Measles vaccination coverage and seroprevalence of anti-measles antibody in south-east Islamic Republic of Iran

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تغطية التطعيم ضد الحصبة والانتشار المصلي للأجسام المضادة للحصبة في جنوب شرق جمهورية إيران الإسلامية

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الخلاصة: كثيراً ما توجد تباينات بين تغطية التطعيم المسجلة وبين المستوى الحقيقي للمناعة في المجتمع. ولتقدير تغطية التلقيح ضد الحصبة في جنوب شرق جمهورية إيران الإسلامية تم إجراء دراسة مقطعية في 3 مناطق خلال صيف عام 2011. فتم اختيار عينة عنقودية من 1368 طفلاً تتراوح أعمارهم ما بين 30-54 شهراً باستخدام الاحتمال المتناسب مع الحجم. وتم فحص عينات مصلية من 663 منهم – ممن تلقوا حقنتين من اللقاح الثلاثي ضد الحصبة والنكاف والحصبة الألمانية (MMR) - للتأكد من وجود الجلوبيولين المناعي G (IgG) المضاد للحصبة. لقد بلغت تغطية التطعيم بالجرعة الثانية من لقاح MMR 93.7٪. وكان انتشار الجلوبيولين المناعي G المضاد للحصبة لدى أولئك الذين تلقوا جرعتين على الأقل من لقاح MMR 94.6٪. وكان هناك ارتباط ذو دلالة إحصائية بين النتائج السيرولوجية وبين المتغيرات التي تعكس ضعف إمكانية الوصول إلى الخدمات الصحية. وبجمع النتائج السيرولوجية مع بيانات التغطية قُدِّرت نسبة الجمهور المحمي ضد الحصبة بـ 88.6٪، والتي كانت أقل من الحدود المعينة لأهداف التخلص من الحصبة.

ABSTRACT Discrepancies often exist between recorded immunization coverage and the real immunity level in a community. To estimate the vaccination coverage against measles in south-east Islamic Republic of Iran, a cross-sectional study was conducted in 3 districts during summer 2011. Using probability proportional to size cluster sampling, 1368 children aged 30–54 months were selected. Serum samples of 663 who had received 2 injections of mumps-measles-rubella (MMR) vaccine were checked for anti-measles IgG. Vaccination coverage for the second dose of MMR vaccine was 93.7%. The prevalence of anti-measles IgG in those who had received at least 2 MMR vaccine doses was 94.6%. There was a statistically significant association between the serological results and variables that reflected poor accessibility to health services. Combining serological results with coverage data, the proportion of the community protected against measles was estimated as 88.6%, which was below the limits defined for the measles elimination goals.

Couverture vaccinale antirougeoleuse et séroprévalence des anticorps antirougeoleux dans le sud-est de la République islamique d'Iran

RÉSUMÉ Il existe souvent des écarts entre la couverture vaccinale enregistrée et le niveau réel d'immunité d'une communauté donnée. Afin d'estimer la couverture vaccinale antirougeoleuse dans le sud-est de la République islamique d'Iran, une étude transversale a été menée dans trois districts durant l'été 2011. En appliquant l'échantillonnage en grappes avec probabilité proportionnelle à la taille, 1368 enfants âgés de 30 à 54 mois ont été sélectionnés. Des prélèvements de sérum de 663 enfants de l'échantillonnage ayant reçu deux injections du vaccin contre la rougeole, les oreillons et la rubéole (ROR) ont été analysés à la recherche d'anticorps IgG antirougeoleux. La couverture vaccinale pour la deuxième dose de vaccin ROR était de 93,7 %. La prévalence des anticorps IgG antirougeoleux chez les enfants ayant reçu au moins deux doses du vaccin ROR était de 94,6 %. Il existait une association statistiquement significative entre les résultats sérologiques et les variables qui reflétaient l'accès insuffisant aux services de santé. En combinant les résultats sérologiques et les données de couverture, la proportion de la communauté protégée contre la rougeole a été estimée à 88,6 %, soit un taux inférieur aux seuils définis pour les objectifs d'élimination de la rougeole.

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Introduction

Measles as a highly communicable viral disease resulting in high morbidity and mortality mostly in children of developing countries and is one of the most important vaccine-preventable diseases (1). As of 2008, a delivery strategy to offer 2 doses of measles vaccine has been used by 192 of the 193 Member States of the World Health Organization (WHO). Presently, 4 WHO regions, including the Eastern Mediterranean Region have adopted measles elimination as their regional goal. Since population immunity needs to be at least 95% in all districts of a country in order to prevent measles epidemics (1) most Member States are trying to reach and keep their immunization coverage at or above 95%, overlooking the fact that there usually is a gap (small or large) between immunity and coverage.

