PERIODICUM BIOLOGORUM
VOL. 114, No 2, 149–166, 2012

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Overview

Childhood vaccinations in Croatia

GORAN TEŠOVIĆ

Head, Pediatric Infectious Diseases Department
University Hospital for Infectious Diseases
University of Zagreb School of Medicine
E-mail: gtesovic@bfm.hr

Abstract

The vaccination of children is an extremely important public health measure which significantly reduced the morbidity and mortality from various infectious diseases in the last sixty years in Croatia. The Childhood Vaccination Program in Croatia is based on mandatory vaccinations that are purchased by the state free of charge. Each year the program is announced by the Minister of Health based on the recommendations by the Croatian National Institute of Public Health. Today all Croatian children are compulsory vaccinated against ten different infectious diseases. Although the program has experienced significant quality improvements in the recent years, including the introduction of modern, combination vaccines, room for further improvement and inclusion of new vaccines, despite the complex economic situation, certainly exists.

Received July 12, 2012.

INTRODUCTION

Vaccines have been an indispensable and generally cost-effective tool in controlling infectious diseases for more than a century *(1)*. Since their introduction, vaccines have played a leading role in the process of reducing and eliminating vaccine-preventable diseases thus significantly diminishing human suffering and death. Thanks to active immunization around 2 million deaths globally have been averted every year during the last decade *(2)*. Dynamic developments in the field of vaccine manufacturing in the last 60 years with the introduction of numerous new effective vaccines not only contributed to the improvement of general health and quality of life but also set the tasks to medical professionals and regulatory bodies responsible for the implementation of active immunization and approval of vaccination program changes *(3)*. The number of vaccines that are mandatory or recommended for use in pediatric age almost doubled during the last twenty years *(3)*. The incorporation of any new vaccine into already crowded immunization schedule is a challenging task. The fact that nowadays the application of practically all vaccines requires parenteral route of administration makes this process even more complicated *(4)*. However, not only technical issues pose a difficulty in modernisation and expansion of current vaccination schedules. With immunization programs long established and accepted by health professionals and the public, reasons for changes have to be well thought-out and documented. Taking into account primary care facilities and vaccine availability, demonstrable public health benefit should be the only professionally and scientifically acceptable reason for making changes *(5)*. High safety requirements, respect for human rights as well as strengthening of antivaccinal movements and the presence of false interpretation of vaccinal side effects further aggravate the process of

changing the current vaccination practice, especially in developed countries *(5)*.

Many vaccines whose long lasting general use was meritorious for a significant reduction in the morbidity and mortality caused by infectious diseases were developed with scarce knowledge on antigenic and immunological interplay between vaccine and vaccinee *(6)*. Effectiveness in the protection against clinical disease and safely application have been the essential criteria for implementing a novel vaccine in the vaccination schedule *(6)*. Modern vaccine development relies on better understanding of the etiology, epidemiology and pathogenesis of target disease, as well as the target population *(7)*. Broadening the knowledge in immunobiology is essential for the creation of effective and protective new vaccines (8). Vaccine design builds on a principal concept of induction of protective immunity against specific pathogen through mimicking of naturally occurring immune response without inducing the disease *(8)*. In contrast to older vaccines based largely on whole microbial pathogens, and to a lesser extent on bacterial toxoids, the new ones are composed with limited number of highly purified antigens/epitopes *(8)*. Although »historical vaccines« (some of them like BCG, OPV and MMR are still on the market) supply a broad repertoire of different epitopes to the macroorganism of which a certain number provoke immunological response that is not essential for the protection against the disease, they in general ensure protective immunity that could be measured through a significant reduction of morbidity caused by infections the vaccines were designed against *(8)*. On the other hand, designing the new, on specific epitopes »concentrated« vaccines, may pose the risk of insufficient interaction with individuals missing the adequate immune-receptor repertoire *(8)*. This fact further underlines the need for careful and meticulous approach to new vaccine development based on profound understanding of the cellular and molecular elements of the human immune system involved *(7, 8)*. Antibody-mediated protection is the cornerstone of successful protective immunity induced by a vaccine. The quality of antibody-mediated protection exhibits through affinity and avidity of vaccine induced antibodies (priming) as well as through persistence of specific antibodies and capability of vaccine to induce immune memory cells able to reactivate effectively and rapidly in the presence of new antigen challenge *(9)*. However, the production of specific, avid and protective antibodies is not sufficient for the control of all human pathogens. Stimulating the specific type 1 T-cell response has considerable role in effective combat against intracellular pathogens like viruses and *Mycobacterium tuberculosis (6, 9)*. In those situations antigen-specific T cells exhibit effector function through targeted removal of infected host cells establishing control over the replication of pathogen thus preventing clinically manifest disease *(6, 8, 9)*.

A better understanding of the human immunological mechanisms that are crucial for proper response to vaccinal antigens and resulting in forming adequate protective immunity to certain infections is a task to be solved in the process of creation of a new vaccine. Besides the impressive progress in immunology, a huge number of significant technical improvements in biotechnology processes of fermentation and purification in the last few decades have been done thus rendering possible production of safer/less reactogenic products *(8)*. Technological attainments upgraded human vaccinology with numerous new products such as split and subunit influenza vaccines, acellular pertussis vaccines as well as purified bacterial polysaccharides *(4, 6-9)*. Although higher purification of antigens resulted in less reactogenic vaccinal products, the benefits of high purification are somewhat lessened through reduced immunogenicity *(8)*. Application of multiple doses, adjuvantation and protein-conjugate technology have became standard procedures for overcoming this problem and attaining sufficient and persistent immunological response that could guarantee adequate and long-lasting specific protection *(9)*.

All aforementioned achievements in better understanding of biological and immunological nature of successful vaccination as well as technological improvements in the production process made possible the changes in routine immunization programs that happened around the turn of the century. However, the success of this process does not depend only on the quality of immunizing agents but also on the raising of awareness on benefits of vaccination among medical professionals and common people *(7)*. Control measures organized and conducted by national regulatory bodies are essential for maintenance of the attained levels of safety, efficacy, purity and potency of the vaccines and they are not less important than previously listed achievements *(7, 10)*. The role of regulatory authorities should be even more conspicuous in new vaccines approval process with special emphasis on vaccine safety and possible side effects *(7, 10)*.

Finally, the importance of well-designed and carefully implemented communication interventions in support of immunization should be also emphasized. Communication is particularly needed to achieve vaccination coverage in hard-to reach populations and to build trust in vaccines among those who question them. The impact of well-designed, research-based communication interventions on achieving health outcomes is indispensable. Without a well-planned, adequately funded strategic communication, immunization programs fall short of meeting and sustaining coverage goals *(11)*.

Recommendations for use of a vaccine depend on the balance between benefits and risks of vaccination *versus* risk of disease. The balance must be periodically accessed *(12)*. Although the development of vaccine schedules and recommendations begins with prelicensure studies of a vaccine, an experience gained through its widely use is immense and should serve as a source of valuable information that are sometimes decisive in the process of changing of current vaccinal practice (withdrawal of rotavirus vaccine in the USA after data on intestinal intussusception were obtained, interruption of further

vaccination with mumps vaccine containing Urabe Am9 strain in England after observation that vaccinal strain causes aseptic meningitis) *(12-14)*.

