Measles seroepidemiology in 3 cities in Turkey

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ABSTRACT

Objective: To evaluate immunity against measles and its relation with some variables among healthy subjects in 3 cities in Turkey.

Methods: We carried out a cross-sectional study on measles antibody titers in the serum samples of 712 people from Antalya, 696 from Diyarbakir and 667 from Samsun, Turkey using particle agglutination test between February 2000 and October 2001. The study groups, informed and asked for their consent by midwives, consisted of randomly selected subjects of all ages older than 6 months. We implemented the study in 3 steps: physical examination, interview and blood collection.

Results: We considered titers of ≥1:16 as positive and we observed lower seropositivity in Diyarbakir (90.8%) than Antalya (95.9%), and Samsun (94.9%) (p=0.040). We also observed that seropositivity was lower among preschool group than older groups (p=0.006). The number of doses of measles vaccine (p=0.001) and measles infection history (p=0.003) were found as a factor increasing the seropositivity ratio. There was no statistically significant between age groups (p=0.219), gender (p=0.148), residence (p=0.537), and number per household (p=0.983) among the provinces.

Conclusion: Based on the findings, measles infection still has a high incidence in Turkey and the second dose of measles vaccine is extremely important in the prevention of measles transmission among school children and the community. Furthermore, we must improve our regional differences in routine vaccination services.

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The vaccination schedule in Turkey includes 2 doses of measles vaccine. Children should receive the first dose of measles vaccine at 9 month of age and the second dose during the first school year (6-7 years of age). In 1970, Turkey initiated measles vaccination. In 1985, a national mass vaccination campaign started and achieved 94% measles coverage among children less than one year old, and 83% among children less than 5 years old. In 1998, second dose measles vaccination was introduced.[1-4] Before the initiation of measles vaccination, a high measles incidence had been noted in 1969, there were 66,111 cases (incidence rate 192/100,000), and 523 deaths. Within 2 years following the first national vaccination campaign, there was a decrease of 2267 and 2194 measles cases (incidence rate of 4/100.000) and no death. Since 1990's, 15,000-30,000 annual cases were noted usually below the age 15 years.[3-4] In 2001, the percentage of vaccinated cases among all cases was 52.8% while the vaccination coverage rate increase.[5] Although herd immunity can be estimated by vaccination coverage and data regarding incidence of the illness, seroprevalence studies are accepted to be the most important data to have accurate estimations. Findings gathered by these studies are accurate data sources for decision making for the vaccination programs.[6-8] Therefore, determination of age specific susceptibility levels has special importance nowadays where elimination of measles in the European continent is a common goal. Serological surveillance has been carried out for the

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immunity against measles in 7 Western European countries those constitute European Sero-epidemiology Network (ESEN) between 1994-1998. In this large international study, a country's susceptibility profile was highly associated with vaccine coverage for the first dose and this study confirmed the high vaccination coverage to be the most important factor in the control and prevention of measles infection.[8] Our study aims to evaluate population immunity against measles and its relation with age older than 6 months, gender, residence, educational status, number of household, history of measles infection and vaccination in 3 selected cities Turkey (Antalya, Diyarbakir and Samsun).

Methods. Aiming to establish a laboratory based surveillance system Refik Saydam National Hygiene Center and General Directorate of Primary Health Care of Ministry of Health of Turkey and Japan International Cooperation Agency (JICA) carried out a project; namely "Infectious Diseases Control Project in Turkey" between 1997 and 2002. In frame of the Project, seroepidemiological surveys, which were designed as a cross-sectional study, were carried out in order to evaluate the immunity against some vaccine preventable diseases (diphtheria, pertussis, tetanus, polio, measles and hepatitis B), which are included in the Extended Vaccination Program of WHO. The community-based survey was performed in 3 cities, which were from the different geographical region (Antalya, Samsun and Diyarbakir) between February 2000 and October 2001.

