

to poliovirus (PV) elimination and marked reductions in other vaccine-preventable diseases (VPDs) [1]. During 2000–2010, the percentage of children receiving all the vaccines recommended in the national immunization schedule by age 12 months increased from 31% to 74% [2]. However, significant immunity gaps likely exist in Cambodian adults for many VPDs because of low vaccination coverage in the

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country before 1993. Recent global experience has shown that large pockets of adult susceptibility can hinder achieving and maintaining VPD elimination and control goals [3, 4]. This paper focuses on polio, rubella, and measles, VPDs that have regional or global eradication, elimination, or control goals.

Cambodia has been identified as one of the countries in the Western Pacific Region (WPR) at increased risk for widespread PV transmission after wild-type poliovirus (WPV) introduction, or emergence of a circulating vaccine-derived poliovirus (cVDPV) [5] as happened in Cambodia during 2005–2006. WPV outbreaks in Albania, Namibia, Cape Verde, Tajikistan, Republic of Congo, and China [3, 4, 6–10] have exposed underlying immunity gaps in adults that necessitated wide age-range outbreak response vaccination campaigns and put global polio eradication at risk. Understanding adult PV susceptibility in Cambodia is required for accurate polio risk assessment and preparedness planning.

Like many countries approaching measles elimination, Cambodia has seen a shift in the age distribution of reported cases towards older children, adolescents, and adults. The proportion of laboratory-confirmed measles cases in persons aged  $\geq 15$  years increased from  $<6\%$  in 2010 to  $>16\%$  in 2011 after a nationwide campaign targeted at children age  $<5$  years (National Immunization Program, Ministry of Health, Kingdom of Cambodia, unpublished data). Assessment of the impact of this post-campaign shift on overall measles incidence in Cambodia is limited by variable case ascertainment and classification over time and multiple geographically staggered supplementary immunization activities (SIA). How much the age-distribution shift could potentially hinder progress towards measles elimination depends on the extent of underlying measles susceptibility in the adult population, which is currently unknown in Cambodia.

Rubella is currently endemic in Cambodia. In 2013, the Global Alliance for Vaccines and Immunization (GAVI) supported the introduction of rubella-containing vaccine (RCV) into Cambodia via a nationwide campaign of children aged from 9 months to 14 years and in the routine childhood immunization schedule – a major step towards rubella control in the country. However,  $>25\%$  of reported rubella cases in Cambodia in 2012 were in women of child-bearing age (WCBA). Knowledge of age-specific rubella immunity can generate national estimates of congenital rubella syndrome (CRS) cases and help design a

comprehensive vaccination strategy, which includes routine childhood vaccination and protection for WCBA from rubella virus infection [11].

We conducted a nationwide serological survey in 2012 to determine the population immunity to polio, rubella, and measles in Cambodian women aged 15–39 years. The objectives of this survey were to determine age-group-specific seroprevalence of antibodies to WPV types 1–3 and identify potential gaps in PV immunity in adolescent and adult women, generate age-group-specific measles immunity profiles, assess susceptibility to rubella virus infection in WCBA and estimate CRS incidence in Cambodia.

## METHODS

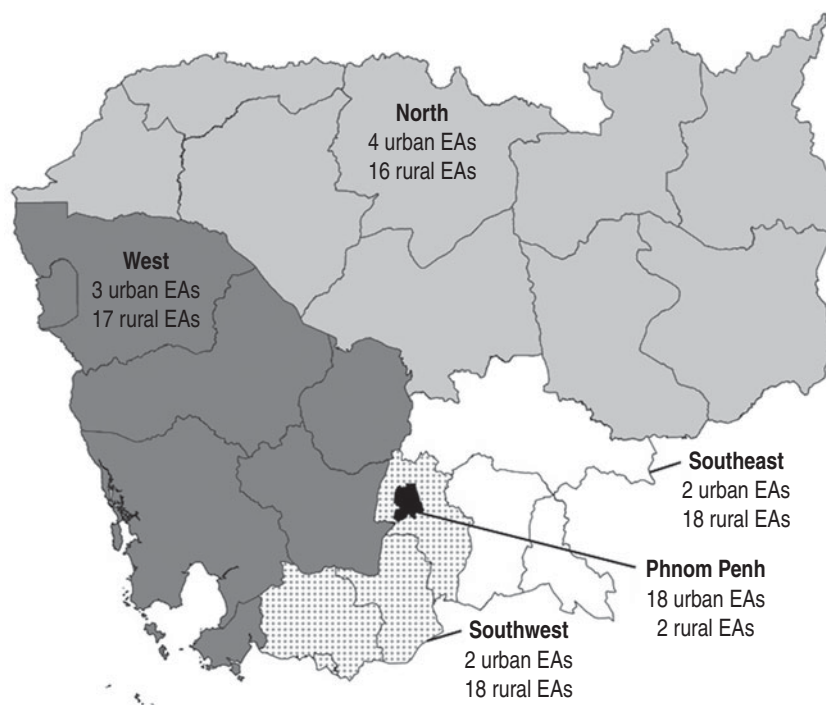
During November–December 2012, we conducted a nationwide cross-sectional serological survey of women aged 15–39 years in Cambodia. Cambodia's 24 provinces were divided into five regions: the capital, Phnom Penh, and four geographical regions with approximately equal population sizes (Fig. 1).

