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ETIOLOGY OF MEASLES SUSPECT CASES REPORTED IN 2006-2007 IN POLAND

ETIOLOGIA PRZYPADKÓW ZGŁASZANYCH JAKO PODEJRZENIA ODRY W POLSCE W LATACH 2006-2007

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STRESZCZENIE

Realizacja Programu Eliminacji Odry wymaga skutecznego systemu nadzoru nad zachorowaniami przebiegającymi z wysypką. Celem prezentowanej pracy było ustalenie, które wirusy, oprócz wirusa odry, powodują zachorowania wysypkowe (*measles-like illnesses* - MLIs) w Polsce. W próbkach surowic (n=278) pobranych w latach 2006-2007 od osób z podejrzeniem MLI i przesłanych do Krajowego Laboratorium Referencyjnego oznaczono obecność przeciwciał w klasie IgM dla wirusa odry (MeV), różyczki (RUBV), parwowirusa B19 (B19V), wirusa Epstein'a-Barr (EBV) i herpeswirusa typu 6 (HHV-6). Zaobserwowano, że etiologia przypadków MLI była ściśle związana z wiekiem badanych osób. W najmłodszej grupie wieku (0-4 lata) dominującym zakażeniem było MeV i HHV-6, podczas gdy w grupie 5-9 lat – RUBV i B19V. Potwierdzenie laboratoryjne odry uzyskiwano znamiennie częściej u pacjentów z wysoką gorączką ($p<0,001$) i wysypką utrzymującą się dłużej niż 5 dni ($p<0,001$). Rodzaj obserwowanej wysypki nie miał istotnego związku z obecnością przeciwciał anty- MeV IgM. Uzyskane wyniki wskazują, że zgodnie z założeniami planu strategicznego WHO EURO, Polska jest bliska fazy eliminacji zachorowań na odrę. Etiologia znacznej liczby przypadków MLIs nie była związana z wirusem odry. Włączenie oznaczeń obecności przeciwciał anty--B19V IgM do rutynowego protokołu badań laboratoryjnych MLIs mogłoby poprawić nadzór w Programie Eliminacji Odry.

Słowa kluczowe*: choroby wysypkowe, etiologia wirusowa, Program Eliminacji Odry*

ABSTRACT

To achieve measles elimination, an efficient surveillance system for rash illnesses is necessary. The aim of the present study was to ascertain which viruses, other than measles, are causing measles-like illnesses (MLIs) in Poland**.** Serum samples (n=278) collected from MLI cases and submitted to the National Reference Laboratory during 2006-2007 were investigated for anti-measles (MeV), rubella (RUBV), parvovirus B19 (B19V), Epstein-Barr (EBV) and herpesvirus type-6 (HHV-6) IgM presence. Age was strongly associated with MLIs etiology. In the youngest age group, 0-4 years, MeV and HHV-6 infection were prevailing, while in group of 5-9 years – RUBV and B19V. Measles was confirmed more often in patients with high fever ($p<0.001$) and with rash lasting longer than 5 days ($p<0.001$). The type of rash was not significantly associated with MeV infection. Our results strongly suggest that according to WHO EURO strategic plan, Poland is close to elimination phase. High number of MLIs were caused by pathogens other than measles. Addition of anti-B19V IgM testing to routine MLIs screening protocol may improve system performance in the more advanced stages of measles elimination.

Key words: *rash illness, viral etiology, measles elimination programme*

INTRODUCTION

Strategic plan for measles and congenital rubella infection for WHO European Region assumes their elimination by 2010 (1). The success of this plan depends on efficiently operating surveillance of measles suspect cases. The system aims to document measles elimination by screening a certain number of rash-febrile illnesses, reported as measles suspicions. When measles is still common in a country, a high proportion of measles suspect cases will be confirmed as measles. In a phase of disease elimination, a decreasing number of measles within all measles-like illness (MLI) cases is observed and most of them will be caused by other infectious and non-infectious factors. Despite the clinical course of classic measles include quite characteristic symptoms (Koplik spots, characteristic maculo-papular rash with high fever), in the elimination phase atypical course of disease is increasingly observed (2). To assure appropriate surveillance performance, WHO developed a series of indicators. One of the most important is screening of at least 2 cases per 100,000 population in each country, with minimum 80% sub-national administrative districts achieving this goal.