CHILDHOOD IMMUNIZATION PROGRAMS

In all developed countries the recommendations about vaccination of children have been created under the auspices of advisory and/or regulatory national bodies *(5, 10, 12)*. In the United States vaccine recommendations for children have been developed by two advisory bodies: (1) the Advisory Committee on Immunization Practices (ACIP) and (2) the American Academy of Pediatrics (AAP) Committee on Infectious Diseases *(12)*. The recommendations issued by those two committees are revised every two to four years and published as supplements to the *Morbidity and Mortality Weekly Report* or as a *Report of the Committee on Infectious Disease* (Red Book) *(12)*. In the United States immunizations for children are provided through both the private and public health sectors *(12)*. Although no mandatory, but only recommended vaccination exists, high vaccination coverage is achieved and on the national level at the beginning of the 21st century it ranged from 76.3% for varicella up to 94.3% for diphtheria, tetanus and pertussis *(12)*. However, it should be noticed that all American states have laws requiring immunization before school or day care entry in effect, that, although not officially, in fact convert recommendation into obligation *(12)*. The efficacy of such organized system of universal continuous vaccination, as in the United States, can be also judged through the analysis of data on morbidity dynamics of certain vaccine-preventable diseases through the last century. Comparing the morbidity of seven different infectious diseases (diphtheria, pertussis, tetanus, paralytic poliomyelitis, measles, mumps and rubella) at the end of the 20th century to figures from 1900, a 94% (pertussis) to 100% (paralytic polio) reduction can be observed *(15)*.

In European countries a great difference among vaccination schedules, as well as a variety of legislative processes can be observed. The wide variation in childhood vaccination schedules encountered throughout European Union suggests that they are the result of national traditions and historical vaccine registrations at the national level. Immunisation programs are often designed following the agenda of the overall prevention programme carried out by family pediatricians or well-baby clinics. Moreover, even the organisation of the school system can play a role, especially for scheduling boosters in late childhood and adolescence *(16)*. In the United Kingdom (UK) all vaccines for childhood vaccinations are free, purchased by the government and provided by general practitioners *(5)*. In Scandinavian countries the situation with the fundings and purchase of vaccines is the same as in UK. The main difference in comparison to UK is that in most cases the vaccines are being provided by primary care nurses *(5)*. In France, pediatricians provide most childhood vaccines while the cost of vaccination is reimbursed from government funds *(5)*. In Germany, vaccines are also provided mostly by pediatricians and reimbursement comes from health insurance funds *(5)*. Each European Union member state has a national advisory body responsible for vaccines recommendation *(5)*. The effect of those recommendations varies according to the centralization of immunization programs and the balance between public and private sector provision. In member states with high level of decentralization like in Austria, Germany, Italy and Spain, national recommendations are modified at the level of autonomous regions/states because they are the bearers of responsibility for public health *(5)*. However, not only the degree of the decentralization plays a role in the final appearance of vaccination program. Even in unitary state like Sweden is, although national committee recommends schedule and type of vaccine, local health authorities have the right to provide the product they want *(5)*.

In Central and Eastern European countries previously belonging to the communist block the situation has dramatically changed at the beginning of the 1990s *(5, 17)*. Some of the countries, like Hungary, Czech Republic and Slovakia successfully overcame the new circumstances and switched vaccines' procurement from »Soviet-based« to »Western-oriented« without interruption in continuous immunization program *(5, 17)*. In Baltic States the collapse of the Soviet Union caused dissolution of the immunization system. Since immunization program was based on vaccines produced in the Russian Soviet Republic, the downfall of the Soviet federation caused vaccine shortages. The lack of hard currency further complicated situation with vaccine purchase which caused temporarily interruption of immunization program, fortunately without significant reemergence of any vaccine-preventable disease. Although significant changes have occurred in the field of active immunization in Central and Eastern European region during the last twenty years, they remained primarily on technical (progressive shift to western-produced vaccines) and socio-economic (move from public to private sector-based medical care) level *(5, 17, 18)*. National control over designing immunization program and immunization policy based primarily on mandatory vaccines remained the two main characteristics of vaccination schedules in those countries *(18)*.

In a situation where citizenship of the European Union includes the right to travel, live and work anywhere within the territory of every member state, a great diversity in vaccination schedules represents an additional difficulty *(16)*. Starting the vaccination program in one country and continuing it in another can bring some challenges: the number of doses, timing, vaccine combinations are different *(16)*. Although, due to the fact that interchangeability of the vaccines is allowed in most situations, current differences in schedules are not a real obstacle to free movement. However, a common schedule can ease the compliance to the national schedule for foreign citizens and can facilitate the work of health care personnel that could vaccinate foreign children just according to the age, without following complex algorithms

and recommendations *(16)*. Currently, according to the European Union regulations, vaccination policies are exclusively under the patronage of member state authorities, and no central legislation exists *(16, 19)*. Although, it seems, especially from the doctrinal point of view, that differences in vaccination schedules between member states do not represent a significant obstacle in providing quality health care and maintaining low vaccine-preventable diseases morbidity, a convergence process with common schedule as a final goal is highly desirable *(16)*. The establishing of the European Centre for Disease Prevention and Control (ECDC), a new European agency with special mission to identify, assess, and communicate current and emerging threats to human health posed by infectious diseases, would maybe be the right address for commencing activities on harmonization of European vaccination schedules *(20)*.

In Croatia the program of continuous universal active immunization of children has been based on mandatory vaccination against ten vaccine-preventable diseases *(21)*. The childhood vaccination program that covers life span from 0 to 19 years of age is based on the recommendations provided by the Croatian National Institute of Public Health *(10)*. Vaccinations that are enclosed in the program are obligatory for all children in the defined target population and are purchased free of charge by the Croatian Institute for Health Insurance *(10)*. Besides mandatory vaccination that is perceived as a public health intervention carrying individual as well as common benefit through the induction of individual and herd immunity against certain infectious diseases, in Croatia there are also recommended vaccinations whose primary goal is to provide specific protection to people belonging to specified high-risk populations *(10, 22)*. For pediatric population this category includes vaccination against influenza, pneumococcal disease and rota virus gastroenteritis *(10, 22)*.

