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RESEARCH PAPER

Seroprevalence of antibodies to measles and rubella eight months after a vaccination campaign in the southeast of Iran

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ABSTRACT

Eight months after the mass immunization campaign of November 2015 against measles and rubella in the southeast of Iran, in order to evaluate the sero-immunity level of the people living in the mentioned region, a serosurvey study was performed. Using a multi-stage probability proportional to size cluster sampling, the sera of 1,056 participants, ranging from 15 months to 20 years old, were tested for measles and rubella IgG antibodies in the National Reference Laboratory at Tehran University of Medical Sciences, Tehran, Iran. The seroprevalence rates of antibodies against measles and rubella in the age groups below 16 years were respectively 98.4 and 93.2%. In the age group of 16 to 20 years, who was not the target of the mass immunization campaign, the said rates were respectively 91.7% and 87.4%. The herd immunity of the age groups below 16 years, who were the target of the campaign, is favourably high and reassuring both for measles and for rubella. Campaigns of supplementary vaccination play a substantial role for filling the gaps in the herd immunity.

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Introduction

By implementing the vaccination campaign of December 2003, which covered about 33 million people aged between 5 and 25 years all over the country, Iran stepped more purposefully onto the road to measles elimination.[1]

That campaign largely raised the herd immunity all over Iran, and the aftermath of the campaign showed a substantial drop in the occurrence of measles outbreaks across the country, at least within the first few years after the campaign. However, since then, some self-restrained sporadic outbreaks have occurred in different parts of Iran every year, especially in three less developed provinces, i.e. Sistan-va-Baluchestan, Hormozgan and Kerman.[2-4] The climax of these outbreaks was reached in 2015, when based on the officially published surveillance data, the number of measles cases in Iran was more than the total number of cases during the preceding four years (2011 to 2014), i.e. 840 cases vs. 580.[5] This trend prompted health authorities to take some actions for filling the gaps in the herd immunity and returning onto the track. Based on a report about the measles outbreaks of the years 2009 and 2010 in the southeast of Iran, out of a total number of 92 serologically confirmed cases, 3 cases were above 20 years old, 12 cases were below 12 months of age, and the rest were younger than 14 years; and totally 42 cases (45.6%) had a positive vaccination history.[3] In another study on the reported measles cases of the years 2012 and 2014 in Iran, respectively 27.2% and 11.3% of the cases had a positive history of receiving MCV; and the rest were either under the age for vaccination (respectively 21.5% to 23.2% for 2012 and 2014) or unvaccinated.[6] It is worth mentioning that based on the unpublished reports of the Surveillance System of Measles, more than 91% of the outbreaks and sporadic cases of measles within the years 2010–2014 occurred in the age groups below 16 years.

The large measles/rubella campaign of 2015 in the southeast of Iran, which was implemented from November 14th to 22nd, tried to reach all children of 9 months to 15 years of age living in the campaign area (in the three above-mentioned south-eastern provinces). During the campaign, the target population was invited to receive a measles/rubella vaccine shot, regardless of the vaccines they had received based on their National Immunization Schedule. Approximately, one million and six hundred thousand children in the mentioned age range took part in the campaign. Based on the National Immunization Programme of Iran, every child should receive two injections of MMR (measles, mumps and rubella) vaccine when they reach 12 and 18 months of age; and based on the (unpublished) reports of the measles surveillance system (Ministry of Health) in 2015, the vaccination coverage of the first and second doses of MMR in the study area have been 99% and 98% respectively. It might also be worth mentioning that even though there is no published work about MMR vaccine effectiveness in Iran, comparison of the attack rates between vaccinated and unvaccinated people, recorded in the surveillance system, usually shows an effectiveness of more than 95% both for measles and for rubella.

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The bivalent measles/rubella (MR) vaccines used in the campaign of November 2015 were of two origins: The vaccine produced domestically by Razi Institute of Serum and Vaccine Production (RIS), which uses the AIK-C measles vaccine strain and Takahashi rubella vaccine strain in its products; and the imported vaccine produced by the Serum Institute of India (SII), which uses Edmonston-Zagreb measles virus strain and Wistar RA 27/3 rubella vaccine strain in its products. It must be noted that except for the 'mumps' moieties, which were not provided in the mentioned vaccines, these are the same vaccines that are in common use in the National Immunization Programme of Iran.

