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SEROPREVALENCE OF MEASLES-SPECIFIC ANTIBODIES IN THE GROUP PREDOMINANTLY AFFECTED BY MEASLES IN 2006-2009 IN POLAND

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ABSTRACT

INTRODUCTION. Irrespective of the high vaccination coverage against measles, sporadic measles outbreaks still occur in Poland. In 2006-2009, a slight increase in the number of measles cases was observed. Of these cases, people born in 1976-1989 were predominantly affected.

AIM. The aim of the study was to evaluate the immunity to measles in the aforesaid age group.

MATERIAL AND METHODS. The serum samples were selected from the serum bank in which material collected from the general population living in 5 provinces in Poland is stored. These samples were collected from patients hospitalized due to emergencies in 2010-2011. The antibody titre against measles was determined in each serum sample by ELISA test (Genzyme Virotech). Linear regression models using log-transformed antibody titres were used to compare the values.

RESULTS. The serum samples collected from 483 persons, including 111 females and 372 males were tested. All patients had antibody titres exceeding 0.5 IU/ml. The antibody titre was statistically significantly associated with the vaccination coverage in each age group in particular province.

CONCLUSIONS. The results of this study suggest good immunity to measles in the general population in Poland. The disparities between randomly selected provinces demonstrated a relation with the coverage rates as well as the differences in measles incidence which is observed recently between these provinces.

Key words: *measles, antibody titre, Polish population*

INTRODUCTION

Measles is an infectious disease, accompanied by i.a. high fever and maculopapular rash. Complications such as otitis media, gastroenteritis and pneumonia are common in the course of measles. Neurological complications are particularly severe conditions, including subacute sclerosing panencephalitis (SSPE) which may occur many years following the infection with measles virus.

Prior to 1974 when the vaccination against measles was introduced, ca 200,000 measles cases were reported annually in Poland. The recommendation to administer the second dose of vaccine, which was introduced to the immunization schedule in 1991, resulted in an increase of the percentage of immune persons which consequently contributed to the stabilization of the epi-

demiological situation of measles. It contributed to the decrease of incidence from 300-400 cases per 100,000 in years preceding the introduction of vaccination to 0.3 per 100,000 in 2001. Furthermore, the periods between the compensatory epidemics began to extend from 2-3 years to 5-7 years prior to and following the introduction of vaccination, respectively. The epidemiological situation in Poland began to be more compatible with the criteria required for a country which is close the elimination of measles. Irrespective of this success, single measles outbreaks involving lower number of cases and being of local character still occur. Simultaneously, the span between the periodical increase of the incidence is extending. Recently, large outbreaks occurred in 1990 and 1998 (56,471 and 2,419 cases, respectively) (1). In 2002, only 34 measles cases were registered. In recent years, a slight increase in the number of measles cases

was reported in Poland: 120, 37, 97 and 115 cases were notified in 2006, 2007, 2008 and 2009, respectively (2, 3, 4, 5). The persons aged 20-30 years were predominantly affected (38 %).

Since many years, the global elimination programme of measles is conducted by WHO. One of the criteria of this programme consists in the achievement of high vaccination coverage in a given population to obtain herd immunity. If the number of immune persons is on the increase, the risk of contracting the disease is decreasing. WHO recommends that the percentage of children vaccinated with one or two doses of vaccine against measles should reach at least 95% and 90% in each country and territorial unit, respectively (6). If herd immunity is achieved, the majority of persons in the population is immune to measles. Therefore, it is feasible to stop the transmission of the measles virus and consequently eliminate this disease (7). Another criterion to eliminate the measles consists in the constant evaluation of susceptibility of persons in given population which enables to assess the risk of disease occurrence. If the susceptibility to measles virus in particular groups living in given territory is higher, it is of importance to conduct catch-up vaccination (8). Serologic testing enables to assess the effectiveness of vaccination campaigns and determine the susceptibility of population (9).

The assessment of susceptibility of Polish population to measles complemented the molecular testing, including genotyping and establishing the relation between wild-type measles viruses circulating in Poland in 2006-2009 (research: NN 404 11 39 39).

