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INFECTIOUS DISEASES

Immunity to measles in the Croatian population

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Abstract. In the frame of measles elimination activities, sera from 1205 Croatian citizens from all parts of the country and of all ages were tested, using Gull Laboratories ELISA, for measles IgG. Equivocal results were found in 50 subjects. Of the remaining 1155 participants, 118 or 10.2% were negative and 1037 or 89.8% positive. The proportion of seronegatives ranged from zero (age groups 41–50, 51 and more) up to 21.4% (1 year of age). As for their distribution into age groups suggested by the European Regional Office of WHO, there were 12.7, 8.9, 9.5 and 8.8% negatives in age groups 1–4, 5–9, 10–14 and 15+ years, respectively. According to these results, only the first two age groups meet WHO criteria, indicating that vaccination coverage higher than the reported 90–94% should be attained if one is to expect measles elimination.

Key words: Croatia, Herd immunity, Measles

Abbreviations: Anti-MV = antibodies to the measles virus; ELISA = enzyme linked immunosorbent assay; IgG = immunoglobuline class G; REV = relative ELISA values; WHO = World Health Organization

Introduction

Measles vaccine has been available for more than 30 years, during which all European countries have introduced it into their vaccination programmes. As a consequence, the number of measles cases in the European Region has declined from over 300,000 cases in 1991 to approximately 36,000 in 2000 [1].

In Croatia, all vaccinations offered to children are mandatory. Measles vaccination was introduced into the childhood vaccination programme in 1968. After a short time, universal vaccination of children against measles has shown its beneficial effects in substantial reduction of measles morbidity. If one compares the mean annual morbidity in the 5-year period preceeding introduction of vaccination (1963–1967), which was 15,183, with the mean annual morbidity in the last 5-year period (1997–2001), which was only 187, the reduction rate is 98.8%. In 2001 only eight cases have been reported [2, 3]. This favourable trend is no doubt in large extent due to vaccination and can be compared with trends in other low incidence European countries (Denmark, Netherlands, Finland, etc.) [1, 4, 5].

The European region of WHO has set a target for eliminating measles from the region by year 2007 [6, 7]. One of the activities within this initiative is also the assessing of population immunity to measles as an indicator for the possible reaching of this goal. According to WHO Euro criteria there should not be over 15% of susceptibles up to 4 years of age, 10% among those from 5 to 9, and not more than 5% among those from 10 to 14 and each cohort above 14 years of age [8, 9]. This is calculated to be the minimum immunity level in the population guaranteeing interruption of measles virus transmission. With the aim to determine the herd immunity level of the Croatian population and to compare age-specific immunity levels to WHO criteria, we undertook this investigation.

Materials and methods

Vaccination programme structure and coverage

Immunization against measles was introduced into the official immunization schedule in Croatia in 1968 [10] as a primary vaccination at the age of 11 months and in the first grade of elementary school. At the same time, some earlier cohorts of children in kindergartens and nursery schools were also vaccinated, so as to cover quickly the whole segment of the preschool population. The primary vaccination in the first grade of school gradually became revaccination but it was not discontinued; though at the time it was believed that a single shot of measles vaccine confers life-long immunity. It was maintained primarily as a 'second chance' for those having missed vaccination earlier. In 1973 the primary vaccination shifts to children 1-year old and rubella is added. In 1974 the mumps component is added [10]. The immunization

schedule is not subject to any changes until 1997 when the revaccination from the first grade is moved into the sixth. All the vaccinations are performed with vaccines containing the Edmonston–Zagreb strain, manufactured by the Institute of Immunology, Zagreb, Croatia.

Vaccination coverage in Croatia is monitored by the administrative method, meaning all vaccinators report on their vaccination performance to Epidemiology departments/field offices in their respective county Institutes of public health, which summarize received reports at the local level and forward summarized reports to the Croatian National Institute of Public Health. The denominator for calculating coverage are all children of appropriate age, eligible to receive the specified vaccination, under the responsibility of the reporting health care provider (pediatrician, school medicine specialist, general practitioner etc.). The numerator is the number of children who actually received the vaccination for which they are eligible, according to their age.

During the last decade (1992–2001) the primary vaccination coverage ranged from 90 to 94% at the national level, thus being somewhat lower than the compulsory 95%. At the same time the revaccination rates were somewhat better, ranging from 93 to 98% [2]. There are 113 epidemiology field offices, corresponding to the same number of epidemiologic areas, and vaccination coverage does not vary significantly from one to other.