The main objective of the present study was to evaluate the sero-immunity level of the communities (i.e. estimating the seroprevalence of anti-measles and anti-rubella antibodies in the communities) in the southeast of Iran, involved in the above-mentioned campaign, after about eight months following the campaign.

Results

The total number of participants having taken part in interviews was 1,135 (male/female = 565/570 ≅ 1), and the number of the participants with blood samples was 1,056 (male/female = 524/532 ≅ 0.985). Only 79 participants refused their consent for blood sampling (male/female = 41/38), and there was no difference in characteristics (such as age, sex, and vaccination history) between these 79 participants and those who took part in the study.

Due to some mistakes regarding the ages of the participants, the total number in each age group does not add up exactly to 264; however, as far as the dimensions of the mistakes are concerned, they do not seem to have distorted the results. The quartiles of ages in each age layer (group) has been presented in Table 1 in order to make certain that there has not been any problem in taking an even and fair sample in each layer.

Out of the 1056 blood samples, 1022 (96.8%; 95% CI: 95.7% to 97.8%) were seropositive for anti-measles antibodies and 969 (91.2%; 95% CI: 90.1% to 93.4%) were seropositive for anti-Rubella antibodies. If age groups above 15 years, who were not the target groups of the mass immunization campaign of November 2015, are excluded from the calculations, the seroprevalence of antibodies against measles and rubella in the remaining 803 participants below 16 years will respectively come to 98.4% (95% CI: 97.5% to 99.2%) and 93.2% (95% CI: 91.4% – 94.9%).

Table 1 and Table 2 report the results and characteristics of the participants by age layers respectively for measles and rubella. The mean age of mothers on the participants' birth dates was calculated by subtracting mothers' present ages from the participants' ages at the time of the interview. They did not vary by sero-immunity status of the participants.

Afghan refugees composed only 1.4% (15 participants) of all of the participants with blood samples, and the rest of the participants with blood samples were Iranian (98.6%).

With regard to the question about the past history of eruptive (exanthematous) diseases, 82.1% of those with blood samples reported a negative history; and among the remaining with a positive history, about 15.0% (158 participants) mentioned a history of chickenpox. Only 6 participants reported a history of catching measles during their life (none of them in the first age group). With regard to rubella, only 3 participants reported a positive history, and only one of them was in the first age group.

Table 3 shows the participants' vaccination history by age group based on the records on their Vaccination Cards. It is worth mentioning that during the immunization campaigns vaccine shots are not usually recorded on the Vaccination Card; therefore, Table 3 mostly reports the vaccines that have been received in the course of routine vaccination activities. When studying the numbers in Table 3, it might also worth noticing that many of the participants in higher age groups have not been in possession of their Vaccination Cards during interviews, so their vaccination history has not been recorded in their questionnaire.

Discussion

After the campaign of December 2003, in which all of the population ranging from 5 to 25 years was invited to take part in the immunization campaign against measles and rubella, the campaign of November 2015 was the largest one in the country.[2] The results of the study show a very high, promising and

Table 1. Characteristics of the participants by their age groups (layers) and the results of their serologic tests for anti-measles antibody. No = 1,064.

	Measurement units	15 months to 5 Years No = 265		6 to 11 Years No = 273		12 to 15 Years No = 265		16 to 20 Years No = 253	
Total sero-immunity proportion in each age layer	%(CI*)	98.9 (97.6 –100.0)		98.9 (97.6 –100.0)		97.4 (95.4 –99.3)		91.7 (88.3 –95.1)	
Age composition of participants (Years)	Mean ± Sd (1st quartile; median; 3rd quartile)	3.7 ± 1.2 (2.8; 3.7; 4.6)		8.7 ± 1.7 (7.2; 8.6; 10.1)		13.8 ± 1.1 (12.9; 13.8: 14.8)		17.8 ± 1.2 (16.8; 17.6; 18.5)	
		Negative No = 3	Positive No = 262	Negative No = 3	Positive No = 270	Negative No = 7	Positive No = 258	Negative No = 21	Positive No = 232
Sex	males No. (%)	3 (2.3)	128 (97.7)	2 (1.4)	138 (98.6)	4 (3.2)	121 (96.8)	11 (8.6)	117 (91.4)
	females No. (%)	0 (0.0)	134 (100)	1 (0.8)	132 (99.2)	3 (2.1)	137 (97.9)	10 (8.0)	115 (92.0)
Participation in the campaign of November 2015	Yes No. (%)	2 (0.8)	247 (99.2)	2 (0.8)	261 (99.2)	6 (2.4)	242 (97.6)	4 (6.4)	59 (93.7)
	No No. (%)	1 (6.2)	15 (93.8)	1 (10.0)	9(90.0)	1 (5.9)	16(94.1)	17 (9.0)	173 (91.0)