MATERIAL AND METHODS

Serum samples. Study sample was composed of 483 serum samples collected from healthy persons aged 20-30 years in 2006-2009. These persons were born between 1976-1989. The samples were obtained from the serum bank formed within the research "Prevalence of HCV infections with utility analysis of screening" (research: NN 404 19 16 36). In 2010-2011, serum samples from patients hospitalized on trauma-orthopedic and surgical wards in Lubelskie, Mazowieckie, Świętokrzyskie, Warmińsko-mazurskie and Wielkopolskie provinces and laryngological ward in Mazowieckie province were collected. The hospitals were selected randomly from the Health Care Units Register.

The patients who met the following criteria were offered to participate in the study:

- Females and males aged 18+ admitted to wards due to emergencies (accidents, injuries) or hospitalized due to appendicitis, hernia and cholecystitis.
- Patients with preserved consciousness at the day of study.

- Patients who signed an informed consent to participate in the study.

The cluster sampling was applied and 21 hospital wards located in 5 aforesaid provinces were selected.

As a reference sample, 2nd International Standard Anti-Measles Serum was used, containing 10 international units (IU) in 1 ml, code 66/202, which was accepted by the National Institute for Biological Standards and Control in Great Britain (10).

Detection of measles-specific IgG antibodies. IgG antibody titre to measles virus in serum was determined by ELISA method pursuant to the instruction of test producers Genzyme Virotech GmbH. ELISA test results were presented in IU/ml, based on the results of the 2nd International Standard Anti-Measles Serum.

Data on the vaccination coverage of the Polish general population. The data on the number and percentage of Polish general population vaccinated against measles in 1976-1985 in provinces enumerated in the table are presented in "Report on the vaccination coverage of children, adolescents and adults" (MZ/E II-8), collected by the Ministry of Health. The data concerning persons born in 1985-1989 were published by NIH in annual bulletin "Vaccinations in Poland" (MZ 54).

Analysis of epidemiological data. Arithmetic mean, standard deviation, mean and interquartile range were used to present numerical variables. Spearman's rank correlation was used to assess the relation between variables. The comparison between numerical variables in two or more groups included the confrontation of arithmetic means (exclusively for variables of normal distribution) by student's t-test or ANOVA test. The variables which were not normally distributed were compared using Mann-Whitney -Wilcoxon test and Kolmogorov-Smirnov test. The effect of vaccination coverage (by province and date of birth) on the mean antibody titres against measles was assessed using linear regression with multiple exogenous variables. Due to the type of data, in addition to the analysis of antibody titre, the log-transformed values were tested as the endogenous variables. As the majority of authors consider the protective level against measles to be 0.5 IU/ml, the proportions of persons with low (0.5 IU/ml - 1IU/ml) and very high titres, 10-fold higher compared to protective level (>5IU/ml) were analyzed.

RESULTS

Characteristics of antibody titres against measles virus in the study population. The study sample comprised 483 persons, including 111 females and 372 males. The antibody titre against measles virus was obtained for all participants. The mean antibody titre was: 1.77+/-0.90 IU/ml, median: 1.532 IU/ml, interquartile

Table I. Results of serologic testing for measles virus (IU/ml) by province, gender and date of birth.

Variable		Number (%)	Mean (\pm SD) (IU/ml)	Median (IU/ml)
Province	Lubelskie*	132 (27.3%)	1.9072	1.6715
	Mazowieckie	131 (27.1%)	1.79135	1.478
	Świętokrzyskie*	104 (21.7%)	1.6201	1.4105
	Warmińsko-mazurskie	55 (11.4%)	1.77827	1.568
	Wielkopolskie	61 (12.6%)	1.67762	1.514
Gender	females	111 (77%)	1.88762	1.649
	males	372 (23%)	1.73527	1.487
Date of birth	1976 - 1978	99 (20.5%)	1.79355	1.451
	1979-1981	98 (20.3%)	1.73413	1.4465
	1982 - 1984	100 (20.7%)	1.70966	1.469
	1985 - 1987	123 (25.5%)	1.65163	1.541
	1988 – 1989*	63 (13%)	2.11786	1.964
	Total	483 (100%)	1.77+/-0.89638	1.532

*p=0.0199

x –significant difference in Multiple Range Tests based on 95% LSD

range: 1.053, range: 5.067. The results were presented in Table I. Standard deviation exceeding 50% of mean value suggest skewed distribution. Thus, having considered as insignificant, it was not presented in other elements of Table I.