### Sample size and sample selection

A target sample size of 400 women within each age group (15–19, 20–24, 25–29, 30–34, 35–39 years) was calculated based on an estimated rubella seroprevalence of 80% [12–14], a desired precision of  $\pm 5\%$  with 95% probability of achieving that precision, a design effect of 1.5, and a 10% non-response rate based on results from previous surveys.

The 2010 Demographic and Health Survey (DHS) selected 611 enumeration areas (EAs) from the 28 764 EAs enumerated in the 2008 Cambodia General Population Census by probability proportional to size (PPS); our sampling frame consisted of these EAs. In each region 20 EAs were selected by PPS. The DHS survey oversampled urban EAs; we corrected this by allocating the number of selected urban and rural EAs within each region based on the proportion of the population living in urban and rural areas.

During the DHS listing, teams drew maps of the selected EAs delineating boundaries and identifying all households (HHs). For our survey, teams used these maps and updated the HH listings in the field to reflect any changes since the 2010 DHS. In each EA, 22 HHs were selected by systematic sampling using previously described survey methods [15]. Although we aimed to enrol 20 women per cluster,



**Fig. 1.** Map of Cambodia illustrating the five regions for the 2012 vaccine-preventable disease serological survey and the number of urban and rural enumeration areas (EA) included the survey for each region. Cambodian provinces by region: West (Battambang, Kampong Chhang, Kampong Speu, Koh Kong, Pailin, Preah Sihanouk, Pursat provinces); North (Banteay Mean Chey, Kampong Thom, Kratie, Mondolkiri, Otdar Mean Chey, Preah Vihear, Ratanakiri, Siem Reap, Steung Treng provinces), Southeast (Kampong Cham, Prey Veng, Svay Rieng provinces); and Southwest (Kampot, Kandal, Kep, Takeo provinces).

22 HHs were visited to adjust for the estimated number of age-eligible women per HH and non-participation rate. All eligible women in selected HHs were invited to participate, and teams revisited HHs up to three times.

#### Data and specimen collection and testing

Field teams visited selected HHs and enrolled eligible women after providing written information about the survey and obtaining consent from participants. A brief questionnaire, including demographic information and tetanus vaccine history, was completed and 5 ml of blood was obtained by venepuncture. Serum was stored at 4–8 °C and transported to the National Institute of Public Health (NIPH) laboratory in Phnom Penh within 96 h of collection. In Phnom Penh, samples were centrifuged and aliquoted into two cryovials, one for measles and rubella antibody testing at NIPH and one for polio antibody testing at CDC (Atlanta, GA, USA). Cryovials were stored at –80 °C until tested at NIPH or shipped by air on dry ice to CDC. Measles and rubella testing was performed

by anti-measles and anti-rubella virus IgG enzyme-linked immunosorbent assay (Enzygost, Siemens, Germany) according to the manufacturer's recommendations. Samples with corrected optical density (OD) values >0.2 were considered positive, samples with values <0.1 negative, and samples with values of 0.1–0.2 were considered equivocal and retested.