On a global scale, there were 8 years of decreasing numbers of reported measles cases during 2000–08 and stable numbers in 2009. Subsequent large outbreaks led to an increase in reported cases in 2010, when 40% of Member States did not meet the reported incidence target of < 5 cases per million population (2). In the Islamic Republic of Iran, after the mass vaccination against measles in 2003 and changing the administered vaccine from monovalent measles vaccine to the presently administered measles-mumps-rubella (MMR) vaccine, there was a remarkable decrease in the number of measles cases for several years (3). However, the occurrence of some measles outbreaks in Chabahar district of Sistan-va-Baluchestan Province during the winter and spring of 2010, led the authorities of the Iranian Ministry of Health to consider the real protection level of children against measles (4). Because the risk of measles outbreaks is determined by the rate of accumulation of susceptible people in the population, programmes should use data on vaccination coverage to monitor the

accumulation of susceptible people and conduct follow-up supplementary immunization activities (1). Within the past few years there have been several seroprevalence studies that have shown discrepancies between the real immunity level in communities and the immunization coverage and these are sometimes large enough to produce concern about imminent outbreaks. This suggests that recorded immunization coverage information is probably are not the best choice for planning the approach against upcoming measles outbreaks (5–9).

In this study we aimed to estimate the vaccination coverage of MMR in the population of 3 districts of 3 provinces of the Islamic Republic of Iran and the seroprevalence of anti-measles IgG among children who had received at least 2 doses of MMR vaccine.

Methods

Study setting

The districts included in the study (Ghale-Ganj in Kerman Province, Jask in Hormozgan Province and Chabahar in Sistan-va-Baluchestan Province) are located in subtropical areas of the south-east of the Islamic Republic of Iran near the borders with Pakistan and India. The climate of these areas is similar to that of the Indian peninsula. The population of the 3 districts involved in the study totalled around 96 500 and included urban and rural area residents and nomadic people.

Based on the vaccination schedule of the Islamic Republic of Iran, each child should receive 2 doses of measles vaccine during his/her life, the first at 12 months and the second 6 months later at 18 months of age. All immunization services as part of primary health care (PHC) in the Islamic Republic of Iran are free of charge. In the urban regions, the urban health centres provide PHC services and in the rural areas, there are the so-called "health houses" and

mobile teams under the supervision of rural health centres to provide PHC services. To facilitate PHC provision, the activities of the health system are organized in the rural areas as follows. The main village has a health house facility and at least 1 (and usually 2) trained health workers. These health workers provide health services not only for the population of the main village but also for 3 to 7 other villages, known as satellite villages located within a range of about 5 km from the main village. In a stepwise manner, every 3 to 5 health houses are under the supervision of a rural health centre. Mobile teams are health teams in charge of provision of PHC services for hard-to-reach small villages and nomadic people. These teams continuously and regularly travel in the field and visit those in need of their services, such as children and pregnant women.

Study sample

Vaccination coverage survey

The study population comprised all 30- to 54-month-old children living in the above-mentioned districts. The age range was selected and defined based on WHO recommendations (10). All children in the age range at the time of the interview were eligible for the study.

Based on the results of an unpublished survey about vaccination coverage of MMR performed on 2007 in Chabahar, we decided to estimate a seroprevalence of about 90% in the study population. Considering the estimation confidence interval (CI) of 95% and precision equal to 0.02, the required sample size was estimated to be about 900 children. However, since we were using a probability proportional to size cluster sampling method, which is recommended by WHO for the evaluation of vaccination coverage, we considered a sampling design effect of 1.5 and by using this method, the final sample size was 1350 (11).

The size of each cluster in the main survey was defined as 10 children, so

there were 135 clusters. To prepare the sampling scheme of the field, we used the results of the 2007 national census (*12*). To locate the clusters and find the participants, we used the information of the household logbooks of the urban and rural health centres. The information of these logbooks is updated on an annual basis. We considered the households as the primary sampling units. Each household with at least 1 offspring in the specified age range could be included in the clusters. We did not sample more than 1 eligible child from each household and so if there was more than 1 eligible child in a household, the youngest was allocated to the study.

Serological survey

For the serological part of the study there were 2 further inclusion criteria in addition to the criteria for the coverage survey: having a recorded history of receiving at least 2 doses of MMR vaccine (based on the vaccination record card of the child); and a minimum time interval of 3 weeks between the second dose of vaccine and the sampling date.