MANDATORY VACCINATIONS FOR CHILDREN IN CROATIA

Vaccination against tuberculosis

About one third of the world population is infected with *Mycobacterium tuberculosis (22)*. Tuberculosis (TB) causes around 2 million deaths each year, of which 450 000 among persons of childhood or adolescent age. The incidence of TB is highest in developing nations, ranging from 60 to 380 newly diagnosed cases per 100 000 population per year. The number of patients is particularly high in countries with an uncontrolled epidemic of HIV infection, in which up to 50% of those infected with HIV also suffer from tuberculosis. In developed countries the incidence of TB is continuously decreasing and does not exceed 5 to 8 new cases per 100 000 population yearly. In Croatia a reduction in TB incidence has been observed since 1955 when the highest number of newly diagnosed cases (20 000 cases) has been registered since the end of World War II *(23)*. The regressive trend has been stopped during the war time period (1991-1995), although no increase in the incidence has been observed *(23)*. The number of patients with TB in Croatia in the last two decades decreased by three fold – the incidence has declined from 60/100 000 in the early 1990s, to 20/100 000 in 2009 *(24)*. Comparing morbidity rates from different parts of the country, somewhat higher numbers could be found in the mainland (15.4 to 39.9 / 100 000) than in the coastal part of Croatia (9.8 to 21.3 / 100 000) that could be explained by the differences in climate and longer indoor stays during winter which stimulates the transmission of mycobacterium *(24)*. While in developing countries a significant number of patients with TB are children and adolescents, in developed countries the incidence of disease among this age group is the lowest *(22)*. In Croatia, the incidence of TB in children and adolescents in 2009 ranged from 0.8 to 7.0 / 100 000 persons (Table 1) *(24)*. A favorable dynamics in TB epidemiology in Croatia in the last half century is certainly the consequence of improvements in socioeconomic conditions, but at least partly it has to be also attributed to continuous mandatory vaccination using BCG (Bacillus Calmette-Guèrin) vaccine for all children which has been on the market since 1948 *(23)*.

The vaccination against TB in the majority of countries around the Globe began in 1948 after the First International BCG Congress in Paris brought the conclusion that BCG is effective and safe vaccine despite the lack of clinical trials in favor of this statement *(25)*. The data that later on studies gave us mentioned the protection against less severe forms of disease ranging from 24% to 75% depending on vaccine used, while against severe forms of disease (disseminated TB and TB meningoencephalitis) BCG shoved immediate protection in 60% to 95% of vaccinees with duration of protection not more than 15 years *(25, 26)*. Although the use of the BCG vaccine has been followed by a certain degree of the distrust from the very beginning, in the first 25 years of its usage more than 1.5 billion individuals have been vaccinated *(25)*.

In Croatia, according to the Childhood Vaccination Program (CVP) active immunization against TB is mandatory for all children. Primovaccination is performed by intradermal application of vaccine in the left deltoid area within the first year of life *(21)*. All newborns born in hospitals are vaccinated before they are discharged home. For those born »*extra muros*« BCG vaccine is applied before the end of the 2nd month of life. Those who are not vaccinated at birth or up to two months of age must be vaccinated with BCG vaccine by the age of 12 months *(21)*. Revaccination is performed at the age of thirteen years only for children with negative tuberculin skin test *(21)*.

Although vaccination with BCG vaccine is safe and effective against severe forms of disease, in a situation of stable declining of TB incidence in Croatia, further vaccinations need to be reconsidered when TB incidence falls to the levels of those in developed countries.

TABLE 1

The incidence of TB in Croatian children and adolescents (2000 – 2009)*

Age	The incidence of TB in Croatian children and adolescents aged 0 – 19 years (number of patients/100 000)									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
0-4 y	4,9	2,6	6,3	2,5	2,9	0,8	2,1	2,1	2,1	0,8
5-9 y	6,3	6,7	7,6	10,9	6,0	4,0	2,8	3,6	2,0	3,2
10-14 y	11,5	11,2	12,7	11,5	8,2	5,2	6,3	3,7	6,3	5,6
15-19 y	15,2	16,2	12,4	13,1	14,4	11,1	9,4	7,4	6,4	7,0

*According to data from: CROATIAN NATIONAL INSTITUTE OF PUBLIC HEALTH 2010 Croatian Health Service Yearbook 2010. Available from: http://www.hzjz.hr/publikacije/hzs_ljetopis/index.htm

Vaccination against hepatitis B

Hepatitis B (HB) is a major global public health problem due to its widespread prevalence and a clear association of chronic hepatitis B virus (HBV) infection with liver cirrhosis and hepatocarcinogenesis. The World Health Organization (WHO) estimates that HBV-associated acute or terminal chronic liver disease causes death in 500 000-700 000 people worldwide annually. WHO also assess that two billion of people around the Globe have markers of present/past HBV infection of whom 300 to 500 million remain chronic carriers *(27, 28)*. Population of chronic carriers (infected people without any discernible clinical sign or biochemical marker of disease activity) represents a significant threat to global health as a silent but ubiquitous source of infection *(27)*. The intensity of the »HB problem« varies widely from country to country. Depending on the percentage of chronically HBV infected people among general population three different groups of countries can be distinguished: a) countries with high prevalence of carriers (≥8% HBs positives), b) medium-prevalence countries (2–7% of carriers) and c) low prevalence countries (less than 2% of carriers) *(29)*. In high-prevalence countries (Sub-Saharan Africa, Pacific region, Southeastern Asia, China, and Central Asian states) the risk for acquisition of HBV infection during the life-span exceeds 60%. The infection is mainly acquired in perinatal period by vertical transmission from chronically infected women of generative age, or during the early childhood through the contacts with chronically infected family members (27-29). In medium-prevalence countries the overall risk for acquisition of infection ranges from 20% to 60%. The predominant route of transmission is through close household contacts and during medical interventions, although sexual and perinatal transmission should be also kept in mind. Therefore, the infection could be acquired at any time during lifetime. In low-prevalence countries (North America, Western Europe) the overall risk for acquiring HBV infection does not exceed 20%. Infection mostly occurs among young adults, and the most important route of transmission is through sexual intercourse *(27)*. In those countries HBV infection more frequently affects people belonging to certain risk groups (chronic carrier's sexual partners and household members, parenteral drugs users, promiscuous heterosexuals and male homosexuals) as well as persons that professionally or during medical treatment (especially patients on hemodyalisis, hemophiliacs and cancer patients) come into contact with blood and blood products. A special risk occurs for newborns born to chronically infected mothers in whom vertically transmitted infection in more than 90% evolves into chronic disease/carrier state *(27-29)*.

Croatia belongs to low-prevalence countries with less than 2% of chronically infected population *(30, 31)*. In the last decade the number of newly diagnosed HBV infected patients did not exceeded 220 patients per year, with the lowest number registered during 2010 (58 subjects) *(24)*. The number of newly registered chronic carriers in the same period ranged also around 200 cases annually *(30, 31)*.

The best way to prevent HBV infection is active immunization *(32)*. Since previously declared WHO doctrine of vaccination for just those subjects belonging to high-risk populations for acquisition of HBV infection didn't show any significant decrease in the morbidity, a new recommendation on universal vaccination in childhood has been issued *(33)*. The majority of nations adopted WHO recommendation starting with infant immunization, while a lesser number, including Croatia, decided to start immunization at a preadolescent age *(32)*. The decision was based on epidemiological data showing a small number of HB cases in children and a peak incidence of disease among young adults *(32)*. The pre-existing practice of vaccinating all newborns born to HBs positive mothers was taken as an additional evidence for the justification of this decision. However, clinical data showed that the implementation of such a strategy has some faults and that certain number of young children, mainly those with chronic conditions and hematooncologic disorders remained unprotected and acquired HBV infection *(34)*. The vaccination against HB in Croatian children started in 1999 with vaccination of all sixth graders using three doses of recombinant DNA vaccine schedule *(34)*. However, starting with the year 2007 the vaccination was moved to the neonatal age *(35)*. According to the Croatian National Institute of Public Health (CNIPH) data for 2010, the vaccination coverage for HB in neonatal age was declared 97.0% *(24)*.