Antalya is a touristic city from Mediterranean Region of Turkey with a population of 1,719,751 according to the census in 2000. Diyarbakir is a city from Southeastern Region, which has low vaccination coverage and socioeconomic status with a population of 1,362,708 according to the census in 2000. Samsun is a well-industrialized city from the Northern Region where vaccination coverage is high and its population is 1,209,137 according to the census in 2000.[9] Vaccination coverage against measles at the years of 2000 and 2001 were 87-89% in Antalya, 50-67% in Diyarbakir, and 93-85% in Samsun.[10-11] For study purposes, sample sizes of 672 for each province were required, using seroprevalence rates of measles in some selected regions of Turkey. A representative, stratified random sampling was established using age, gender and residency strata. Sampling was disproportionate to size and included 16 individuals from each stratum. Eight health centers from Antalya (4 rural and 4 urban), 8 health centers from Diyarbakir (4 rural and 4 urban) and 10 health centers from Samsun (5 rural and 5 urban), a total of 26 health centers were selected by Provincial Health Directorates of each city by practical considerations such as which health centers are willing and capable of participating at the required level. Sixteen with additional 3 individuals were selected randomly from each age group among ages older than 6 months (0,1,2,3,4,5,6,7,8,9,10-14, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60+) using a randomization list in which all household members are systematically registered by health center. Midwives of related region informed the selected individuals through door-to-door visits and asked them for their consent. A total of 672 individuals were aimed to be selected for each city; but due to the participation of some additional individuals the number increased. Individuals could participate to the survey as an eligible subject if they were free of infectious diseases at the time of the survey.

The following information was gathered by filling out a questionnaire during an individual face to face interview: some sociodemographic data, vaccination status, and history of past infectious diseases. Data for vaccination status were verified with vaccination cards, or health center vaccination records if there were no cards or records individual's declaration were accepted. Data of children younger than 18 years were recorded according to the information from their parents. After the interview, 10 milliliters of venous blood samples were collected with vacutainer tubes. Serum samples with small quantities were not included in the laboratory test. Serum samples were divided into aliquots and distributed to the laboratories for specific antibody testing; a total of 2075 serum samples were tested to evaluate the immunity status for measles (**Figure 1**). A total of 2465 individuals (826 from Antalya, 894 from Diyarbakir and 745 from Samsun) were selected. Among the selected individuals, measles antibody titers were studied in the serum samples of 712 (86.2%) individuals from Antalya, 696 (77.9%) from Diyarbakir and 667 (89.5%) from Samsun. Serum was separated and allocated under sterile conditions at the Regional Branch Laboratories after keeping it overnight at 4^0C; the aliquots were then transported to the Refik Saydam National Hygiene Center under cold chain condition.

Particle agglutination test (PA) (Serodiameasles, Fujirebio Co. Japan) was applied as indicated in the manufacturer instructions and could detect the antibodies quantitatively. Lyophilized gelatin particles that are covered with H and F antigens of the measles virus were suspended with the dilution solution of the kit. Two-fold serial dilutions of each blood sample were carried out (25µl) from 1/2 titers to 1/2048 titers on the U shaped microplate with 96 wells. Sensitive particle suspension was added in equal volume and studied after an incubation period

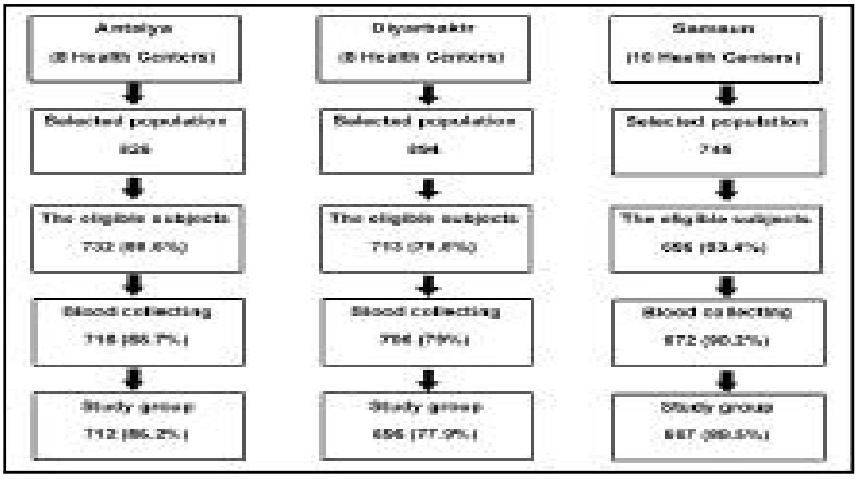

Figure 1 - Study group; Antalya, Diyarbakir and Samsun.

of 2 hours at room temperature. The highest dilution in which agglutination was observed was accepted as particle agglutination titer of the sample. The positive control of the kit with a titer of 1/128 was used as standard control. Antibody titers of ≥1:16 against measles virus were grouped as positive (presence of antibody) and <1:16 as negative.[12-14] All analyses were corrected for sampling strategy, using sampling weights, calculated as inverses of sampling fractions. Weighted and unweighted analyses were compared and contrasted to check for the robustness of sample weights.