Samples were tested using a standard microneutralization assay for antibodies to PV types 1–3 according to established protocols at the Global Polio Specialized Laboratory, CDC [16, 17]. Briefly, 80–100 CCID<sub>50</sub> of each PV serotype and twofold serial serum dilutions were combined and pre-incubated at 35 °C for 3 h before adding HEp-2(C) cells. After incubation for 5 days at 35 °C and 5% CO<sub>2</sub>, plates were stained with Crystal Violet and cell viability measured by OD via spectrophotometry. Each specimen was run in triplicate, with parallel specimens from each participant tested in the same assay run. Neutralization titres were estimated by the Spearman–Kärber method [18] and reported as the reciprocal of the calculated 50% endpoint. Each run contained multiple replicates of a reference antiserum

pool starting at 1:32 dilution to monitor performance variation. A serum sample was considered positive if antibodies were present at  $\geq 1:8$  dilution.

### Data management and analysis

Statistical analysis was conducted using SAS v. 9.3 (SAS Institute Inc., USA). Sampling weights were calculated to take into account each stage of selection, including the sampling probability in the original DHS EA selection. A non-response adjustment within each of the 12 original design strata was included using the weighting class approach. Post-stratification methods were used to adjust weights to conform to the population distribution of the 2008 Cambodian Population Census. Seroprevalence estimates and 95% confidence intervals (CIs) were calculated taking into account the stratified cluster sampling design and sampling weights. Point estimates  $<0.20$  or  $>0.80$  were reported with Wilson CIs, otherwise Wald CIs were reported. Second-order Rao–Scott  $\chi^2$  tests were used to assess differences in seroprevalence across age groups, regions, and rural/urban residence.

For estimates of CRS incidence and annual number of CRS cases in Cambodia, age-structured catalytic models were fitted as described previously [19] to the observed age-stratified serological data overall and for the different regions using maximum-likelihood to estimate the average force of infection (rate at which susceptibles are infected) for rubella for those aged  $<13$  and  $\geq 13$  years, denoted by  $\lambda_y$  and  $\lambda_o$ , respectively. The equation for the proportion of people that were susceptible by age  $a$  ( $s(a)$ ) assumed that the force of infection differed between those aged  $<13$  years and those aged  $\geq 13$  years. For the age groups represented in the data, which referred to people aged  $\geq 13$  years, the equation was:

$$s(a) = \exp(-13\lambda_y) \exp(-\lambda_o(a - 13)).$$

The CRS incidence per 100 000 live births in 5-year maternal age groups up to age 44 years ( $A_i$ ) in each region was then calculated using the expression

$$0.65 \times s(A_i) \times (1 - \exp(-16\lambda_o/52)) \times 100000,$$

where  $s(A_i)$  is the estimated proportion of women in age group  $A_i$  that are susceptible and  $\lambda_o$  is force of infection estimated for the given region. The CRS incidence per 100 000 live births in women aged 15–44 years was then calculated as the average of the corresponding value in age group  $A_i$ , weighted by the number of live births occurring in that maternal age group in 2010. As in previous analyses [12, 19], the risk of a child being born with CRS was assumed to be 65% if the

mother was infected during the first 16 weeks of pregnancy and zero thereafter. The CRS incidence was then multiplied by estimates of the number of live births in women in the corresponding age group to obtain the number of CRS cases. The number of live births was calculated by multiplying age-specific fertility rates by the number of women in each age group in 2010, both extracted from the United Nations population database, 2008 revision. CIs on the force of infection and CRS incidence for each region and catalytic model were obtained by bootstrapping, using 1000 bootstrap datasets generated using the approach employed by Shkedy *et al.* [20].

### Human subjects' protection

We obtained written informed consent from participants and from parents or guardians of women aged  $<18$  years. The protocol was reviewed at CDC and determined to be a programme evaluation and therefore exempt from Institutional Review Board review. The survey protocol was approved by the Cambodian National Ethics Committee for Health Research.