Recombinant vaccine that is in use in CVP in Croatia is immunogenic, effective and safe vaccine *(36)*. The protection against HBV infection is directly related to the creation of anti-HBs antibodies. Antibody titer of >10 mIU/mL measured 1-3 months after administration of the last dose of vaccine is considered a reliable marker of short and long-lasting protection against natural infection *(37)*. Immunological response to vaccination could be modified even by vaccine injection site. In newborns and infants vaccine should be applied by intramuscular injection in mediolateral aspect of the thigh *(32)*. The application of HB vaccine concomitantly with other inactivated and live-attenuated pediatric vaccines seems not to have an effect on the immunogenicity of HB vaccine as well as the coadministered vaccine *(36)*.

The exact duration of protection after vaccination with HB vaccine is not known, although 15% to 50% of children vaccinated in infancy lose detectable antibody levels within 5 to 15 years after vaccination *(32, 36)*. However, no clinically manifested disease caused by HBV among immunocompetent children has been observed, while only few cases of chronic HBV infection have been documented *(36)*. Basing on those data, currently there are no recommendations for routine booster doses of HB vaccine as well as for periodic serologic testing *(36)*.

Generally, recombinant HB vaccine is a safe vaccine. More than 90% of vaccinees don't manifest any side effects. Pain at the injection site (3% to 5%) and mildly elevated body temperature (1% to 6%) are the most frequently reported side effects. Rarely fatigue, nausea, flu-like illness, vomiting, dizziness, pruritus, arthralgia and diarrhea have been registered (36-38). Although a variety of chronic diseases have been reported following HB vaccination, including demyelinating disorders (multiple sclerosis, optic neuritis, transverse myelitis), rheumatoid arthritis, type 1 diabetes and chronic fatigue syndrome, no definitive findings supporting these hypotheses have been found *(36, 38)*.

Vaccination against diphtheria

Diphtheria is among rare infectious diseases that is eliminated in Croatia, and since the last case of this indigenous disease was registered almost 40 years ago (1974) there is also a belief, although unsupported by results of research, that its causative agent is even eradicated *(39)*. A favorable trend that led to the disappearance of diphtheria from Croatian population is certainly the result of continuous implementation of universal vaccination of children with diphtheria toxoid (D) since 1947 *(39)*. A similar epidemiologic pattern has been observed in all other countries that implemented D in their vaccination schedule *(40)*. Although no modern designed study dealing with immunogenicity and efficacy of D has been available, it is obvious from epidemiologic data that D has good protective effect against clinically manifested disease *(40)*. It is generally accepted, partly based even on the results from animal studies, that diphtheria antitoxin level of less than 0.01 IU/mL is not protective. Vaccinees with antitoxin level ranging from 0.01 to 0.09 IU/mL are partly protected, while the concentration ≥0.1 IU/mL provides full protection *(39, 40)*. The other important question is the duration of protective immunity. According to data from England, less than 30% of people aged more than 60 years have detectable protective level of antitoxin *(39)*. A Croatian study, although limited with low number of collected sera, showed the presence of partly protective levels of antitoxin in 66%, while fully protective level was found in 33% of subjects older than 60 years *(39)*.

There are two important questions regarding the vaccination against diphtheria: 1) is there a need for booster doses in elderly people who lost specific protection and 2) do we still need continuous vaccination against diphtheria if the disease has been eliminated from the population. Regarding boostering the adults or elderly people it is postulated that in spite of low levels of immunity among those age groups, diphtheria will remain well controlled with effective childhood immunization *(40)*. On the other hand, since the causative agent of diphtheria is still present in certain populations, ceasing the immunization could result with reemerging of diphtheria among populations with long-lasting state of elimination. »Russian epidemics« in the early 1990s with more than 100 000 cases after the interruption of vaccination due to dissolving Soviet immunization program is a relatively recent argument for continuing the vaccination *(39, 40)*.

Vaccination against tetanus

Tetanus is unique among vaccine-preventable diseases because it is not communicable *(41)*. Around 1 million of people every year acquire tetanus worldwide *(42)*. In developing countries, especially in some parts of Eastern Africa and Middle East, neonatal tetanus accounts for 50% of cumulative number of cases *(42)*. The causative agent, *Clostridium tetani*, is ubiquitous microorganism, widespread in the environment. Clinical manifestations of disease develop as a consequence of entering clostridial spores into a devitalized human tissue usually in the vicinity of cutting or stab injuries. Anaerobic/microaerophylic conditions typical for such injuries stimulate germination of spores to vegetative bacili that elaborate toxin responsible for the development of disease symptoms *(41, 42)*. Isolation and purification of toxin and its inoculation into experimental animals facilitates further studies of disease pathogenesis, but also the production of animal sera to be used in prevention/treatment of tetanus in humans *(41, 42)*. First attempts to stimulate specific immunity against tetanus in humans were made in 1917 when Vallee and Bazy immunized seven heavily wounded French soldiers of African ancestry with chemically modified (iodine treated) tetanus toxin *(43)*. Although effective, further investigations on iodine inactivated tetanus toxoid were abandoned *(43)*. Preparation of »anatoxin«, latter called »toxoid« (T) – with formaldehyde inactivated but immunogenic tetanus toxin, marked the beginning of an era of modern active immunization against tetanus *(41-43)*. Clinical application of formal-

dehyde inactivated T began in 1926. Preventive widespread usage of this vaccine during the World War II decreased the incidence of tetanus among wounded American soldiers by 30-folds (from 13,4 cases of tetanus per 100 000 wounds in World War I to 0,44 cases per 100 000 wounds in World War II) *(42)*.

Tetanus toxoid was introduced in many vaccination schedules during the 50s *(43)*. Immunization against tetanus has been implemented in Croatia since 1955 (42). In 1965, the WHO standardized the calibration of the potency of T containing vaccines and established the first international standard for T *(41)*. New WHO potency standards for T-based vaccines were set in 1982, since when 40 IU preparations (60 IU when in combination with D and/or pertussis vaccine) have been used *(41)*. Although widespread use of T began with single-component vaccines, today tetanus toxoid when used in childhood immunization schedules is usually incorporated in diphtheria-tetanus-pertussis combinations or even more advanced versions *(41-43)*. T-based vaccines used as a single vaccines or in combinations are stable, immunogenic and with low reactogenicity *(41)*. National-based Danish study dealing with the problem of duration of specific protection after completing primoimmunization against tetanus in childhood showed persistence of protective levels of antitoxin for 14 years in 96% of vaccinees and for even 25 years in 72% of them *(41)*. Studies from Sweden and USA found a 10-year persistence of protective antitoxin levels in around 90% of vaccinees. Based on these results, the majority of nations worldwide advise life-long boostering every 10 years *(41)*. CVP in Croatia uses in total eight doses of tetanus for primovaccination and boostering – after finishing primary series in their first year of life, Croatian children will be vaccinated with one dose in the second and fourth year of life and boosted twice during the elementary school and finally at the age of 19 *(21)*.