The Statistical Package for Social Science version 10.0 for windows and a special statistical analysis programs prepared by the JICA expert using visual basic 6.0 and access were used for the statistical analysis. Analysis included bivariate, stratified analyses and multiple logistic regression modeling. In the logistic model, the dependent variable was seropositivity for measles (positive versus negative) and covariate variables included age group, city, gender, residence, number of household, educational status, history of measles infection, and doses of measles vaccine. Stratified analyses suggested potential effect modification of age groups (0-29 and ≥30) and history of having infection on the association between measles seropositivity. Therefore, interaction terms for such factors were tested in multivariate logistic regression models. Inter-variable correlations were evaluated prior to modeling: none of the correlations were above 0.25. Dummy variables were created to study various risk factors. Odds ratios and relevant 95% confidence intervals (CI) were presented for potential risk factors of interest.

Results. The study groups were included 2075 subject, 712 (86.2%) of them were from Antalya, 696 (77.9%) from Diyarbakir and 667 (89.5%) from Samsun. Among the 712 samples surveyed in Antalya 50.6% (n=360) were from rural areas, 49.4% (n=352) were from urban areas; 45.5% (n=324) were males, 54.5% (n=388) were females. Among the 696 samples surveyed in Diyarbakir 50.4% (n=351) were from rural areas, 49.6% (n=345) were from urban areas; 48.3% (n=336) were males, 51.7% (n=360) were females. Among the 667 sample s surveyed in Samsun 49.9% (n=333) were from rural areas, 50.1% (n=334) were from urban areas; 48.6% (n=324) were males, 51.4% (n=343) were females.

The positivity of the measles antibody titers was found as 95.9% in Antalya, 90.8% in Diyarbakir and 94.9% in Samsun. The positive antibody titers at the age of 30 years or older in Antalya were 100%, 99.1-100% in Diyarbakir, and 97.8-100% in Samsun. The distribution of the antibody titers against measles among the study group by some parameters are shown in **Table 1**. A 6-8 months of age neonates was evaluated as a group who could have only maternal antibody. The positive antibody levels against measles among 6-8 months infants in Antalya were 33.3%, Diyarbakir 47.4%, and Samsun 63.6%.

Illiterates and just literate are included to the never admitted to a school group and evaluated together. With this acceptance, educational status was evaluated by grouping into 5 as illiterates and just literates, preschool, primary school, primary and junior high school, and high school and over. The positive antibody levels among preschool children and primary children are shown in **Table 1**. In Antalya, at least one dose of measles vaccine had 74.3% of the 0–29 age group, 54.5% in Diyarbakir, and 81.5% in Samsun based on the health center's registration. Distribution of persons who had at least one dose of the vaccine according to residence in

Table 1 - Distribution of the measles antibody titers by some parameters: Antalya, Diyarbakir, and Samsun (2000-2001).