The difference between results in Lubelskie and Świętokrzyskie provinces was found to be statistically significant. The higher values were observed in the youngest group (born in 1988 – 1989).

Effect of the number of measles cases and vaccination coverage of the population on the antibody titre against measles virus. In the linear regression model accepted, logarithm of antibody titre was endogenous variable while the exogenous variables were: categorical variable: province of residence and continuous variables: date of birth, percentage of persons vaccinated with 1 dose in given year group living in particular province, percentage of persons vaccinated with 2 doses in given year group living in particular province and percentage of unvaccinated persons (Tab. II).

Based on the study results, it was concluded that all of the analyzed variables had an effect on the antibody titre in the population. The greatest impact was attributed to the percentage of unvaccinated persons while the vaccinations with 1 or 2 doses of vaccine were of

comparable effect. Furthermore, it was stated that the earlier the date of birth of persons subject to analysis, the poorer the humoral immune response is. The differences between the percentages of persons immune to measles in particular provinces were also observed. In Lubelskie province, the measles-specific IgG antibody titre was the highest.

The discussed model explicate more than 80% variability of results in serologic testing ($R^2=80.3167\%$).

Measles-specific antibody titre in particular age groups and provinces. Out of the study sample, in 87 (18%) and 2 (1%) persons very low (0.5IU/ml – 1 IU/ml) and high (>5IU/ml) protective levels were detected, respectively. Figures 1 and 2 present the distribution by province and date of birth.

The lowest number of persons with very low IgG antibody titre was detected in Lubelskie province while the highest in Świętokrzyskie province. Furthermore, the persons with the highest immunity: from 5 to 6 IU/ml were observed in Lubelskie province.

Similar analysis was performed by grouping the study sample according to their date of birth.

It was stated that the highest percentage of persons with high immunity (at least doubly exceeding the protective level) occur in early age groups (1976-1983).

Table II. Results of measles-specific antibody titre by date of birth, vaccination status against measles and province.

Analyzed variable	Value	Standard error	95% confidence interval		P ₀
			Maximum limit of value	Minimum limit of value	
Date of birth (continuous variable in years)	-0.03	0.01	-0.02	-0.05	0.0005
% of unvaccinated persons (continuous variable in percentage points)	0.70	0.19	1.09	0.33	0.0003
% of persons vaccinated with 1 dose (continuous variable in percentage points)	0.71	0.20	1.11	0.31	0.0004
% of persons vaccinated with 2 doses (continuous variable in percentage points)	0.71	0.20	1.11	0.32	0.0004
Provinces	-0.6	0.03	-0.12	-0.12	0.0181

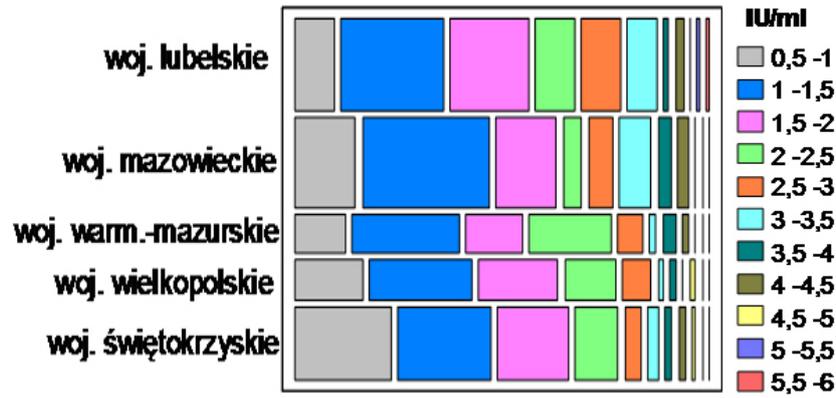


Fig. 1 Measles-specific antibody titre in the provinces as a multiple of 0.5 IU / ml, which is considered to be protective against measles.