Vaccination against pertussis

Despite being greatly reduced by vaccination, pertussis is still present as an endemic and epidemic disease. In countries with long-lasting history of continuous vaccination against whooping cough, the disease mainly occurrs in atypical form in adolescents and young adults or as a severe disease of unimmunized or partially immunized infants accompanied with high rate of complications and even unfavourable outcome *(44-46)*. Pertussis occurs in periodic cycles approximately every two to five years. This typical epidemic pattern is preserved even in countries with low incidence due to high vaccination coverage *(47, 48)*.

Although the first attempts to introduce vaccines against whooping cough occurred immediately after the discovery of the pathogen in the early 1900s, the era of active immunization against pertussis began in 1947 with the introduction of a combined vaccine against diphtheria, tetanus and pertussis that contained D, T and inactivated whole cell of *Bordetella pertussis* (DTwP) in the US childhood vaccination schedule *(49)*. In the next two decades DTwP became part of the recommended immunization schedules for children in all developed nations. Active immunization against whooping cough using domiciliary produced vaccine in Croatia began in 1959 *(47, 50, 51)*. In the early 1960s, the WHO has set international standards to be met by every pertussis vaccine. Each vaccine dose had to contain at least 4 international units (IU) of *B. pertussis*. One IU corresponded to a concentration of one billion microorganisms per milliliter of vaccine *(47, 49, 52)*. The average concentration of inactivated *B. pertussis* in the Croatian vaccine corresponded to the total dose of 12-30 IU for the entire primovaccination, or an average of 20 IU in three doses which made the Croatian DTwP vaccine acceptable for international standard *(52, 53)*. Epidemiological studies that have been conducted prior to the introduction of DTwP in immunoprophylaxis of whooping cough, as well as field studies performed after the introduction of the vaccine into the routine use have shown good effectiveness of vaccines in many countries. Compared with non-immunized population, the vaccine, depending on the country where the research was conducted, showed an effectiveness of 63% to 94.8% if it was applied to infants in three consecutive doses *(47, 52)*. In most countries vaccination schedule in the first year of life comprised application of three vaccine doses starting at the age of 2 or 3 months of life, with 4 to 6 weeks intervals between the doses applied *(47, 52)*. From the beginning the differences in the number and time of application of booster doses existed. Some countries along with an early booster at 18 months of age applied the second one at the age of 4 years (Argentina, Croatia, The Netherlands, United Kingdom) while others disposed the second booster to the time of primary school enrollment (Australia, Canada, USA, Spain, Switzerland) *(48, 50)*. Regardless of the differences in the vaccination schedule and number of booster doses, active immunization against whooping cough using DTwP vaccine has dramatically changed the classical epidemiological shape of the disease and has achieved an impressive reduction in morbidity and mortality *(47, 50)*. In Croatia, the continuous application of DTwP with coverage rate of ≥80% in the primovaccination and ≥90% for booster doses reduced the number of cases of whooping cough in the first 20 years of universal vaccination for 94.4% in comparison to the prevaccinal period *(50)*. Although doubtless effective in preventing disease, systematic monitoring clearly showed that the universal use of DTwP modified conventional epidemiological pattern of disease with a shift of morbidity in younger and older age groups. Frequent side effects, even some infant deaths in the US, UK and Japan were also observed *(47, 48, 54-57)*. Although neither naturally acquired infection doesn't leave lifelong immunity, specific immunity after vaccination with DTwP, regardless of the number of booster doses applied, is limited to the period within no more than 12 years after the application of the last dose *(58)*. A direct consequence of the limited duration of protection in highly vaccinated populations is the frequent occurrence of pertussis among adolescents and adults. Adolescents and young adults thus serve as a

reservoir of infection for unimmunized or partially immunized young infants *(56, 58)*.

DTwP reactogenicity was observed soon after the introduction of vaccine in general use. Local reactions at the site of vaccine application were the most common while the generalized side effects such as fever, prolonged unexplained crying, seizures and hypotonic-hyporesponsive episodes (HHE), were less frequent and had not discouraged further implementation of DTwP *(47)*. A continuous decline in the incidence of pertussis in countries with high vaccine coverage further justifies this perception *(45, 47)*. In the early 1960s the first reports linking the use of DTwP with progressive encephalopathy, infantile spasms, and sudden infant death syndrome (SIDS) appeared *(47, 59, 60)*. Although these side effects were very rare, and there is even a reason to doubt their direct connection with the vaccines, their occurrence in some countries (Japan, Sweden, United Kingdom) completely disrupted the implementation of DTwP *(47, 61, 62)*. The direct consequence was reemergence of epidemic disease with a high number of hospitalizations, and even deaths *(61, 62)*. Faced with the renewed threat of epidemic pertussis, but also with the resistance of the public and the medical authorities to DTwP, during 1970s and early 1980s vaccine manufacturers have invested considerable effort and resources into creating a new, acellular pertussis vaccine (aP) *(63)*. The first country that introduced the aP into routine immunization was Japan in 1981. After a large number of field trials, from the beginning of 1990s the aP became a part of the immunization schedule in many European countries, Canada and the United States *(63, 64)*. The development of aP vaccines went through several phases, starting from monocomponent vaccine containing only pertussis-toxin (PT), through two-component vaccines containing PT and filamentous hemaglutinine (FHA) to modern aP containing three – PT, FHA and pertactine (PRN) to five components (PT, FHA, PRN and two types of fimbriae – FIM) *(63, 64)*. The effectiveness of these vaccines, depending on the number of included components ranges from 59% to 93% *(63)*. Since the reactogenicity of aP vaccine is much lower than that of wP, effectiveness approximately equal, and duration of protective immunity not significantly shorter (6 years after the last dose), multicomponent aP vaccine is now preferred to wP-based vaccines *(65, 66)*. However, the introduction of aP did not resolve the question of displacement of pertussis morbidity to the youngest and older age groups *(54)*. A possible solution is the vaccination of adolescents, as well as vaccination of certain adult populations, such as young mothers and newborn's household members („cocoon strategy"), health professionals and day-care staff members *(54, 58)*. Another possibility for better control of disease burden in a population is periodic boostering of the whole adult population. However, even in most developed nations this strategy seems far from implementation.

Vaccination against poliomyelitis

Although the first written description of poliomyelitis dated from very ancient times of Egyptian XVIIIth dynasty, poliomyelitis has remained for long time an endemic disease that crippled limited number of subjects *(67)*. At the turn of the 19th into 20th century, a change in the epidemiology of poliomyelitis from endemic to an epidemic form was observed in many industrialized European nations, and then in the US and Canada *(67)*. The epidemics of polio reached their peak after the end of Word War II with more than 20 000 cases of disease reported annually in the US associated with high case-fatality rate *(68)*. In Croatia at the beginning of the 1960s major epidemics were also registered with more than 600 cases occuring annually *(69)*.