In order to determine the seroprevalence of anti-measles antibody, we based the sample size on a seroconversion rate in similar conditions of about 80% (*13*). Considering an estimation CI of 95%, precision equal to 0.04 and a design effect equal to 1.5, the required sample size to cover the serological objectives of the study came to about 576. However, to simplify the field protocol, we decided to take blood samples from half of the total sample, i.e. 675 children.

Therefore in each cluster of 10, in addition to filling the questionnaire, a blood sample was taken from the first 5 participants who met the eligibility criteria for the serological part of the study.

Data collection

Interviews and blood sampling began on 10 September 2011 in Ghale-Ganj and Jask districts in Kerman and Hormozgan Provinces and on 24 October

2011 in Chabahar district in Sistan-va-Baluchestan Province. The last case was sampled on 26 November 2011 in Chabahar.

Interviews

A questionnaire with 23 questions about vaccination history and some important demographic characteristics were filled by an interviewer adept in the local language during a face-to-face interview with one of the child's guardians (usually the mother), at the door of the participants' house. The vaccination history was defined only based on records of the vaccination cards of the participants, i.e. those children who had no vaccination card were considered as not vaccinated and those who had a vaccination card with the times of MMR vaccinations were defined according to the number of MMR vaccine injections recorded on their cards. After the interview and a brief explanation about the objectives of the project in the local language, if the guardian consented and if the child was eligible for the serosurvey, a blood sample was taken from the child by a well-trained laboratory technician.

All interviewers had at least 1 training session about how to find the participants in the field, how to fill in the questionnaire and how to take verbal informed consent. Two phone numbers were provided in each district for solving any problems that might be occur in the field during implementation field days.

Laboratory methods

After the blood samples were drawn, the serum was separated and stored frozen at −20 °C within at most 4 hours after collection and sent in a well-preserved cold-chain to the reference national measles laboratory in the School of Public Health, in Tehran University of Medical Sciences, Tehran.

In order to determine the presence of IgG antibodies against the measles virus, we used indirect enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions

(Enzygnost® Anti-Measles Virus/IgG; Siemens). Based on the manufacturer's guidelines, samples containing approximately 150 mIU/mL were found to be within the optimal density range of 0.100–0.200 ΔA. Specimens below 0.100 ΔA were considered as negative.

Data analysis

A computerized database was produced based on the completed questionnaires by using the double-entry validation method using *Epi Info* software, version 6.04. After refinement, the data were analysed using *SPSS*, version 15, and *Stata*, version 9.0. We used descriptive tables, charts, chi-squared tests, odds ratio (OR) and logistic regression modelling to analyse the data. The means and standard deviation (SD) and when appropriate the 95% CI of statistics are reported.

Results

Table 1 shows the sampling profile of the study samples for the coverage survey and serological survey in the 3 districts. The median and mean age of the participants were 44.7 and 44.1 (SD 7.5) months respectively. The male/female ratio of the participants was 51:49.

Some anomalies in the sampling should be noted. There were 18 children aged 2–4 months older than 54 months (the upper limit of the inclusion criteria for age). These children had been replaced with eligible ones during implementation of the study. However, after studying their questionnaires during analysis and since we found no other reasons for excluding them from the study, they were included. It is also worth noting that since the amount of blood taken from some of the participants was less than the amount stated in the protocol, for the sake of confidence, a few more children were sampled for the serological part of the study. This issue mainly occurred in Jask (20 more samples) and in Ghale-Ganj (21 more

Table 1 Sampling profile, vaccine coverage and seroprevalence rates among children aged 30–54 months in 3 districts in the south-east of the Islamic Republic of Iran, September to October 2011

District	Column 1 Clusters in each district	Column 2 Participants in each district	Column 3 Participants who had received 2 MMRs		Column 4 Sera of participants who had received 2 MMRs	Column 5 Ig-positive (participants received 2 MMRs)		Column 6 Seropositive in total study population[a]
	No.	No.	No.	%	No.	No.	%	%
Jusk	20	210	210	100.0	120	111	92.5	92.5
Chabahar	85	855	770	90.1	372	362	97.3	87.6
Ghale-Ganj	30	303	302	99.7	171	154	90.1	89.8
Total	135	1368	1282	93.7	663	627	94.6	88.6

[a]*Product of multiplication of the rate of coverage (Column 3) by the rate of seroprevalence (Column 5).*
MMR = measles-mumps-rubella vaccination; Ig = immunoglobulin.

samples). However, later all the blood samples were tested and so the number of prepared blood samples increased above the requested number, which was not harmful to the internal validity of the study. There were only 7 participants (5 in Chabahar and 2 in Ghale-Ganj) who refused blood sampling. Finally, the blood samples of 53 participants were unsuitable for doing laboratory tests. However, since the questionnaires of these participants had been completed appropriately we did not exclude them from the study of vaccination coverage.