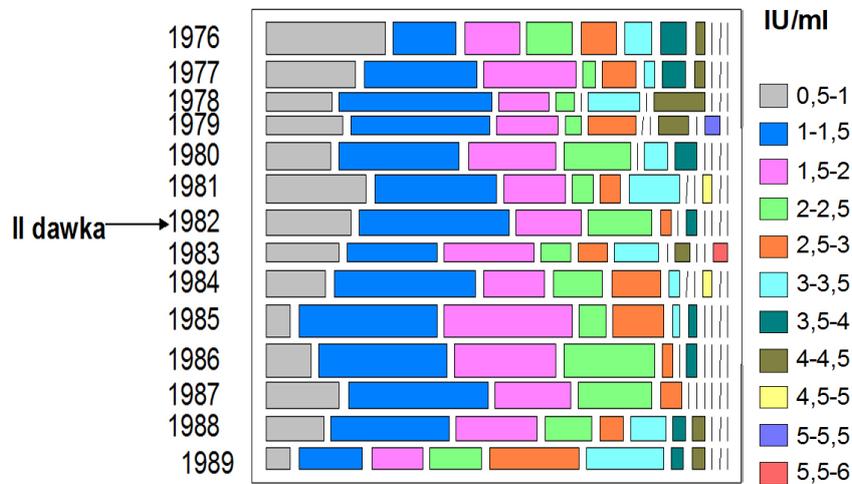


Fig. 2 Measles-specific antibody titre in particular age groups as a multiple of 0.5 IU / ml, which is considered to be protective against measles.

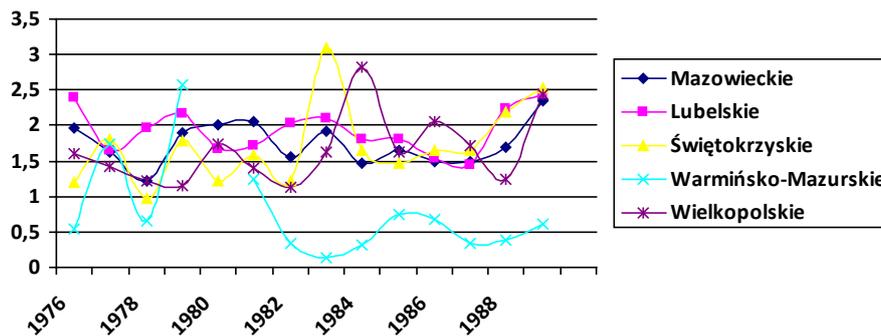


Fig. 3 Measles-specific antibody titre in persons born in 1976-1989 in particular provinces.

Simultaneously, relatively high number of results of the lowest antibody titre was noted in this group. It was claimed that beginning from 1984, the lowest antibody titre (0.5-1 IU/ml) occur less often compared to persons born earlier. In persons born later, the distribution of results is more balanced (Fig. 2).

The antibody titre in particular age groups demonstrates statistically significant differences ($P_0 = 0.0041$) between particular provinces (Fig. 3). The lowest antibody titre against measles virus was noted

in Warmińsko-mazurskie province in persons born after 1982.

DISCUSSION

This paper presents the results of serologic testing for measles in healthy persons aged 20-30 years in 2006-2009, i.e. born between 1976-1989. Persons living in the following provinces: Świętokrzyskie, Wielkopolskie,

Warmińsko-mazurskie, Mazowieckie and Lubelskie were tested. The results of serologic survey suggest that high percentage of Polish population has antibody titre which is sufficient enough to protect them against measles.