Serum survey collection

The collection and testing of sera specimens were undertaken between October 1999 and February 2000. The number of sera collected was in total 1205.

The use of residual sera collected during routine laboratory sampling was chosen as a method of sampling. Samples were collected from a variety of geographical locations within Croatia (cities Zagreb, Rijeka, Varaždin, Osijek and Slavonski Brod) to provide a reasonably representative estimate of the general population immunity. For each specimen age, city of origin and date of collection were gathered. There was no exclusion criterion, therefore sera were collected regardless of vaccinal status, immune status, or history of measles.

Serum survey testing

Serum specimens were stored at −20 °C and transported frozen to the Institute of Immunology Inc., Zagreb, where they were tested for measles-specific IgG antibodies. Commercial enzyme immunoassay was used (Rubeola IgG ELISA, Ref/ Code 4872031/ RBE 100, Gull Ltd., distributed by TRE-GI s.a.s.). Rubeola IgG ELISA is intended for qualitative detection of IgG antibody to measles virus in human serum. For comparisons of data between different test runs, test absorbance values were converted to relative ELISA values (REV). The conversion was done by expressing the test serum absorbance as a ratio to the mean absorbance value of the reference serum. Qualitative IgG results were classified as positive, equivocal or negative according to the REV values: $\geqslant$1.000 positive, 0.901–0.999 equivocal and $\leqslant$0.900 negative. Unfortunately, the sera that tested equivocal have not been retested and for statistical analysis and comparison with WHO criteria, these sera were considered low positive.

Results

The results are summarized in the following few tables and diagrams. In total, 1205 sera were processed, stratified by age groups from the age of one to the age group of 51 years and more, with every group consisting of more than 40 (from 41 to 72) sera (Table 1). Equivocal results were found in 50 participants, with the proportion of equivocals ranging from 0 to 11% by age group. Seroprevalence data according to age and vaccination history of each cohort is shown in Figure 1. The highest proportion of negatives was found in 1-year olds, many of whom probably did not receive their first dose of measles vaccine and surprisingly, in adolescents and young adults (12, 16–30 years) most of whom received two doses of vaccine. In age groups above nine, susceptibility levels are higher than those recommended by WHO Euro. The deviation from the WHO Euro target is higher in age group 10–14 than in the 15+ age group (Figure 2). Despite high susceptibility levels in persons

Table 1. Results of anti-MV testing by age/age group

Age	Positive	Equivocal	Negative	Total
1	51	4	15	70
2	59	0	9	68
3	51	1	7	59
4	48	5	1	54
5	48	4	7	59
6	53	3	3	59
7	39	4	6	49
8	48	0	3	51
9	42	6	5	53
10	41	0	5	46
11	42	3	2	47
12	42	0	10	52
13	52	0	2	54
14	54	3	6	63
15	38	4	5	47
16–20	55	6	11	72
21–30	59	5	12	76
31–40	70	1	9	80
41–50	74	0	0	74
51+	71	1	0	72
Total	1037	50	118	1205

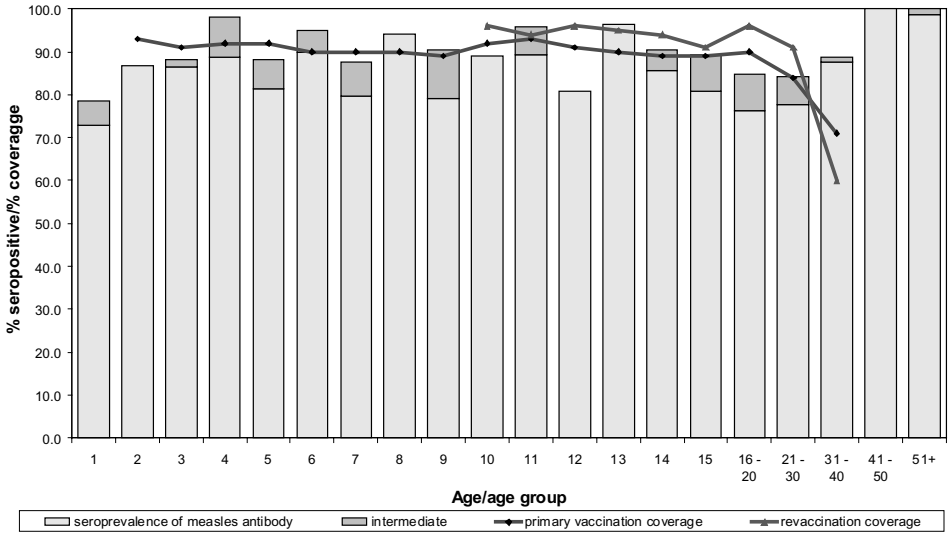

Figure 1. Seroprevalence of measles antibody and vaccination experience of cohorts.