In Poland, measles-like illness cases (MLI) surveillance was established in 1998. Physicians are required to report suspect measles cases to Territorial Health Departments and to obtain samples for confirmatory testing. An interview is collected from each case, compatible with WHO measles case investigation form. Confirmatory IgM testing is required for all MLIs. Currently, the only laboratory in Poland accredited by WHO for serologic testing within the enhanced rash illness surveillance is located in the National Institute of Public Health (NIPH).

In view of the goals of enhanced surveillance, it is important to understand which etiological factors, other than measles, are most often reported as MLI (3). The Australian example shows, that following a thorough review of most common etiologies of rash-febrile illnesses, significant improvement of MLI surveillance was achieved by adding parvovirus B19 testing to measles and rubella (4, 5).

The aim of this work was to ascertain which viral etiological factors, other than measles, are causing measles-like illnesses in Poland, in a phase close to elimination, based on samples tested during 2006-2007.

MATERIAL AND METHODS

The material used in the present study were serum samples collected from MLI cases and submitted to the National WHO Measles/Rubella Laboratory during 2006-2007. The sera were investigated for the presence of anti-measles virus (MeV) and anti-rubella virus (RUBV) IgM antibodies with the Enzygnost Anti-Measles-Virus/IgM (Simens, formerly DadeBehring; Germany) and ETI-RUBEC-M reverse PLUS (DiaSorin; Italy) enzyme-immunoassay (EIA) tests. As a part of the WHO elimination strategy, assessment of the serologic confirmation of MeV and RUBV is regularly performed by means of external quality assurance organized by Regional WHO Measles/Rubella Laboratory at the Robert Koch Institute (Berlin, Germany). Following routine testing for MeV and RUBV, the samples were aliquoted and stored at -70°C for further determinations. Samples were subsequently tested for anti-parvovirus B19 (B19V) IgM presence. Anti-B19V antibodies were detected by Parvovirus B19 IgM Enzyme Immunoassay (Biotrin International Ltd.; Ireland). This test is based on the μ-capture sandwich technique, where total serum class M antibodies are immobilized onto microtiter wells and pathogen specific IgM is detected using labeled viral protein. This technique allows avoiding non-specific reactions. If no etiologic factor was confirmed in the first phase of virological investigation, the samples were additionally tested for anti-Epstein-Barr virus (EBV) IgM using ETI-EBV-M reverse (DiaSorin; Italy) EIA test. As in the case of parvovirus B19 test, this test was using the μ-capture sandwich technique. In all EIA tests, positivity was determined by comparison with a reference serum (cut-off control) always running in duplicate. Results above the grey zone (+/- 10% of cut-off value) were defined as positive, results below as negative, and results within grey zone as equivocal. All tests were performed under the same laboratory conditions using an automatic-open system (ETI Star, Italy).

In sera negative for the four above-mentioned viruses, anti-human herpesvirus type 6 (HHV-6) IgM was evaluated with Human Herpesvirus-6 IgM Immunofluorescent Assay (Biotrin International Ltd.; Ireland) by qualitative immunofluorescence (IF) technique. Serum samples were incubated with HHV-6 infected cells immobilized on a glass slides, and then stained with a fluorescein conjugated goat-anti-human IgM. Slides were examined under a fluorescent microscope. A HHV-6 IgM positive reaction was denoted when bright green fluorescence was observed (fluorescent intensity $\geq$+2). Assay results were considered valid if Positive Control was also yielded a fluorescent intensity $\geq$+2 and Negative Control was yielded no specific fluorescence. Serum samples indicated weak anti-HHV-6 IgM responce (fluorescent intensity $\leq$+1) were retested.

The information about socio-demographic, clinical and epidemiological factors was obtained from MLI case reports, routinely collected for each notified MLI case. Stata ver. 9 was used in the analysis.