*CI = Confidence interval.
**Standard deviation.

Table 2. Characteristics of the participants by their age groups (layers) and the results of their serologic tests for anti-rubella antibody. No = 1,064.

	Measurement units	15 months to 5 Years No = 265		6 to 11 Years No = 273		12 to 15 Years No = 265		16 to 20 Years No = 253	
Total sero-immunity proportion in each age layer	%(CI*)	94.3 (91.5 –97.1)		96.3 (94.1 –98.6)		88.7 (84.8 –92.5)		87.4 (83.2 –91.5)	
		Negative No = 15	Positive No = 250	Negative No = 10	Positive No = 263	Negative No = 30	Positive No = 235	Negative No = 32	Positive No = 121
sex	males No. (%)	10 (7.6)	121 (92.4)	6 (4.3)	134 (95.7)	14 (11.2)	111 (88.8)	21 (16.4)	107 (83.6)
	females No. (%)	5 (3.7)	129 (96.3)	4 (3.0)	129 (97.0)	16 (11.4)	124 (88.6)	11 (8.8)	114 (91.2)
Participation in the campaign of November 2015	Yes No. (%)	15 (6.0)	234 (94.0)	10 (3.8)	253 (96.2)	28 (11.3)	220 (88.7)	10 (15.9)	53 (84.1)
	No No. (%)	0 (0.0)	16 (100.0)	0 (0.0)	10 (100.0)	2 (11.8)	15 (88.2)	22 (11.6)	168 (88.4)

*CI = Confidence interval.
**Standard deviation.

reassuring level of sero-immunity first and foremost against measles and then against rubella. In total, the results of the present study's post-campaign serosurvey evaluation are pretty similar to the results of evaluating the campaign of December 2003; i.e. 98.4% vs. 97.4% for measles, and 93.2% vs. 94.6% for rubella.[2] In the road to eradicating measles, in addition to efforts for achieving ≥ 95% coverage with both doses of MCV, intermittent vaccination campaigns with the aim of covering those who for any reason have not achieved the required protection during routine vaccination activities or have not received the vaccines at all, have been recommended by WHO.[7,8]

Considering the even distribution of age and sex variables in all four age layers of the sample, it might be speculated that the sampling procedures have been effective in taking a fair and representative sample of the community. Hence, generalization of the results to the communities under investigation might be considered logical, and it might be speculated that the campaign of the year 2015 has been successful in raising the general level of the herd immunity against measles and rubella.

Previous studies in Iran show that there is usually a gap between the vaccination coverage and real immune level of the community. For instance, in a study implemented in the same provinces as the ones being the subjects of the present one, during summer 2011, in children ranging from 2.5 to 4.5 years old, who had received two shots of measles-containing vaccines, the seroprevalence of anti-measles antibodies, on average, was about 94.6%, and the results were variable from one district to another from 90.1% to 97.3%.[9] Whatever the reason for the occurrence of such a gap between the reported coverage and the real seroprevalence, the presence of the gap per se highlights the importance of studies like the present one in the evaluation of the real state of immunity. It is worth mentioning that even though there are a few studies that, based on IgG avidity measurements, suggest waning immunity as one of the reasons for secondary vaccination failure of measles, this does not seem to play any important role in outbreak transmissions.[8,10,11]

Table 3. Vaccination history for measles-containing vaccines received by the participants based on the records on their Vaccination Cards.