The discovery of the virus, definition of three different serotypes, confirmation that neutralization antibodies protect against the disease and finally demonstration that all three serotypes could be grown in cell cultures of non-neuronal origin were crucial steps in the development of the first effective vaccine against poliomyelitis *(67, 68)*. In 1954 the first trivalent poliovirus vaccine inactivated by formalin (inactivated polio vaccine – IPV) was designed by Jonas Salk. Its effectiveness and reactogenicity were tested in one of the largest vaccine field trials that comprised 419 000 vaccinees and 330 000 controls. Since the results of the study were promising, IPV was licensed for widespread use in the US next year *(68)*. After the IPV introduction in pediatric vaccination schedule in US, a sharp decline in the number of cases has been observed very soon – three years after the beginning of the widespread use of IPV an 86% reduction in the incidence of poliomyelitis in USA was registered *(67)*. Although the impact of IPV on the epidemiology of polio was impressive, in the early 1960s IPV was eclipsed by new attenuated live oral polio vaccine (OPV), except in some northern European countries (Finland, Sweden, the Netherlands) *(67)*. The OPV era started in the US with licensure of monovalent OPV in 1961, and then trivalent OPV in 1963 *(67, 68)*. However, the first large-scale production, the first large safety and efficacy field trials, as well as the first mass immunization campaign took place in the former Soviet Union *(67)*. First results were impressive – incidence decreased from 10.6/100 000 population in prevaccine era to 0.43/100 000 population in the third year of OPV universal implementation *(67)*. In Croatia, compulsory vaccination against polio was introduced in 1961 using monovalent Koprowsky's OPV vaccines. During the two year period the whole population aged less than twenty years of age were fully vaccinated *(69, 70)*. In 1964 the compulsory boosters were introduced for all children in the 3rd and 5th year of life, as well as for elementary school 1st and 4th graders *(69)*. In 1965 a new booster for 14-year-old children was introduced while booster in the 4th grade was abandoned *(69)*. In 1983 Koprowsky's vaccine was switched to trivalent Sabin OPV *(69)*. Systematic and universal use of OPV in Croatian population, with coverage rates between 73% and 80% for primovaccination and 90% for

boosters, has very soon shown impressive results – twelve years after the introduction of OPV for the first time not a single case of paralytic polio was diagnosed *(69)*. The last epidemic was registered in 1984 and the last sporadic case in 1989 *(69, 71)*.

Although there is no doubt that systematic and widespread use of OPV was the main contributory factor in the eradication of wild polio infection from three major WHO world regions – Americas, Europe and Western Pacific, soon after the introduction of OPV in national immunization programs the first cases of vaccine-associated disease were recorded *(67, 69, 71)*. Although rarely seen, vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived poliovirus (VDPV) disease pose continuous risk associated with OPV use *(72, 73)*. That was the main reason why WHO suggested that OPV should be replaced by IPV in post-eradication era *(74)*. Good effectiveness and appropriate duration of specific immunity showed in some European countries with long-lasting experience in IPV usage, as well as its ease incorporation into combination vaccines further facilitated the (re)introduction of inactivated vaccine into immunization schedules of numerous nations *(67)*. In Croatia OPV was completely replaced by IPV in 2008 *(35)*.

Vaccination against *Haemophilus influenzae* type b disease

Before the introduction of effective vaccine against the *Haemophilus influenzae* type b (Hib) this bacteria was the leading cause of acute bacterial meningitis among infants and children younger than 5 years of age worldwide *(75)*. At the end of the 1980s 20 000 to 25 000 US children developed invasive Hib disease, mainly acute meningitis, annually. The incidence and mortality of Hib disease among US children were thus similar to those caused by polio virus before the introduction of universal vaccination *(75)*. Data on invasive Hib disease from Croatia are scarce and incomplete primarily due to the fact that significant number of microbiologic laboratories had never performed serotyping of *Haemophilus* spp. isolates from primary sterile sites. According to data collected from the University Hospital for Infectious Disease (UHID) in Zagreb that covers Zagreb metropolitan area (around one fourth of the total Croatian population) the incidence of invasive Hib disease before the introduction of universal immunization was around 22 cases per 100 000 children aged less than 5-years, with mortality of 3.3% *(76)*. After the introduction of mandatory vaccination against Hib in Croatia, although the »catch-up« immunization of all susceptible subjects (younger than 5 years of age) was not performed, the incidence sharply decreased as was previously seen in other populations that implemented universal vaccination of all children *(76)*. In 2009 no Hib invasive disease was diagnosed at UHID, and in 2010 one case of bacteremic pneumonia in nonimmunized 10-year-old girl was registered *(76)*.

Although several surface structures of Hib appear to be important in its pathogenicity, the outermost structure, polysaccharide capsule that consists of a repeating polymer of ribosyl and ribitol phosphate (polyribosylribitol phosphate, PRP) is crucial for invasiveness of Hib *(75)*. Natural immunity to Hib resists on anticapsular antibodies. Maternally acquired antibodies still present in infants younger than 6 months of age, and the natural acquisition of antibodies in toddlers and children aged 2 to 5 years are responsible for typical epidemiologic pattern of invasive Hib disease with peak incidence in the age range from 6 months to 2 years of age, as well as the disappearance of disease after the age of five *(75)*. Although a precise minimal level of anti-PRP antibody that is protective has not been established, data from passive protection of agammaglobulinemic children as well as from studies on naturally acquired antibodies, suggest that minimal serum concentration of anti-PRP antibody that provides protection in humans is 0.15 μg/mL, while the concentration of more than 1.0 μg/mL guarantees protection for a minimum of one year *(75)*. PRP, as a typical polysaccharide that consists of repeating oligosaccharide units, is primitive antigenic unit that elicits weak immune response involving minimal T-cell influences *(75)*. Upgrading of PRP immunogenicity in infants could be made by switching from T-cell independent to T-cell dependent antigen through the process of conjugation *(75)*. PRP conjugated to protein carrier (diphtheria toxoid, tetanus toxoid, meningococcal outer membrane protein) could elicit adequate immunologic response characterised by switching in immunologlobulin classes (IgM to IgG), appropriate avidity of specific antibodies and memory capacity needed for adequate booster effect *(75)*. Among all Hib-conjugate vaccines, PRP-tetanus toxoid conjugate vaccine (PRP-T) is most widely used. PRP-T showed excellent immunogenicity, with seroconversion rates of 98% to 100% after the third primovaccination dose, achieving mean anti-PRP antibody concentrations of 5 to 10 μg/mL and persistence of good antibody levels a year after immunization *(75)*. The vaccine is generally well tolerated. Local mild side effects were registered in 7% to 15%, while fever higher than 38°C occurred in less than 10% of vaccinees *(75)*. Efficacy of PRP-T is showed on prelicensure studies and during the universal use of vaccine. In all countries that implemented Hib vaccine in their universal immunization program, invasive Hib disease disappeared from the population *(75, 76)*.

DTaP-based combinations

Inauguration of new inactivated vaccines in the pediatric vaccination schedule is firmly connected with the development and promotion of usage of combined vaccines. The development of combined vaccines has been a public health interest and priority, and their use is recommended by the WHO. The usage of combined vaccines minimizes the number of injections, reduces the cost of vaccination program and increases the compliance (77, 78). However, it is important to ensure that safety, immunogenicity and efficacy of combinations are not in-

ferior in comparison to individual components when applied separately *(77, 78)*.