RESULTS

Socio-demographic characteristics of the studied population are presented in table I. Out of 278 tested MLI samples, 83 (29.9%) were anti-MeV IgM positive, 67 (24.1%) were positive for anti-RUBV IgM, and 15 (5.4%) for anti-B19V IgM. Of 115 samples negative for anti-MeV, RUBV and B19V IgM, 5 (4.3%) were positive for anti-EBV IgM. Of 110 samples negative for anti-MeV, RUBV, B19V and EBV IgM, 12 (10.9%) were positive for anti-HHV-6 IgM (table II). Equivocal results which were obtained for anti-MeV (n=9), anti-RUBV (n=2) and anti-B19V (n=1) testing were excluded from final analyses. Samples positive for two or more viruses were obtained from 2 persons. In an unvaccinated 37-year old female, with papular rash covering the entire body and lasting 7 days, accompanied by cough, runny nose and sore throat, concomitant anti-MeV and anti-RUBV antibodies were detected. Samples from this subject were taken 17 days after symptom onset. The course of the illness in that patient necessitated hospitalization. Another subject was a 24-year old male, with 1-day lasting macular rash covering the trunk and upper limbs, accompanied by short-lived high fever, but no other symptoms. This person was anti-MeV, RUBV and B19V IgM positive. He received one dose of monovalent measles vaccine at the age of 2 years. Serum sample was collected at 8th day after symptom onset. He was not hospitalized, and the samples were taken by a general practitioner.

Table I. Description of the measles-like illness cases reported to the Polish enhanced measles surveillance in 2006-2007 and enrolled in this study

Tabela I. Charakterystyka zachorowań przebiegających z wysypką zgłaszanych jako podejrzenia odry w ramach Programu Eliminacji Odry WHO w Polsce w latach 2006-2007, włączonych do obecnego badania

Characteristic	Number	Percent
Gender		
Males	164	59.0%
Females	114	41.0%
Age group		
0-4	49	17.6%
5-9	36	12.9%
10-14	29	10.4%
15-19	32	11.5%
20-24	41	14.7%
25-29	55	19.8%
>30	36	12.9%
Residence type		
Urban	187	67.3%
Rural	91	32.7%
TOTAL	**278**	**100%**

Age was strongly associated with the etiology of reported MLIs (fig. 1 and table II). Presence of IgM specific for particular viruses was significantly associated with age (for all viruses $p<0.0001$), except for HHV-6 ($p=0.116$).

In the youngest age group, 0-4 years, MeV and HHV-6 infection were prevailing, while in group of 5-9 years – RUBV and B19V. In the remaining cases, MeV and RUBV infection were prevailing. In the age groups 10-19 the vast majority of cases were confirmed as RUBV (34 cases, 55.7%). In contrast, in the age groups 20+ MeV was predominantly detected (65 cases, 48.5%).

Table II presents the results of confirmatory testing in relation to clinical and epidemiological factors. Measles was confirmed more often in patients with high fever ($p<0.001$) with rash lasting longer than 5 days ($p<0.001$), and in patients who were either unvaccinated or incompletely vaccinated with the measles vaccine. The type of rash was not significantly associated with anti-MeV IgM, the most commonly observed type of rash in cases confirmed as measles were: maculo-papular and papular rash. Rubella and parvovirus B19 were confirmed more commonly among persons vaccinated against measles. Another factor significantly associated with anti-RUBV IgM presence was rash lasting 3-5 days ($p<0.001$). The type of rash or the presence or absence of high fever did not influence the probability of anti-RUBV IgM detection; non-specific types of rash were most commonly seen. Parvovirus B19 confirmations were significantly associated with absence of high fever ($p=0.001$). No studied factors have influenced detection of neither anti-HHV-6 IgM nor anti-EBV IgM in the studied population.

DISCUSSION

The limitation of the present study could be insufficient representativeness of the screened population. First of all, not all physicians report measles suspect cases within the Polish enhanced MLI surveillance. The WHO surveillance indicator (2 cases of rash illness per 100,000 population) was met only in one province during 2006-2007. To meet the above sensitive indicator, physicians would have to report for virological investigation a minimum of 760 cases of febrile-rash illnesses each year. In 2006-2007, a total of 438 MLI cases were reported, of which 387 were tested serologically. In the studied period 295 samples were sent to the National Reference Laboratory at the National Institute of Public Health, the remaining 92 samples were tested in the Provincial Sanitary Stations (90 samples) and in private laboratories (2 samples). Comparison of

Table II. Results of confirmatory testing (anti-IgM presence) of rash illnesses in Poland, 2006-2007
Tabela II. Wyniki oznaczeń obecności przeciwciał klasy IgM u osób z wysypką w Polsce, 2006-2007