Recorded vaccination coverage

In Jusk and Ghale-Ganj, the vaccination coverage for the first dose of MMR vaccine was 100% and in Chabahar it was 96.8% (95% CI: 95.1–98.6%). Total vaccination coverage for the first dose of MMR vaccine was 98.0% (95% CI: 96.9–99.1%). There were 27 children (2.0%) without any recorded history of measles vaccination and 59 (4.3%) with recorded history of receiving only 1 measles vaccine.

Column 3 of Table 1 shows the vaccination coverage for the second dose of MMR vaccine in the 3 districts. The total coverage for the second dose was 93.7% (95% CI: 91.5–95.9%). The mean ages at receiving of the first and second doses of MMR were 13.0 (SD 3.1) months and 20.1 (SD 4.6 months) respectively.

Anti-measles seropositivity

Column 5 of Table 1 shows the results of ELISA tests for detection of anti-measles IgG in the sera of those children who had received their second dose of MMR vaccine. The total seropositive rate among children who were recorded as receiving 2 MMR vaccine doses was 94.6%. Although Jusk and Ghale-Ganj districts had reported vaccination coverage above 99% for the second MMR dose, they had lower seroprevalence rates (92.5% and 90.1% respectively) than Chabahar district (97.3%), where the reported coverage was only 90.1%.

Based on the study protocol, only participants who had received at least 2 doses of MMR vaccine were to be entered in the serological part of the study; however, there were 18 participants in Chabahar who had been sampled and tested mistakenly without fulfilling this criterion. Among these participants 17 had received only 1 dose of MMR vaccine (2 IgG negative; 15 IgG positive) and 1 child, a 4.5-year-old girl without any recorded history of receiving any kind of measles-containing vaccines, was IgG positive. She also had no previous history of measles or any other kinds of eruptive diseases.

Demographic determinants of seroresponse

Table 2 shows the results of the statistical analysis of the relationship between the presence of anti-measles IgG and some of the demographic variables in the questionnaire. In both the univariate and multivariate analysis (using logistic regression modelling), living in the remote areas (mobile health team areas) increased the chance of non-response to MMR vaccination by a factor of about 3 (OR 2.9; 95% CI: 1.3–6.8) (Table 2). The other important variable in this regard was the material used in housing. Children living in tents or homes built of straw were 3 times more likely to be seronegative than those living in houses built of brick, cement or clay (OR 2.9; 95% CI: 1.3–6.8). The distance from the individual's house to a health centre (< 10 km versus ≥ 10 km) showed a relationship with seronegativity in univariate analysis, but dropped from the model in logistic regression analysis. There were no other statistically significant relationships between any other variables studied and seroprevalence of anti-measles antibodies.

Estimated actual seroprevalence

By assuming that the immunological situation of those who had received only 1 MMR vaccine dose was at most as good as those who had received 2 MMR vaccine doses, we combined the results of the 2 parts of the study (i.e. Columns 3 and 5 of Table 1) and estimated that the antibody seroprevalence of the study population should be about 88.6% (Column 6 of Table 1).

Discussion

In this study, the seroprevalence of anti-measles antibody of the study population (30- to 54-month-old children) who had received at least 2 MMR vaccinations was 94.6% and the vaccination coverage for the second dose of MMR vaccine was about 93.7%. Combining the recorded vaccination coverage with the antibody seroprevalence data we estimated that antibody seroprevalence of the study population was about 88.6%, which, considering the vaccination coverage goals of the elimination phase, is below expectations. It is interesting to note that the 2 districts that had reported vaccination coverage above 99% for the second MMR vaccine had lower seroprevalence rates than the third district with lower coverage (90.1%). In other words, the seroprevalence of anti-measles IgG did not match the vaccine coverage. The analysis of the obtained data gave us no clues for the reason for this. However, there could be many explanations (such as problems with preparation, transportation and the administration of the vaccine by health workers) and almost all of them are out of the scope of the work of

this manuscript. Whatever the reasons behind these findings, they emphasize the importance of validating coverage rates by serological findings, especially in countries that are in the elimination phase of measles.