DTaP-based combinations are cornerstone of modern immunization programs and the majority of nations use pentavalent (DTaP-IPV-Hib) or even hexavalent (DTaP-IPV-Hib-HBV) combinations *(79, 80)*. Pentavalent combination containing D, T, five-component aP (PT, FHA, PRN, fimbriae type 2 and 3), IPV and PRP-T was introduced in the Croatian CVP in 2008 and used until 2011 *(24, 35)*. The introduction of this »new« pentavalent combination represented a major shift in CVP in Croatia – for the first time IPV instead of OPV and aP instead of wP were introduced *(35)*. Although some local experts expressed doubts regarding the effectiveness of these new vaccines, judging through the data provided by the CNIPH, a switch from the »old« to »new« vaccines didn't disarrange the stable epidemiological situation in Croatia *(24)*. Compared to previous period when wP was in use, the number of patients with clinically diagnosed whooping cough continued to fall (45 cases in 2010 *vs* 123 in 2007) *(24)*. Although obviously good vaccine, the usage of pentavalent combination introduced in 2008 was suspended at the end of 2011, and the vaccine was substituted with the »new« pentavalent combination containing the same components as the previous one except the aP which is a two-component (PT and FHA) *(21)*. The currently used combination has been extensively assessed in clinical studies testing its safety and immunogenicity in a primary series of vaccinations as well as a booster in the second year of life *(79, 81)*. The vaccine is well-tolerated and its application is associated with predictable side effects that are generally mild to moderate. Good immunogenicity was shown to all antigens comprised in vaccine in both primary vaccination series and boostering. In national immunization programs where combination with two pertussis antigens is in use, good control of pertussis incidence has been shown *(79, 81)*.

Vaccination against measles

Before the active immunization became available, measles was ubiquitous, highly contagious, seasonal viral disease affecting nearly every person in a given population by adolescence *(82)*. In the ten years period from 1958 to 1967 an average of 12 000 children suffered from measles in Croatia annually *(83)*. First efforts to reduce the morbidity thus preventing early and late complications of disease have been undertaken soon after the discovery of the infectious agent *(82)*. After the unsuccess of formalin-inactivated vaccine that produced the short-lived immunity and placed many recipients at risk for atypical measles, the first live-attenuated vaccines derived from Edmonston B strain of virus have been licensed in the mid 1960s *(82)*. In Croatia lesser campaigns of vaccination with Edmonston B vaccine began in 1964, while universal vaccination using domiciliary produced Edmonston-Zagreb (EZ) vaccine became mandatory in 1968 (83). In the first six years of its usage, Croatian children were vaccinated with one dose of live-attenuated vaccine at the end of first year of life, or in the ages of 7 to 10 years of life *(83)*. From 1975 onward two doses of vaccine were applied – first at the age of 12 months and the second for elementary schools' first-graders *(83)*. The law passed in 1975 provided that vaccinal coverage against measles must be above 85% (84). This demand was in 1980s increased to 95% *(85)*. The success of a universal mass vaccination of children in Croatia against measles soon became clearly visible. The number of cases in the 1976 to 1985 period dropped to 3345 annually which represented a reduction in the incidence for 72,3% *(83)*. Continuous vaccination using two-doses schedule further reduced the incidence – in the 5-year period (1997-2001) the mean annual morbidity dropped to 187 cases that represented a 98.8% reduction in comparison to prevaccinal data (86). In the first decade of the 21st century the disease practically disappeared from Croatian population – less than ten cases annually were registered in seven out of ten years, with no cases diagnosed in 2007 (24). In the same period the vaccination coverage for primovaccination and revaccination exceeded the statutory defined 95% (24, 87). However, in a situation in which WHO's goal laid out in the strategic plan for measles elimination from the European region by 2010 has not been achieved and when despite the high vaccine coverage in some countries the minimum immunity level is still under the defined percentage that guarantees the interruption of virus transmission, the reintroduction of wild measles infection into a population remains a real threat *(87, 88)*. In the last decade several European countries reported high number of cases and outbreaks, mainly in unimmunized or partially immunized people. One fifth of cases were subjects older than 20 years of age *(88)*. In the same period, in Croatia two imported epidemics with 54 and 49 cases were noted *(24, 87)*. The last one that occurred in spring 2008 caused by virus belonging to genotype D4 was probably imported from Italy where significant disease activity was observed during the nine-months period from early autumn 2007 to spring 2008 *(89)*. The majority of Croatian cases, as well as Italian ones were registered among partially immunized or fully unimmunized young adults *(87, 89)*. The data from the last European epidemics thus underscore the need for further strengthening of high two-dose regimen vaccination coverage as a unique strategy in successful combat against measles.

Active immunization against measles in Croatia for many years relied on local vaccine production *(83)*. Domestic vaccine contained EZ strain was prepared from the original Edmonston B strain by additional passage on WI-38 cells *(90)*. Used as a monovalent vaccine or as a component of combined vaccine against measles, mumps and rubella (MMR), EZ showed low level of reactogenicity, seroconversion after primovaccination in 99.3% of vaccinees with HI titers ≥1:8 in 96.1% of them and fair persistence of sustained seropositivity *(83, 91)*. From 2009 a new MMR vaccine containing another Edmonston B derived strain (Schwarz) was introduced for pri-

movaccination, while MMR combination containing Enders strain was implemented for revaccination at the age of 7 years since 2012. According to experience from other countries both vaccines provide good protection against measles when used in two-dose schedule and both show low level of reactogenicity *(82)*. However, further surveillance of epidemiologic situation and side effects of vaccines are necessary *(92)*.

Vaccination against mumps

Mumps is an acute systemic viral disease caused by mumps virus (MuV). In prevaccinal era mumps usually occurs in school-aged children and adolescents *(93)*. Since no etiological treatment of mumps exists, and complications, especially in the CNS, are frequent, active immunization remains as the only possibility to control MuV spread and thus reduce morbidity *(94)*. Continuous universal usage of vaccine with sustained high coverage rates may even lead to complete eradication of the disease from the population *(95)*. The definition of the etiologic agent and then first successful propagation of MuV in chicken embryo and tissue culture allowed cultivation of large amounts of virus and creation of a basic prerequisite for the development of vaccine *(96)*. The first inactivated vaccine developed in 1950 in the United States originated short-lasting specific immunity and in epidemic situation it was proved ineffective *(96, 97)*. Enders and co-workers discovery that during continuous passage in embryonated chicken eggs MuV loses pathogenicity but not immunogenicity enabled the commencement of work on the preparation of the first live attenuated vaccine against mumps *(96)*. The first live attenuated vaccine that has entered into wide use, was produced in 1954 in the Soviet Union and has successfully been applied in children and adults *(98)*. Viral strain contained in this vaccine developed in embryonated chicken eggs, marked with the name Leningrad became the parent strain for all subsequent mumps vaccines produced in the Soviet Union *(96)*. Using Leningrad strain the first vaccine produced in cell culture was subsequently developed. After the initial passages on primary culture of guinea pig kidney, vaccinal strain was adapted to the quail embryo fibroblasts. The first modern live attenuated vaccine against mumps was thus created. This vaccine was highly immunogenic stimulating production of neutralizing antibodies in more than 96% of vaccinees *(96)*. Simultaneously efforts undertaken in the US resulted in the creation of successful vaccinal strain – Jeryl Lynn (JL). First vaccine containing JL was licensed in 1967 *(99)*. Within the next twenty years other vaccinal strains were developed: L-Zagreb, Urabe Am9, Hoshino, Torii, Rubini and RIT 4385 *(98, 99)*. In Croatia vaccinal strain L-Zagreb (LZ) was created during 1970 and 1971 through additional attenuation and adaptation of Leningrad-3 strain on chicken fibroblasts primary cultures *(100)*. The monovalent vaccine contained LZ was introduced into Croatian CVP in 1972, and since 1975 MMR vaccine with LZ as a mumps component became mandatory for all Croatian children *(99, 100)*. In 1994 two-dose schedule with the first dose applied at the age of 12 months and the revaccination at the age of 7 years was introduced *(101, 102)*.