Characteristic	Samples tested	MeV positive n (%)	RUBV positive n (%)	B19V positive n (%)	Tested for EBV		Tested for HHV-6	
					No samples	n (%) positive	No samples	n (%) positive
Age group								
0-4	49	12 (24.5%)	0 (0.0%)	2 (4.1%)	35	0 (0.0%)	35	7 (20.0%)
5-9	36	1 (2.8%)	6 (16.7%)	10 (27.8%)	19	0 (0.0%)	19	0 (0.0%)
10-14	29	2 (6.9%)	15 (51.7%)	1 (3.4%)	11	0 (0.0%)	11	1 (9.1%)
15-19	32	3 (9.4%)	19 (59.4%)	0 (0.0%)	10	3 (30.0%)	7	0 (0.0%)
20-24	41	14 (34.1%)	14 (34.1%)	0 (0.0%)	14	0 (0.0%)	14	3 (21.4%)
25-29	55	39 (70.9%)	8 (14.5%)	0 (0.0%)	8	2 (25.0%)	6	1 (16.7%)
>30	36	12 (33.3%)	5 (13.9%)	2 (5.6%)	18	0 (0.0%)	18	0 (0.0%)
Rash characteristic								
maculo-papular	93	32 (34.4%)	15 (16.1%)	8 (8.6%)	38	2 (5.3%)	36	4 (11.1%)
macular	116	31 (26.7%)	30 (25.9%)	3 (2.6%)	53	3 (5.7%)	50	5 (10.0%)
papular	21	10 (47.6%)	5 (23.8%)	1 (4.8%)	6	0 (0.0%)	6	1 (16.7%)
other	48	10 (20.8%)	17 (35.4%)	3 (6.3%)	18	0 (0.0%)	18	2 (11.1%)
Rash length								
<3 days	26	4 (15.4%)	7 (26.9%)	1 (3.8%)	15	1 (6.7%)	14	1 (7.1%)
3-5 days	133	23 (17.3%)	45 (33.8%)	10 (7.5%)	55	3 (5.5%)	52	9 (17.3%)
>5 days	113	56 (49.6%)	13 (11.5%)	4 (3.5%)	41	0 (0.0%)	41	2 (4.9%)
Fever								
<38,0	65	5 (7.7%)	24 (36.9%)	9 (13.8%)	28	3 (10.7%)	25	2 (8.0%)
≥38,0	213	78 (36.6%)	43 (20.2%)	6 (2.8%)	87	2 (2.3%)	85	10 (11.8%)
Measles vaccination status								
vaccinated acc. do schedule	119	3 (2.5%)	46 (38.7%)	13 (10.9%)	57	1 (1.8%)	56	6 (10.7%)
incompletely vaccinated	31	16 (51.6%)	7 (22.6%)	0 (0.0%)	9	1 (11.1%)	8	1 (12.5%)
not vaccinated	128	64 (50.0%)	14 (10.9%)	2 (1.6%)	49	3 (6.1%)	46	5 (10.9%)
TOTAL	**278**	**83 (29.9%)**	**67 (24.1%)**	**15 (5.4%)**	**115**	**5 (4.3%)**	**110**	**12 (10.9%)**

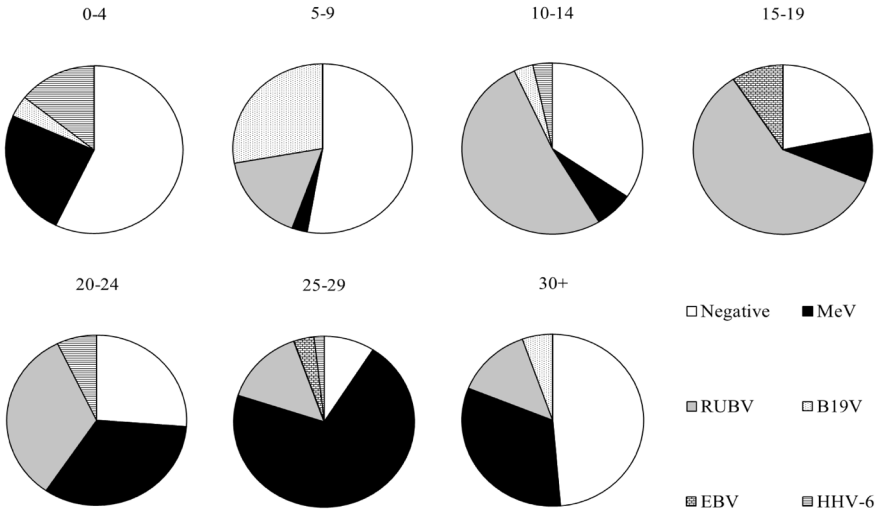

Fig. 1. Distribution of cases positive for anti-IgM presence of particular viruses by age group, Poland, 2006-2007.
Ryc. 1. Rozkład w grupach wieku przypadków zachorowań wysypkowych potwierdzonych etiologicznie, Polska, 2006-2007

the group enrolled in this study with remaining cases indicate however similar socio-demographic patterns.