In Poland, serologic surveys for measles have been already performed several times in the 90s. in which the following age groups: 1984-1989 and 1997 were enrolled. The observed immunity of the population was significantly lower. Probably, such discrepancy resulted from different methodology adopted. In previous years, the authors used haemagglutination inhibition of monkey erythrocytes by virus antigen and sera with titres $\geq 1:8$ were considered to be positive (11). While the antibodies detected by HI test bind to the virus areas vital for recognizing the cell receptors, ELISA enables to detect antibodies for all virus antigens with prevailing epitopes of nucleoprotein. Thus, antibodies detected by HI test are correlated with neutralization of virus while there is no such relation for the antibodies detected by ELISA. They exclusively confirm the contact with antigens of measles virus or other related viruses. For instance, sera stimulated by canine distemper virus react with antigens of measles virus when ELISA is used contrary to HI test. The highest crossreactivity is observed for nucleocapsid of all morbilliviruses (12).

Based on the results of other authors, serum samples with measles antibody titres exceeding 0.5 IU/ml were considered to positive in the present paper. The results of study conducted by Janaszek et al. suggest that antibody titres below aforesaid value may not be sufficient enough to protect against measles (13). Antibody titre equal to 0.5 IU/ml determined by ELISA corresponds to titre between 1:8 and 1:16 by HI test (14). Having converted the results of serology from units of ELISA test producer to international units (IU/ml), none of the sera tested had measles-specific antibodies titre below 0.5 IU/ml.

Thus, none of the study participants were susceptible to the infection with measles virus. Such observation corresponds to the current epidemiological situation where relatively sporadic infections with measles virus are observed (incidence ranging from 0.44 per 100,00 in 2007 to 2.02 per 100,000 in 2009).

Low number of measles cases as well as slight percentage of persons with low antibody titre measured in international units (lack of titres below 0.5 IU/ml) suggest that vaccination is highly effective method and the percentage of unvaccinated persons is rather low (below the detectability threshold for population size tested in the present serologic survey and described in this paper). However, high infectivity of measles virus results in the situation in which even if there are few persons susceptible to infection, measles may still be present in Poland.

The level of antibodies against measles in the analyzed group revealed a relation between the percentage of unvaccinated persons, vaccination coverage in given province and date of birth. For older age groups, the antibody titres were lower ($P_0 = 0.0005$), while the higher vaccination coverage corresponded to higher level of antibodies. Worth mentioning is the fact that the percentage of persons unvaccinated with measles vaccine also had a positive effect on the antibody titres in the general population. Having contracted measles, such persons have higher humoral immunity response compared to those with vaccine-induced immunity (15).

In the majority of provinces, the analysis of serologic response did not reveal any statistically significant differences, excluding Lubelskie province where the lowest number of persons with low antibody titres as well as the highest number of persons with the highest antibody titres to measles virus were observed. Of all provinces from which the serum samples were collected for serologic survey, it was only one province in which the higher number of measles cases occurred, especially in Roma population (16). It may suggest to what extent natural infections by affecting surrounding population play a role in sustaining high antibody level (booster effect).

In Warmińsko-mazurskie province where the lowest titres of antibodies against measles virus were observed, no measles cases were reported in 2006-2009.

In this paper, it was proved that the measles antibody titres are decreasing with the age of study participants. It is pursuant to the observations of other authors which suggest that antibody titres may decrease to undetectable level within 15 years following vaccination (15). It was also noted that in children who do not have detectable level of antibodies by HI test after 1-5 years from vaccination, neutralizing antibodies are still present (17). Several authors claim that the titre of antibodies against measles virus decreases by 6.5% annually which suggests that its half-life amounts to ca 12 years (18). It is presumed that vaccine-induced antibodies persistence is dependent on the genetic, environmental and demographic factors (19).

Due to memory B lymphocytes which produce antibodies following each contact with virus antigen, the waning of antibodies may not necessarily be interpreted as the loss of immunity to measles (20, 21). Repeated contacts with virus circulating in the environment stimulate the immunological system to another humoral response. It occurs in the course of asymptomatic infection with pathogen or related pathogens (20). It should be emphasized that cell-mediated immunity plays an important role in measles infection while the antibodies are the evidence of virus antigenic stimulation.

CONCLUSIONS

From the results of serologic survey transpires that the high percentage of persons born in 1976-1989 in Poland have antibody titre which protects against measles. The high immunity is affected by both vaccinations and number of measles cases.

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