In statistical analysis of the data, among those children who had received 2 doses of MMR, 3 variables showed a significant association with the presence or absence of anti-measles-IgG in the univariate analysis. In a closer look at these variables, it is clear that they all represent the same problem, i.e. poor accessibility to health services. In other words, living more than 10 km away from rural health centres was associated with living in the areas under coverage of mobile health-care teams and in such areas the most common type of housing are straw cottages and tents. Mobile teams, as described in the Methods section, are regularly travelling to remote (hard-to-reach) areas and performing health services such as vaccination for the people who need them. Since in this study we included only those children who had received at least 2 documented MMR vaccine doses, the reason for the remaining seronegative cases could not be a shortage in vaccination services,

and we have to consider other aspects of the vaccination process such as maintenance of the cold-chain and regular retraining of the health staff involved. However, we did not do any research about any of these possibilities. So at this point we may only propose that in future studies such problems need special attention.

Among our participants, we had a 4.5-year-old girl with negative MMR vaccination and a negative history of any eruptive diseases who was positive for anti-measles IgG. The number of reported asymptomatic measles cases in the literature is not large. In an outbreak investigation report in Texas in the spring of 1985, there were 3 seronegative students who had seroconverted without experiencing any symptoms (14). However, the simplest explanation might be the failure of a health worker to record the child's vaccination details in the vaccination card, even though we found no evidence for that in our in departmental investigation.

As described in the Methods section, we used cluster sampling to select the participants. Although to compensate for the possibility of spatial clustering of seroprevalence we multiplied the

Table 2 Association between seropositivity and demographic variables of participants in the serological study (663 children aged 30–54 months) in 3 districts in the south-east of the Islamic Republic of Iran, September to October 2011

Variable	Seropositive		Seronegative		Univariate analysis	Logistic regression	P-value[a]
	No.	%	No.	%	OR (95% CI)	OR (95% CI)	
Distance from health centre (km)							
< 10	558	95.2	28	4.8	1	–[b]	0.023
≥ 10	59	88.1	8	11.9	2.7 (1.2–6.2)	–	
Residential area							
Urban/rural health centre areas	393	95.9	17	4.1	1	1	0.006
Mobile health-care teams areas	69	87.3	10	12.7	3.4 (1.5–7.6)	2.9 (1.3–6.8)	
House build materials							
Brick/cement/clay	527	95.5	25	4.5	1	1	0.026
Straw/tent	99	90.0	11	10.0	2.3 (1.1–4.9)	2.9 (1.3–6.8)	

[a] Fisher exact text; [b] Exit from model.
OR = odds ratio; CI = confidence interval.

sample size by a factor of 1.5, there still might have remained some clustering effect that we have to consider as a limitation of this type of study.

Presently, the criteria for achieving the elimination goal of measles have been defined based on the vaccination coverage for the second dose of measles vaccine, i.e. a vaccination coverage of more than 95% for each district. Using this criterion the condition of 2 of the 3 districts involved in this study could be categorized as satisfactory (Column 3 of Table 1) (1,10,15,16). However, considering the antibody seroprevalence of those who had received 2 MMR vaccine doses, the protection level of none of the districts could regarded as satisfactory (Columns 5 and 6 in Table 1). Ignoring such evidence will lead to accumulation of susceptible individuals within the community year by year, until there is occurrence of a real outbreak. In fact, some researchers believe that broad-scale vaccine coverage goals are unlikely to have the same impact on the interruption of measles transmission in all demographic settings. It seems that the achievement of elimination goals might require vaccine coverage objectives tailored to local conditions (such as demographic, logistic and economic factors) (17).

There are experiences with vaccination coverage reports in other countries that again could be regarded as evidence for the importance of surveillance of the seroprevalence of antibodies in planning and management of anti-measles campaigns instead of surveillance of vaccination coverage. Quebec in Canada has reported a vaccination coverage similar to that in our study (95–97% for the first dose and 90% for the second dose of MMR), yet in 2011 it experienced the largest measles outbreak in North America in a decade; 56% of cases were adolescents aged 12–17 years. More than 22% of the adolescents in that study had received 2 vaccine doses, even though they had milder illness and a significantly lower risk of hospitalization than those who were unvaccinated or had received 1 vaccine dose (18). These findings suggest that even those who have received 2 vaccine doses might be susceptible during an epidemic. There are some other experiences similar to that in Quebec which are worthy of note (5–9,14,19–21).

The findings of our study defined the immunological situation of the study population with respect to measles and, in line with previous reports, emphasized the importance of validation of vaccination coverage by serological surveys in defining the real protection of the community against measles outbreaks. Based on these findings we highly recommend the implementation of periodic serosurveys as an integral part of measles surveillance systems in countries in the elimination phase of their campaign.

Acknowledgements

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