Characteristics	Antalya -ve (%)	Antalya +ve (%)	Antalya n	Diyarbakir -ve (%)	Diyarbakir +ve (%)	Diyarbakir n	Samsun -ve (%)	Samsun +ve (%)	Samsun n
Gender									
Male	(4.6)	(95.4)	324	(9.8)	(90.2)	336	(5.9)	(94.1)	324
Female	(3.6)	(96.4)	388	(8.6)	(91.4)	360	(4.4)	(95.6)	343
Age group									
6 – 8 months	(66.7)	(33.3)	15	(52.6)	(47.4)	19	(36.4)	(63.6)	11
9 – 11 months	(27.3)	(72.7)	11	(57.9)	(42.1)	19	(57.9)	(42.1)	19
1 years	(5.6)	(94.4)	36	(58.6)	(41.4)	29	(11.4)	(88.6)	35
2 years	(2.9)	(97.1)	34	(27.6)	(72.4)	29	(8.8)	(91.2)	34
3 years	(8.8)	(91.2)	34	(16.7)	(83.3)	30	-	(100)	29
4 years	(6.7)	(93.3)	30	(12.5)	(87.5)	40	(12.5)	(87.5)	32
5 years	(5.7)	(94.3)	35	(8.6)	(91.4)	35	(6.3)	(93.8)	32
6 years	(3.1)	(96.9)	32	(3.6)	(96.4)	28	(3)	(97)	33
7 years	(7.1)	(92.9)	28	-	(100)	29	-	(100)	32
8 years	-	(100)	31	(2.9)	(97.1)	34	(3.3)	(96.7)	30
9 years	-	(100)	31	(6.9)	(93.1)	29	-	(100)	21
10-14 years	-	(100)	32	-	(100)	35	-	(100)	34
15-19 years	-	(100)	30	-	(100)	32	(6.7)	(93.3)	30
20-29 years	(3.6)	(96.4)	84	-	(100)	75	-	(100)	70
30-39 years	-	(100)	80	-	(100)	61	-	(100)	70
40-49 years	-	(100)	73	-	(100)	65	-	(100)	64
≥50 years	-	(100)	96	(0.9)	(99.1)	107	(2.2)	(97.8)	91
Residence									
Rural	(4.4)	(95.6)	360	(8.3)	(91.7)	351	(3.3)	(96.7)	333
Urban	(3.7)	(96.3)	352	(10.1)	(89.9)	345	(6.9)	(93.1)	334
Educational status									
Illiterate + just literate	-	(100)	74	-	(100)	224	(3)	(97)	67
Preschool*	(6.5)	(93.5)	199	(24.3)	(75.7)	202	(12.2)	(87.8)	196
Primary school	(2.6)	(97.4)	115	(3.6)	(96.4)	111	(1.9)	(98.1)	105
Primary/junior high school	(0.4)	(99.6)	225	(0.9)	(99.1)	113	(1)	(99)	198
High school and over	(2.5)	(97.5)	81	-	(100)	26	-	(100)	90
Number of household									
1-4	(3.7)	(96.3)	491	(11.9)	(88.1)	109	(7.1)	(92.9)	295
5-9	(5.3)	(94.7)	209	(7.6)	(92.4)	394	(3.7)	(96.3)	327
≥10	-	(100)	4	(11.4)	(88.6)	185	(2.4)	(97.6)	41
History of measles infection									
Non	(6.1)	(93.9)	410	(16.3)	(83.7)	332	(7.1)	(92.9)	410
Yes	(2.2)	(97.8)	182	(1.1)	(98.9)	262	(1.3)	(98.7)	156
Unknown	-	(100)	119	(6.9)	(93.1)	102	(3)	(97)	101
History of vaccination against measles									
Unvaccinated	(14.1)	(85.9)	78	(11.8)	(88.2)	229	(5.8)	(94.2)	103
1	(5.6)	(94.4)	250	(9.7)	(90.3)	207	(8)	(92)	276
2+	(1.1)	(98.9)	95	(10.2)	(89.8)	49	(2.3)	(97.7)	87
Unknown	(1)	(99)	289	(5.7)	(94.3)	211	(2)	(98)	201

*does not include 6-8 months year of age.

rural and urban regions were 52.4% and 46.2% in Antalya, 42.1% and 32.9% in Diyarbakir, 54.3% and 56.4% in Samsun. The percentages of being vaccinated at least one dose of measles vaccine in preschool children were 91.4% in Antalya, 64.8% in Diyarbakir, and 95.9% in Samsun. Individuals who had 2 or more measles vaccine doses among primary school students consisted 54.8% in Antalya, 21.6% in Diyarbakir, and 53.3% in Samsun. The interaction of age groups (0-29 and ≥30) and history of having infection was studied and resulted in a non-statistical significant interaction (Beta: -1.803, SE: 1.343, Wald test: 1.804, SD: 1, and p=0.179). Results of the bivariate logistic regression are presented at **Table 2**. The number of the people who were positive for measles antibody in Diyarbakir was significantly lower than in Samsun and Antalya (OR: 0.514, 95% CI: 0.272-0.971, p=0.040). Seropositivity among preschool group was lower (p=0.006) than other groups of educational status. The number of doses of measles vaccine (p=0.001) and measles infection history (p=0.003) were found as a factor increasing the seropositivity ratio. There was no statistically significant between age groups (p=0.219), gender (p=0.148), residence (p=0.537), and number of household (p=0.983) among the provinces.