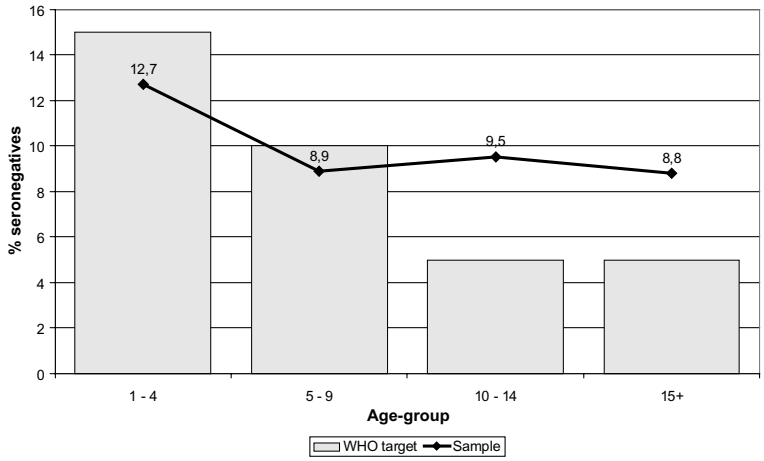

Figure 2. Age-specific susceptibility levels.

above 9 years of age, measles epidemic cycles have been interrupted since 1985 (Figure 3). On the other hand, as a result of the accumulation of susceptibles in persons above 9 years of age, the case age group distribution has changed in the last decade (Figure 4).

Discussion and conclusion

A comment on the size and quality of the sample studied should be given first. The size of the sample was limited by financial reasons, nevertheless, given the size of the population (4.5 million) this sample should be sufficient. Its representativeness is assured by the allocation of participants, which is not exactly at random, but is nearly so. The quality is also guaranteed by the geographical distribution of participants, originating practically from all over the country. For all these reasons the results of this study may be with minimal reservations extrapolated to the population of Croatia.

As for the laboratory technique, it can be said that it has repeatedly proved to be highly specific and sensitive and the manufacturer of the kits is of recognized quality, which both guarantee reproducible and relatively comparable results [11–14]. Unfortunately, standardisation by testing a reference panel was not done. In the absence of standarisation, the classification of equivocal sera into low positives, rather than negatives, is based on the likelyhood that an equivocal result indicates vaning of the antibody level, but not vaning immunity. The first rationale for this conclusion originates in individual reports of measles cases, which contain information on the vaccination status of cases, demonstrating that there are no laboratory confirmed cases of measles who received two doses of measles-containing vaccine. Another rationale for this conclusion arises from the

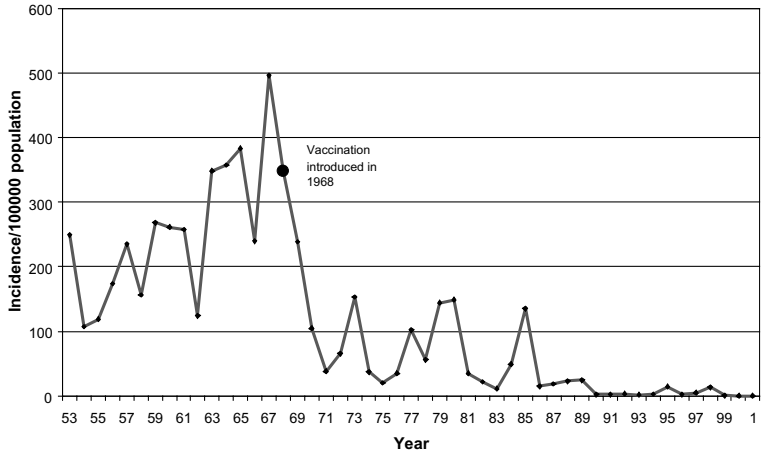

Figure 3. Measles incidence rates per 1,00,000 population in Croatia (1953–2001).