Age Groups	Total Participants in the Age Group	Possession of Vaccination Card	Age Groups
15 months to 5 years	(265)	260 (98.1%)	256 (98.5%)
6 – 11 years	(273)	245 (89.7%)	240 (98.0%)
12 – 15 years	(265)	174 (65.7%)	155 (89.1%)
16 – 20 years	(253)	90 (35.6%)	35 (38.9%)

WHO-recommended measles elimination strategies include achieving and maintaining ≥ 95% coverage with two doses of measles-containing vaccine in every district, delivered through the routine immunization programme or through supplementary immunization activities.[7]

Usually the minimum inhibitory level of herd-immunity required for eliminating an infectious disease is calculated based on the "basic reproductive number" (depicted as R_0) of the infectious agent. The R_0 values for measles and rubella are respectively 9–18 and 2–8; and the milestones of vaccination coverage and the minimum sero-immunity level of a population for achieving elimination is usually calculated based on these values.[12,13] For measles, the mentioned minimum inhibitory level of the herd-immunity is about 94.4%; and for rubella, this value comes to 87.5%.[8-10] In fact, the herd protection threshold for measles is the highest of all vaccine-preventable diseases and may even vary in different settings.[8,12]

Based on the results of the present study, the lowest level of immunity was in the same age groups not covered in the mass immunization campaign of November 2015. This difference is additional evidence in favour of the effectiveness of the campaign in enhancing the level of immunity in the target age groups. However, with regard to the seroprevalence rates of the age group 16 to 20 years, it should be kept in mind that in December 2003 these people had been children aged 4 to 8 years, i.e. most of them (those who then had been 5 to 8 years old) should have taken part in the nationwide measles/rubella campaign of the same year.

As another point of concern, in the last two columns of Tables 1 and 2, i.e. the age group above 15 years, there are 63 participants that have mentioned a positive history of taking part in the campaign of November 2015. The mean age of these participants was 17.2 years (SD = 1.0; Median = 16.9 years). These participants were not supposed to take part in the campaign; however, allegedly they have taken part and have received the vaccine. Even though we did not gather the required information which can justify this, our best suggestion is that these people have received the vaccine in their schools during the campaign of November 2015. Still, there were no statistically significant differences between seroprevalence rates of those who allegedly had taken part in the campaign of

November 2015 and those who had not, either for measles (P-value = 0.519) or for rubella (P-value = 0.376).

Even though, based on the findings presented in Tables 1 and 2, the level of herd-immunity against rubella is not as high as that for measles, as it was described before, the minimum inhibitory level of herd-immunity is much lower than the level required for measles (about 87.5%). Therefore, the situation for rubella, even for those parts of the population with sero-immunity below 90%, seems even more reassuring in terms of achieving elimination goals. Before the introduction of rubella vaccine, the incidence of congenital rubella syndrome (CRS) in Iran, like most other countries, varied from 0.1- 0.2/1000 live births during endemic periods, and from 0.8 – 4/1000 live births during rubella epidemics.[14,15] This is while based on the latest reports of the rubella surveillance system, during 2016, the incidence of rubella (not congenital rubella syndrome) in Iran has been about 0.32 per million total population.[5]

Based on the findings of the present study, it might be speculated that within the next few years, the frequency of measles outbreaks in the study area will decrease substantially. Before the mass immunization campaign of November 2015, these areas were the most problematic regions with regard to measles elimination efforts. The immunity level of the study population with regard to rubella is reassuring and favourable too.

No doubt, in order to achieve the elimination goals, in addition to efforts undertaken for keeping a high immunization coverage as well as routine surveillance and well-organized sero-surveys, campaigns of supplementary vaccination play a substantial role.

Materials and methods

The survey was implemented from July 24th to August 26th, 2016, about eight months after the vaccination campaign of November 2015 mentioned above. The provinces involved included parts of Sistan-va-Baluchestan and Hormozgan provinces as well as the southern regions of Kerman Province. Even though the vaccination campaign of 2015 did not include the age group above 15 years in this survey, the age group of 16 to 20 years was also included and blood sampled. In Iran, provision of health services, especially primary healthcare services and immunization activities, is governed by state medical universities; and in each province, there is at least one state medical university. In this study, four medical universities took part: one in Hormozgan Province, one in Kerman Province, and two in Sistan-va-Baluchestan Province.