## RESULTS

Field teams visited 2200 HHs; 1829 (83%) had at least one eligible woman, 271 (12%) had no eligible women, and 100 HHs (4.5%) were not able to be contacted to determine if any eligible women lived in the HH despite up to three visits being made. These 100 HHs were treated in the weighting adjustment and the non-response calculation as if they had one non-responding eligible woman. Of the 2167 women enrolled, 2154 had samples available for serological testing and are included in the analysis. Overall response rate was 92%, response rate by region ranged from 85% to 98%. Table 1 shows the distribution of participants by age group and region. Median age of participants was 26 years, and 63% of participants were pregnant or had been previously. Twenty-one of 24 provinces had clusters that were selected for inclusion in the survey; the three provinces without clusters included in the survey (Kep, Mondolkiri, Pailin) constitute 1.2% of Cambodia's population according to the 2008 Census.

### PV antibody serological results

Overall, 29.8% (95% CI 27.4–32.3) of women were seronegative for antibody to  $\geq 1$  PV serotypes. PV antibody seropositivity ranged from 83.3% for PV3 to 93.4% for

Table 1. *Distribution of serosurvey participants by age group and region, Cambodia, 2012*

Age group (yr)	Phnom Penh	North	West	Southwest	Southeast	Total (%)
15–19	93	87	89	95	71	435 (20.2)
20–24	120	89	96	78	88	471 (21.9)
25–29	94	74	107	101	107	483 (22.4)
30–34	106	86	87	85	86	450 (20.9)
35–39	58	58	66	64	69	315 (14.6)
Total (%)	471 (21.9)	394 (18.3)	445 (20.7)	423 (19.6)	421 (19.6)	2154

PV2. Antibody seropositivity did not differ by location for any of the PVs, but did differ significantly by age for both PV1 and PV3 ( $P < 0.001$ , Table 2). PV1 antibody seropositivity increased with increasing age; from 67.9% in those aged 15–19 years to 97.2% in those aged 35–39 years. PV3 antibody seropositivity also increased with age; from 72.9% in those aged 15–19 years to 92.6% in those aged 35–39 years. In the 15–19 years age group, 48.2% (95% CI 42.4–54.1) were susceptible to WPV types 1 or 3; 35.4% (95% CI 30.5–40) of those aged 20–24 years were susceptible to  $\geq 1$  type. Antibody seropositivity for all PVs did not differ between rural and urban areas (data not shown).

#### Measles antibody serological results

Of 2154 women tested for measles antibodies, 21(1%) had samples with repeated equivocal results and were considered to be seropositive. Analysing these results as seronegative or excluding equivocal results did not change the seroprevalence estimates significantly for any subgroup (data not shown). Overall measles seroprevalence was 95.9% (95% CI 92.8–96.4), and the estimated seropositivity was  $>95\%$  for every subgroup except those aged 15–19 years. Seroprevalence was significantly lower for this age group than for all other age groups (89.6% vs. 97.4%,  $P < 0.001$ ). Seroprevalence did not differ by region or rural/urban location (Table 3).

#### Rubella antibody serological results

Rubella antibody seronegativity was 26.7% overall. Women aged 15–19 years were significantly less likely to be seropositive for antibody to rubella virus (59.7%) than women in all other age groups (71.4–79.0%,  $P < 0.001$ ). A significant decreased seropositivity was apparent in women aged 20–24 (71.4%) compared to those aged 25–34 (78.9–79.0%,  $P < 0.05$ ). Women from rural areas were less likely to be seropositive for antibody to rubella virus (71.7%) than women

from urban areas (78.7%,  $P = 0.015$ ). Women in the southwest and southeast provinces had the lowest estimated antibody seropositivity, but this difference was not significant (Table 4).

#### Model-based estimates of CRS incidence

For Cambodia overall, the best-fitting value for the force of rubella virus infection was 76/1000 persons per year (95% CI 65–87) for children aged  $<13$  years and 33/1000 persons per year (95% CI 21–44) for those aged  $\geq 13$  years. These estimates varied between regions, with the highest force of infection in those aged  $\geq 13$  years estimated for Phnomh Penh (61/1000 persons per year (95% CI 31–97)). The CRS incidence for Cambodia overall was estimated therefore to be 167/100 000 live births in women aged 15–44 years (95% CI 107–220). Predicted CRS incidence ranged from 76/100 000 live births in West Cambodia to 270/100 000 live births in Southeast Cambodia (Fig. 1), reflecting differences in the force of infection between these areas. Incidence decreased with increasing age group from 222/100 000 births in women aged 15–19 years to 115/100 000 in women aged 35–39 years (Table 5). For 2010, 645 babies (95% CI 416–859) were estimated to be born with CRS in Cambodia.