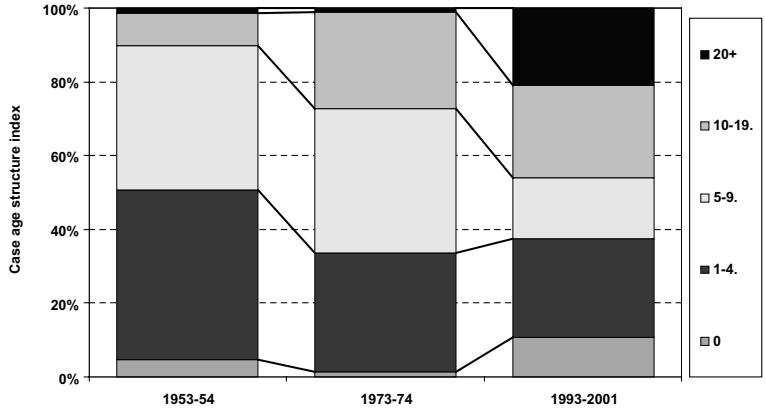

Figure 4. Case age group distribution of measles in Croatia. Comparison of the prevaccination period, postvaccination high incidence period and the latest low incidence period.

distribution of low positives in the population. Namely, the highest proportion of equivocal sera is in 4–9-year old children, who had a lesser chance to be exposed to the boosting effect of natural infection. The absence of this boosting effect has consistently been shown to cause vaning antibody levels in published studies, but the influence of vaning antibody levels on susceptibility to measles infection is not so consistent [15–20].

The results of this survey demonstrate an insufficient level of herd immunity of the Croatian population. Cohorts which received only one dose of measles vaccine (1–9 years), with some 90% vaccination coverage are more susceptible than older age groups. If we take into consideration that up to 5% of vaccines are nonresponders, we cannot expect to find higher immunity levels in these cohorts with the demonstrated vaccination coverage [21, 22]. To deal with their high susceptibility, in year 2001 the second dose of measles vaccine is shifted back to the age of 6 years as it was until 1996. Children who did not receive the second dose in first grade and would not receive it in the sixth grade because of the change in the schedule, are receiving the second dose in the fourth grade. Therefore, at the moment we vaccinate three cohorts of children: 1-year olds, 6-year olds and 9-year olds. This way children who belonged to the first two age groups at the time of the serosurvey (1–4 and 5–9) should reach WHO criteria by 2004, after which revaccination will be continued at the age of six. As for the third age group (10–14 years) with 9.5% of seronegatives, they should have received two doses of measles containing vaccine and at the moment we are not planning catch-up campaigns for these cohorts. This group has a lower proportion of seronegatives than age group 1–4 (9.5 vs. 12.7%, respectively), which might in some extent be due to the fact that this group was eligible for two doses of measles vaccine, but further analysis hampers any effort to bring such conclusions. Namely, age group 5–9 was also eligible to only one dose of vaccine, but still has a lower proportion of seronegatives than age group 10–15 (8.9 vs. 9.5%, respectively. On the other hand, in the oldest age group 15+ susceptibility levels are quite above WHO Euro criteria, but since this group contains a very wide age span, we observe

two very different subgroups within the group: cohorts above the age of 40, with no susceptibility at all, which is due to natural infection alone, and persons 15–40 years of age with high susceptibility to measles (13.5%). Such susceptibility in this age group is not expected because these cohorts were eligible to two doses of measles vaccine and in addition, had a higher chance for contact with wild measles virus than the younger cohorts. It is difficult to interpret the high proportion of seronegatives among persons 15–40 years of age, especially in view of the fact that in a previously performed study involving the vaccine used in our vaccination programme, a significant decline in immunity over time was not observed in the presence of circulating wild measles virus [23]. Nevertheless, we do not plan any supplementary vaccination for these cohorts at the moment. It is worth noting that regardless of the accumulation of susceptibles among adolescents and adults, and the shift in the case age group distribution, measles cases at this age are rare and the total incidence of measles is declining.

We conclude that our priority is to improve vaccination coverage, in primary vaccination as well as revaccination. We should continue to carefully monitor age-specific incidence in order to recognize further changes in the mean age of infection, which could be a signal to consider supplementary vaccination for filling population susceptibility gaps.

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