Discussion. The PA method used in this study is an appropriate test for seroepidemiological studies as it can determine the antibody levels with low titers, gives results in a short time, and is easy to apply without a need of expensive equipment's in the cases of small serum samples.[12,14] According to data of Ministry of Health the vaccination coverage in 2000 was 87% and in 2001 was 89% in Antalya, 50% in 2000 and 67% in 2001 in Diyarbakir, and 93% in 2000 and 85% in 2001 in Samsun, 81% in 2000 and 84% 2001 in Turkey.[11] In our study, group

Table 2 - Logistic regression analysis of positive antibody levels of the study group in relation with some variables; Antalya, Diyarbakir, Samsun (2000-2001).

Characteristics	Seropositivity (%)	OR (95% Confidence Interval)	*P*-value
City			
Samsun	(94.9)	1	
Antalya	(95.9)	1.376 (0.701-2.699)	0.353
Diyarbakir	(90.8)	0.514 (0.272-0.971)	0.040
Age group			
0-29 years	(90.9)	1	
≥30 years	(99.6)	4.119 (0.431-39.392)	0.219
History of infection			
Non	(90.6)	1	
Yes	(98.5)	4.362 (1.654-11.506)	0.003
Age in years versus measles infection		0.165 (0.012-2.289)	0.179
Gender			
Male	(93.2)	1	
Female	(94.5)	1.437 (0.879-2.350)	0.148
Residence			
Rural	(94.6)	1	
Urban	(93.1)	0.850 (0.509-1.421)	0.537
History of education			
Illiterate + just literate	(99.5)	1	
Preschool	(82.9)	0.086 (0.015-0.490)	0.006
Primary school	(97.3)	0.320 (0.047-2.188)	0.245
Primary school and junior high school	(99.3)	0.682 (0.105-4.409)	0.687
High school and over	(99)	0.231 (0.029-1.837)	0.166
Number of household		1.001 (0.907-1.105)	0.983
Doses of measles vaccine		2.491 (1.430-4.340)	0.001

measles seropositivity were 95.9% in Antalya, 90.8% in Diyarbakir, and 94.9% in Samsun. Persons with positive antibodies against measles were significantly less in Diyarbakir city than Samsun and Antalya (p=0.040). As seropositivity of the people with history of infection in Diyarbakir was similar with the other studied cities, insufficient vaccination coverage in Diyarbakir was found when compared with other study cities and an increase in the susceptible group was seen. There was no statistically significant difference in the measles seropositivity between the age group equaled or above 30 years versus below (p=0.219). This shows the high prevalence of past measles infection in 30 years and older group as they were not covered in the routine vaccination program.

Measles antibody levels between 6-8 months of birth, which were assumed the maternal antibody, were 33.3% in Antalya, 47.4% in Diyarbakir, and 63.6% in Samsun. Some studies demonstrated very low level in maternal measles antibody presence after the 7th month.[15,16] In similar studies conducted in our country, Kanra et al[17] noted a 15.6% measles maternal antibody level in 5-6 months infants, Metintas et al[18] reported a 26% in 4-9 months infants, and Kilic et al[19] found a 50% in 7-9 months infants. In various studies, measles antibody positivity of unvaccinated mothers and their infants reported to be significantly higher than the vaccinated mothers and their infants.[20-22] High levels of maternal seropositivity in our study group can possibly be attributed to the very low levels of vaccination coverage especially before 1985, and therefore, a high possibility of an unvaccinated and hence, passed measles infection of the mother.

We showed the decreasing seropositivity of measles in 9-11 months infants when compared with 6-8 months infants in cities but Antalya. In the study carried out in Turkey by Metintas et al[23] it was shown that 11.4% of the infants at 9-11 months of birth had maternal antibodies against measles and after vaccination for measles just 61.3% of them remained seroconverted on the 30-40 days. Evliyaoglu et al[24] noticed a primary vaccine failure and inappropriate vaccination age. The decrease in seropositivity of infants at 9-11 months in our group is thought to be related to interference of maternal originated antibodies with vaccine in Diyarbakir and Samsun, and furthermore, to the low levels of vaccination coverage in Diyarbakir. Based on these findings, we can note the importance of the second vaccination dose in our country.

In our study, we see the range from positive antibody titer level at the ages between 1-6 as 91.2-97.1% in Antalya, 41.4-96.4% in Diyarbakir, and 88.6-100% in Samsun. The cause of low measles seropositivity in Diyarbakir at this age group can be explained with low vaccination coverage at 9-11 months of age.