#### DISCUSSION

This nationally representative survey demonstrated significant gaps in the immunity of Cambodian women to PV1 and PV3, especially in the late teen years, where around half the cohort was susceptible to  $\geq 1$  serotype. The increasing seroprevalence to PV1 and PV3 with age in our study, as has been seen elsewhere [21, 22], likely reflects increased remote exposure to circulating WPV types 1 and 3 in older persons in our population and a greater opportunity for exposure to shed polio vaccine virus through contact with children. Although we did not measure



Table 2. Antibody seropositivity to polioviruses by age group and region in women aged 15–39 years, Cambodia, 2012

Poliovirus (PV) type	Population	Tested, No.†	Seropositive*			$\chi^2$ P value
			No.	%	95% CI	
PV1	Overall‡	2152	1838	85.9	84.1–87.5	—
	By age group (yr)					
	15–19	435	305	67.9	61.7–74.2	<0.001
	20–24	469	386	82.7	78.3–86.5	
	25–29	483	426	88.8	85.6–91.4	
	30–34	450	416	93.5	90.7–95.5	
	35–39	315	305	97.2	94.3–98.6	
	By region					
	Phnom Penh	470	399	86.0	80.9–89.8	0.841
	North	394	336	84.7	80.4–88.2	
	West	445	384	86.7	83.3–89.6	
Southwest	423	360	85.1	81.2–88.2		
Southeast	420	359	86.9	82.6–90.2		
PV2	Overall‡	2152	2015	93.4	91.6–94.8	
	By age group (yr)					
	15–19	435	404	93.8	90.2–96.2	0.203
	20–24	469	439	94.8	91.7–96.8	
	25–29	483	455	92.1	88.1–94.9	
	30–34	450	418	92.4	88.3–95.1	
	35–39	315	299	94.1	89.4–96.7	
	By region					
	Phnom Penh	470	431	91.3	88.4–93.5	0.646
	North	394	380	95.1	85.6–98.4	
	West	445	418	93.7	91.1–95.6	
Southwest	423	402	95.5	92.4–97.3		
Southeast	420	384	90.7	87.5–93.1		
PV3	Overall‡	2152	1773	83.3	81.1–85.2	
	By age group (yr)					
	15–19	435	304	72.9	68.1–77.6	<0.001
	20–24	469	361	76.0	71.0–81.0	
	25–29	483	409	85.7	80.9–89.5	
	30–34	450	400	89.9	86.8–92.4	
	35–39	315	299	92.6	85.3–96.5	
	By region					
	Phnom Penh	470	375	80.8	76.5–84.4	0.192
	North	394	332	84.6	77.9–89.6	
	West	445	378	85.0	81.4–88.0	
Southwest	423	332	79.1	75.5–82.6		
Southeast	420	356	85.0	80.1–88.8		

CI, Confidence interval.

\* Estimates adjusted to account for sampling weights and survey design.

† Two participants without sample available for polio testing.

‡ Design effect (DE). P1: DE = 1.3; P2: DE = 1.1; P3: DE = 1.4. Estimated intra-class correlation (ICC) = (DE – 1)/(b – 1), where *b* is the average number of responses per cluster. P1: ICC = 0.016; P2: ICC = 0.006; P3: ICC = 0.019.

susceptibility in men, previous studies have demonstrated lower PV antibody seropositivity in men than women [23–26], suggesting the overall level of

immunity in the adult population might be even lower than in our survey. Current immunity to polio in women in our survey is less than the population

Table 3. Antibody seropositivity to measles virus by age group, region, and urban/rural residence in women aged 15–39 years, Cambodia, 2012

Population	Tested, No.	Seropositive*			$\chi^2$ P value
		No.	%	95% CI	
Overall†	2154	2057	95.9	93.9–97.3	
By age group (yr)					
15–19	435	384	89.6	85.0–92.9	<0.001
20–24	471	453	96.1	93.1–97.8	
25–29	483	466	96.5	93.2–98.2	
30–34	450	443	98.8	97.3–99.5	
35–39	315	311	98.6	95.2–99.6	
By region					
Phnom Penh	471	445	94.8	90.9–97.1	0.166
North	394	383	98.0	95.1–99.2	
West	445	413	92.2	83.0–96.7	
Southwest	423	413	97.9	95.7–98.9	
Southeast	421	403	96.1	93.8–97.6	
By residence					
Rural	1497	1431	96.0	93.3–97.6	0.769
Urban	657	626	95.6	93.3–97.1	

CI, Confidence interval.