Another limitation of the present analysis is possibility of false laboratory results. If considering potential cross-reactivity, we paid particular attention to this problem, especially in context of anti-B19V IgM testing. Before this study we made evaluation of 3 different, commercially available EIAs (6) and as a result, the

only parvovirus B19 diagnosic test that has been cleared by US Food and Drug Agency was used in present study (7). Furthermore, for anti-RUBV and EBV IgM detection, technique allowing avoiding non-specific reactions was used. In MeV, RUBV and B19V infections, simultaneous reactivity against more than one virus was observed in previous studies, necessitating confirmation of positive results by IgG avidity (8) or testing convalescent samples taken 3-4 weeks after rash onset (9). It should be pointed out, that in the study presented here only 2 persons had IgM antibodies specific for two or three viruses. However we did not undertake further tests in order to confirm these results, it could be connected with mixed infection as such situation were observed by others (10). Futhermore, while the vacination in Poland was performed in 13-14 months old (1st dose) and 10 year old (2nd dose) children, the detected IgM antibodies would be connected with vaccination. It should be pointed that we observed presence of anti-MeV IgM in only one child aged below 5 years who was vaccinated 4 months before illness.

Out of 278 samples included in the study, etiologic factor was identified in 180 (64.7%) cases. Our results are similar to those reported from other countries where etiology of more than 50% rash-febrile illness cases could be confirmed. In a Brazilian study 71% cases with rash, viral etiology was confirmed, with Dengue virus as the principal cause (11). But in Somalia for instance only one third cases of febrile illiness could be identified as caused by viral, mycoplasmal and rickettsial etiology (12). In our study more than half of screened patients were confirmed as measles or rubella. The remaining 35% of cases reported as measles suspicions, were caused by other, probably non-viral etiologic agents. These possibly include bacterial (streptococci, rikettsiae), parasitic or non-infectious (allergic, autoimmune) aetiologies.

As Poland approaches elimination of measles, decreasing contribution of MeV as a cause of febrile-rash illnesses is expected. Furthermore, as revealed by molecular studies, the high number of confirmed measles cases in Poland were imported from other regions (13, 14). Age was strongly associated with the etiology of reported MLIs. As expected, our results shown that measles is more often observed in children below the age of vaccination (13-15 months), and adults above 20 years of age. The high number of measles in adults above 20 years of age recorded in 2006-2007, confirms the effectiveness of 2-dose vaccination which started in Poland in 1991 (15). Present results indicate that rubella virus was causing rash illnesses mostly among school-aged children. This could be expected as 2-dose immunization against rubella was initiated in Poland in 2005. Rubella is still a childhood disease, with highest incidence recorded among 10-14 year old children (16). Parvovirus B19 infections are usually occurring in the youngest age groups, and most persons in Poland have contact with the virus by the age of 20, as documented in a seroprevalence study from 2006 (17). Within MLI cases presented in this study, B19V infection were occurred the most frequently in children aged 5-9 years. Age distribution of cases caused by other etiological factors is more difficult to interpret due to small numbers.

Comparison of clinical symptoms related to diseases caused by different viral pathogens confirms the well-known phenomenon of unspecific measles course in a highly vaccinated society. We observed high number of cases that did not represented typical course of measles such as maculo-papular rash lasting longer than 3 days. On the other hand, the most of unspecific rash illnesses with low fever were caused by other then MeV viruses.

Our results strongly suggest that according to WHO EURO strategic plan for measles and congenital infection, Poland is close to elimination phase. High number of measles-like illness were caused by pathogens other than measles (RUBV, B19V, EBV, HHV-6). Addition of anti-B19V IgM testing to routine MLI screening protocol may improve system performance in the more advanced stages of measles elimination.

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