Sampling scheme: Sampling method was Probability Proportional to Size cluster sampling (PPS) recommended by World Health Organization (WHO) for the evaluation of immunization coverage.[16] The results of the last Population and Housing Census of the year 2016 were used in defining the place of clusters and in preparing the sampling scheme.[17] The details of sampling procedures were similar to those used in our previous works described elsewhere in previous publications.[18]

To make sure that the age structure of the community would not intervene in the even distribution of the sample and that there would be enough sample size from all age groups, four separate continuous age layers were considered in the study, including: 15 months to 5 years; 6 to 11 years, 12 to 15 years, and 16 to 20 years. The primary sampling units were households; and from each household, at most one eligible participant was to be invited to take part in the study. If there were more than one eligible person in a household, only the youngest one was to be invited.

The field teams in the study were composed of an interviewer, a nurse skilled in blood sampling, and a local health worker. All of the interviewers in the study were adept in the local languages and had taken part in a training session in order to learn about sampling methods and working with questionnaires. Interviews were conducted in the participants' houses. If the parents consented for their children to be blood sampled and signed the written consent form, following sterile techniques and standard methods, five millilitres of blood would be drawn from each participant by a nurse skilled in blood sampling. The participants 18 years and older signed the written consent form for themselves. A three-page instruction pamphlet, containing the details of field works and methods of the study, was supplied to each colleague in the field.

Sample Size: To calculate the sample size, the proportion of the population having antibody against measles and/or rubella was considered to be about 0.90; and considering the estimation error equal to 0.09 for a two-sided confidence interval of 95% (alpha = 0.05), the required sample size for each age group came to 43 participants. Considering a design effect equal to 1.5 for cluster sampling, the sample size for each age layer came to 66 and the total sample size for each medical university taking part in the study (as mentioned above) added up to 264; and for the four universities; the total sample size amounted to 1,056. In total, 1,056 participants in 132 clusters of 8 people were devised to be sampled in the study area. The objective of the sampling was to provide blood samples from 1,056 participants in 132 clusters of 8 people, who had been organized in four age layers in such a way that from each age group in each cluster, there would be only and at least two participants.

The team members were instructed to continue invitation and sampling in each cluster by the time when eight blood samples would have been taken. This way, the size of each cluster in terms of the number of invited and interviewed participants could become even larger than eight, but not for the number of blood samples. The interviewers were instructed to keep the questionnaires of those participants who, for any reason, did not agree to give blood samples in order to make comparisons between them and those who had agreed to give blood samples.

Questionnaire: The questionnaire of the study was composed of 19 questions about demographic characteristics of the participant, his/her participation in the vaccination campaign of 2015, immunization history against measles, and medical history of any kind of exanthematic diseases. The questionnaires were filled out by trained interviewers who were adept in the local languages.

Laboratory procedures: The sera were separated from the blood samples in a local laboratory at the district level, and they were kept at -20 centigrade degrees until they were delivered to the National Reference Measles Laboratory in School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. The laboratory is part of the lab network of WHO laboratories.

In order to determine the presence of IgG antibodies against the measles and rubella viruses, indirect ELISA was used according to the manufacturer's instructions (Enzygnost® Anti-Measles Virus/IgG and Enzygnost® Anti-Rubella Virus/IgG; Siemens, Marburg, Germany). Based on the manufacturer's descriptions with Enzygnost Anti-Measles Virus/IgG and Rubella IgG, samples containing approximately 150 mIU/mL were found to be within the range of 0.100 to 0.200 DA. Samples below 0.100 were considered as negative. In this study, like our previous studies, both for measles and for rubella, serum samples with OD levels equal to or above 0.100 were considered as positive.[9,19]

Statistical Methods: The collected data were transferred to a computerized data bank, and they were analysed using Stata (ver. 11.2). In the analysis of the data, the design of the study (the stratified and cluster sampling method) was brought into consideration. The 95% confidence intervals were calculated; and where applicable, the statistics were reported as 'mean ± standard deviation'.

Ethics

Ethics Committee on Medical Research of Zahedan University of Medical Sciences has reviewed and approved the protocol and implementation of the study (Reference No.: IR.ZAUMS.REC.1395.57; 15 May 2016).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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