\* Estimates adjusted to account for sampling weights and survey design.

† Measles (overall) design effect = 2.4 and intraclass correlation = 0.066.

immunity documented prior to recent outbreaks in Tajikistan and China [10, 27].

The recognition of a substantial immunity gap to PV in adolescents and young adults underlines the importance of maintaining good acute flaccid paralysis surveillance, including in persons aged >15 years, and the need to maintain high levels of polio vaccine coverage in young children. Although financially and logistically challenging, proactive polio vaccination campaigns are less expensive than outbreak response campaigns and have been advocated for areas with identified immunity gaps [28]. The WHO has recently broadened the recommended age for polio outbreak response campaigns to at least 15 years, given the increasing role of older unprotected persons in many outbreaks and the need to rapidly boost overall population immunity [29, 30]. Based on the epidemiology of the outbreak, recent response campaigns in China and the Republic of Congo were targeted at an even wider age range [3, 4].

The >95% overall measles antibody seroprevalence in Cambodian women is higher than both the modelled estimates of susceptibility targets necessary

Table 4. Antibody seropositivity to rubella virus by age group, region, and urban/rural residence in women aged 15–39 years, Cambodia, 2012

Population	Tested, No.	Seropositive*			$\chi^2$ P value
		No.	%	95% CI	
Overall†	2154	1602	73.3	70.5–76.1	
By age group (yr)					
15–19	435	275	59.7	52.5–67.0	<0.001
20–24	471	342	71.4	65.9–77.0	
25–29	483	379	79.0	74.7–83.2	
30–34	450	359	78.9	72.5–85.4	
35–39	315	247	76.6	71.9–81.3	
By region					
Phnom Penh	471	379	81.1	74.9–86.1	0.070
North	394	306	75.5	70.9–80.1	
West	445	334	75.8	70.7–80.9	
Southwest	423	287	68.0	62.8–73.3	
Southeast	421	296	70.1	62.1–78.1	
By residence					
Rural	1497	1081	71.7	68.4–75.1	0.015
Urban	657	521	78.7	74.3–83.1	

CI, Confidence interval.

\* Estimates adjusted to account for sampling weights and survey design.

† Rubella (overall) design effect = 2.1 and intra-class correlation = 0.054.

for elimination [31] and the level of population immunity in adults in the USA during the absence of endemic measles [32]. Previous studies in multiple settings have demonstrated no difference in measles seroprevalence between adult men and women [33–35]. This would suggest that in the face of high population immunity in children, there is not a significant enough reservoir of susceptible adults to perpetuate measles virus transmission in Cambodia. Maintaining high vaccine coverage in children aged <15 years would likely effectively eliminate endemic measles, although limited transmission could still occur in groups with lower (<90%) immunity. The recent addition of a second measles dose to the national vaccination schedule and the 2013 measles-rubella vaccine campaign are therefore crucial steps towards achieving the permanent absence of endemic measles in Cambodia.

More than 25% of WCBA are susceptible to rubella virus – a level considered to indicate a high risk for CRS [36, 37]. The overall 26.7% rubella susceptibility is similar to the 29.8% susceptibility in neighbouring Vietnam [12] prior to a large rubella outbreak during

Table 5. Estimates of the age-specific force of infection and the CRS incidence per 100 000 live births (for 5-year age groups) in different regions of Cambodia