After 1998, second dose vaccination for measles was introduced to our country at the first year of the primary school. The ranges of positive antibody levels for measles at the ages between 7 and 9 were found to be 92.9-100%. The high levels of antibody at this age group may be related to the second dose vaccination at the primary school period. In this

group, the percentage of individuals having had 2 or more measles vaccine doses were 54.8% in Antalya, 21.6% in Diyarbakir, and 53.3% in Samsun. Considering that children at the age of more than 9 years old only had a single dose of measles vaccine, percentage of positive antibody titers for measles in the 10-19 years old group were found to be 100% in Antalya, and 100% in Diyarbakir, and 93-100% in Samsun. Many studies report that vaccination before 12 months in infants can result in primary vaccination insufficiency.[15,16,25,26] Additionally, preventive immunity acquired with vaccination, and so without booster effect of naturally contaminated virus, can not be lifelong.[27] Ratnam et al[28] conducted a study in children at the age range from 5-17 and reported a decrement in the immunity until 5 years of age, which was then kept decreasing in a slower manner. In our study, maintenance of high measles antibody positivity percentage in 10-19 years old group is probably reflecting the concurrent presence of measles virus circulation especially among the school children rather than the vaccine's effect.

In our country, 90-95% of all measles cases notified before 1989 were under the age of 15 years. Between 1989 and 2002, 0-4 years of age group consisted 28-52%, and 5-14 years of age group consisted 48-60% of all cases. In the years of epidemics, the occurrence with high incidences at the groups aged younger than one year of age and between 5 and 9 years demonstrates a rapid circulation of the measles virus both at the schools and among the society.[3] In the study regions in 2000 and 2001 measles morbidity rates (per 100,000 population) were recorded to be 31.4 and 11.1 in Antalya, 19.4 and 77.3 in Diyarbakir, and 20.3 and 46.5 in Samsun.[10,11] The high incidence rates in Diyarbakir and Samsun can be explained due to the peak in the year 2001. In the study group, while the measles seropositivity among those vaccinated with a single dose was 94.4% in Antalya, 90.3% in Diyarbakir and 92% in Samsun, it was 98.9% in Antalya, 89.8% in Diyarbakir and 97.7% in Samsun among those who had 2 or more measles vaccines. The results of logistic regression analysis showed that measles seropositivity increased with increasing doses of measles vaccine (p=0.001). Further, we could show that the seropositivity in rural settings varied in the range from 91.7-96.7% and in urban 89.9-96.3%. There was no statistical difference in antibody positivity between rural and urban settings (p=0.537). In a similar manner, there was no statistically significant difference in antibody positivity between males and females (p=0.148). We were not able to show a difference in seropositivity due to different number of household (p=0.983). The morbidity of diseases highly contagious diseases are known to be frequent in crowded families. However, beyond the infection in families, widespread prevalence of measles infection also affects the individuals in the population. It gives rise to close contact of schooled children of the families with the students from different families.

Measles seropositivity among those having measles infection in the study group differed in the range between 97.8% and 98.9%. The measles antibody positivity among those stating of having had an infection were significantly higher than those who had not (p=0.003). The measles infection itself is well known to result in higher antibody titers than to the measles vaccine.[15] Hutchins et al[29] reported that while 99% of those borne in the USA before vaccination period to be immune to the measles, it decreased to 81% in those borne in the vaccination period. In a study of Itoh et al[27] the titer of antibody in those who were vaccinated was 4.9 times lower than those had infection naturally, and it had a significant decrement at approximately 20 years of age. The researchers emphasized the insufficiency of vaccination with a single dose that had been in practice in Japan and noticed the need to a shift to a 2 dose vaccination policy.[27] In our country, with regard to the measles infections seen in the 0 age group, it clearly demonstrates the importance of vaccination against measles at the 9th month of birth. In 1997, WHO reached to the conclusion that the elimination strategies based on the determination and vaccination of the susceptible population to be the most appropriate strategy in the elimination of measles infection in the European region. The Turkish Ministry of Health initiated an elimination program based on both the recommendations of WHO and the analysis of Turkish epidemiological data, in 2002. In our country where measles infection still has a high incidence, putting the measles elimination program into practice is extremely important in the prevention of measles transmission in schools and the community. Furthermore, regional differences in routine vaccination services must be improved.

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