Setting	Force of infection (per 1000 per year)		Estimated number of CRS cases per 100 000 live births				
	<13 yr	≥ 13 yr	15–19 yr	20–24 yr	25–29 yr	30–34 yr	35–39 yr
Cambodia (all)	76 (65–89)	33 (21–44)	222 (130–319)	188 (117–258)	160 (105–210)	136 (94–168)	115 (84.2–136)
West Cambodia	95 (66–120)	15 (0–43)	87 (0–309)	81 (0–249)	75 (0–200)	69 (0–161)	64 (0–131)
Southwest Cambodia	64 (45–89)	26 (5–47)	210 (32–442)	184 (31–349)	162 (30.3–275)	142 (29.5–217)	125 (29–173)
Southeast Cambodia	50 (29–77)	45 (20–69)	399 (146–703)	318 (131–504)	254 (118–360)	203 (106.9–261)	162 (96–194)
North Cambodia	94 (65–125)	26 (0–58)	143 (0–385)	126 (0–290)	111 (0–221)	97 (0–168)	85 (0–130)
Phnom Penh	73 (43–104)	61 (31–97)	379 (154–746)	280 (131–465)	206 (111–291)	152 (94–189)	112 (79–131)

Values in parentheses are 95% confidence intervals.

2011–2012 which resulted in 281 reported CRS cases [1]. While there are, to our knowledge, no previous estimates of rubella seroprevalence or CRS cases from Cambodia, the estimated incidence rate of 176 cases/100 000 live births in our study is very similar to the overall estimate of 173/100 000 in the WHO’s WPR in 1996 [19]. No serological data from Cambodia was available for this previous estimate and it was assumed that the incidence rate was similar to the overall regional rate [19]; if this is true, there has been little change in this rate between 1996 and 2012. Without the introduction of RCV, on the basis of the rubella force of infection and the age-specific fertility rate, at least 400 children are born every year in Cambodia with CRS. The medical, economic and personal burden of continued rubella virus transmission in Cambodia is therefore substantial, as we estimate that ~1 in 600 infants born in Cambodia will have CRS. The introduction of rubella vaccine into Cambodia covering children up to age 15 years should decrease the overall force of infection significantly but will not decrease the number of WCBA susceptible to rubella virus for many years [11]. Fortunately, sentinel CRS surveillance was established in Cambodia in 2011. This surveillance identified 32 CRS cases in 2012 [1], which while only 5% of our estimated CRS burden in the country was >10% of the total reported cases worldwide [38]; this highlights the absence of CRS surveillance in many areas of the world with continued rubella transmission. Maintaining high routine rubella vaccine coverage, monitoring for changes in

rubella epidemiology, and expanding CRS surveillance will be essential after the campaign. Given the significant susceptibility of WCBA, rubella vaccination strategies targeting WCBA, either through routine services for WCBA or a vaccination campaign including men and women should be considered, as recommended by WHO and the Strategic Advisory Group of Experts on Immunization (SAGE) [11, 39], to accelerate progress towards rubella and CRS elimination.

Our survey has certain limitations. First, we surveyed women only, thus inference to the entire adult population should be done cautiously. If anything this would likely underestimate the true immunity gaps to PVs. Second, although we used rigorous methods to randomly select the sample, only 2154 women throughout Cambodia were included. For this reason, we do not provide estimates for individual provinces or for age groups within the regions. Finally, although we selected survey EAs randomly and used PPS methods, the initial sampling frame came from EAs included in the 2010 DHS survey, which was based on the 2008 census. This sampling strategy gave us access to detailed maps and household listings of EAs, helping to ensure that a true probability sample was selected. This improved the representativeness of our survey and permitted appropriate weighting to allow for unbiased population estimates. However, smaller or non-existent settlements in 2008 would be less likely to be included and therefore underrepresented in our survey.



Overall, our serosurvey shows a level of immunity for measles in adult women that is encouraging for sustained elimination of measles in Cambodia if high childhood vaccine coverage is maintained. However, the gap in immunity to PV identified in our survey in young women is of sufficient size that a significant outbreak in this age group is possible if polio were re-introduced into Cambodia. In addition, the high susceptibility to rubella suggests that until this age cohort passes through prime fertility years, a substantial number of CRS cases can be expected in Cambodia unless further action is undertaken to protect these women. This is probably true even with the reduced force of rubella infection expected to follow the MR vaccination campaign. Sensitive rubella and CRS surveillance after this campaign will be essential to monitor progress towards accelerated rubella and CRS control and changes in rubella epidemiology. Periodic repeat seroprevalence surveys, ideally integrated in health or demographic surveys, could provide objective evidence of changes in population susceptibility profiles over time and monitor the impact of changes in immunization programme practices towards achieving and maintaining VPD control goals.

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#### DECLARATION OF INTEREST

None.

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