Live mumps vaccine stimulates the production of a number of antibodies to different MuV epitopes that can be measured by different serological techniques and results are regularly expressed through seroconversion rate *(103)*. Neutralizing antibodies against hemaglutinin (HN) are probably of the paramount importance and their presence correlates with protection against the disease *(103, 104)*. Data on seroconversion for all mumps vaccines showed favourable results ranging from 86.6% to 100% *(103)*. LZ vaccine caused seroconversion in 88% to 94% of vaccinated preschool-aged children. Seroconversion was even better (98%) in children vaccinated with MMR containing LZ (100). A single study performed in Croatia showed the presence of neutralization antibodies against HN in 96% of the vaccinees *(105)*. The duration of specific immunity is also of crucial importance in the protection against the disease. Seroreversion measured by neutralization test developed in 19% of JL vaccine and in 15% of Urabe Am9 recipients four years after the vaccination *(103)*. In infants immunized with RIT 4385 neutralization antibodies persisted in 95% of vaccinees 18 months after the primovaccination *(103)*. For LZ data on antibody persistence are rather scarce. A single study performed in Croatia showed no detectable antibodies against MuV in 17.4% subjects 18 to 19 years of age vaccinated with single dose of LZ vaccine at the age of 12 months *(101)*.

Although data on immunogenicity and duration of specific immunity are somewhat confusing due to different serologic techniques used and different time interval between vaccination and determination of seropositivity, maybe a better picture of the real situation could be obtained through the results of efficacy trials and effectiveness in the field use of vaccine. All widely used vaccines with the exception of Rubini showed efficacy in range 91% to 99% and effectiveness between 79% and 100% *(103)*.

Prelicensure clinical studies of different mumps vaccines reported only mild to moderate local reactions and rarely occurrence of general symptoms, usually mild fever of short duration, rash and salivary glands swelling. However mass usage of vaccines has shown a true scope and intensity of reactogenicity of certain vaccine strains *(106)*. Through more than four decades of widespread live attenuated mumps vaccine usage, many undesirable side effects have been attributed to the vaccine, with more or less clear evidence of causal relationship *(106)*. Insulin dependent diabetes mellitus, inflammatory bowel disease, autism, idiopathic thrombocytopenic purpura, aseptic meningitis, Guillain-Barrè syndrome and cerebellar ataxia have all been associated with vaccination against mumps *(98, 106-111)*. Especially great concern and fear aroused Wakefield's report from Great Britain that vaccination against mumps correlates with the development of inflammatory bowel disease and an increase in the incidence of autism *(107)*. This report

resulted with almost complete cessation of vaccination with MMR vaccine in the UK (106, 107). However, the results of the study were based just on epidemiological observations and studies that followed did not confirm Wakefield's conclusions *(112, 113)*. Analyzing huge number of papers dealing with the problem of side effects that have been attributed to vaccination against mumps, Jefferson and colleagues in a large meta-analysis published in 2003 have found that only two side effects could be undoubtedly causally linked with mumps vaccine: postvaccinal parotitis and aseptic meningitis *(114)*. The incidences of those side effects significantly differ from vaccine to vaccine. Generally vaccines developed from Japanese and Russian strains are significantly more neurovirulent and have significantly higher incidence of aseptic meningitis than JL and RIT4385 based ones *(106)*. The incidence of aseptic meningitis ranged from 1 case per 1000 vaccinated for Japanese strains (Urabe Am9, Hoshino, Torii) to 1 case per 100 000 vaccinated with JL vaccine *(116, 117)*. Neurovirulence of LZ was first observed in Slovenia in 1989 where the incidence of aseptic meningitis after primovaccination with this strain was calculated as 48 cases per 100 000 vaccinated *(118)*. Calculating the incidence of aseptic meningitis after the primovaccination with LZ among the Croatian infants considering only virologically confirmed cases, Tešović found the incidence to be 49 cases per 100 000 vaccinated *(119)*. However, regardless of the severity of acute illness, no permanent neurologic sequellae were found among children suffering from aseptic meningitis after vaccination with LZ *(106)*.

Long-term use of vaccines against mumps in Croatia has significantly reduced the morbidity of this disease and established a stable epidemiological situation. Although reactogenic, LZ is, due to Croatian experience, undoubtedly efficacious strain *(24, 102, 106)*. However, in January 2009 LZ was removed from primovaccination and replaced by the RIT4385 based MMR vaccine *(92)*. From the beginning of 2012 LZ is no longer in use neither for revaccination – JL containing MMR vaccine became constituent part of CVP in Croatia. In conclusion, one efficacious but reactogenic vaccine is replaced by vaccines that showed high efficacy and low reactogenicity through their widely use in many regions of the world. In situation when reemergence of epidemic mumps is reported even in nations with long-term two--doses vaccination schedule, maintaining a high vaccinal coverage is highly desirable *(120-122)*.

Vaccination against rubella

Rubella is a benign systemic viral disease characterised with mild fever, malaise and maculopapular rash. 20% to 50% of infected subjects have minimal or no clinical symptoms and complications of disease (encephalitis, thrombocytopenia) are rare *(123)*. Typically, a mild childhood disease, rubella in pregnancy can result in miscarriage, stillbirth or an infant born with congenital rubella syndrome (CRS) which comprises deafness, heart disease, cataracts and other permanent congenital manifestations *(124)*. The elimination of rubella and prevention of congenital rubella infection in Europe has been a high priority for the WHO European Regional Office for the past 10 years. In 2002 a strategic plan was developed and implemented for the prevention of congenital rubella infection with the target of <1 case of CRS per 100 000 live births by 2010 *(125)*. Three years later the strategy was revised to include rubella elimination by 2010, defined as <1 indigenous case per 1 000 000 *(126)*. In September 2010 the WHO regional committee for Europe renewed its commitment to the elimination of rubella and prevention of CRS with the new target of 2015 *(127)*. A highly effective attenuated live rubella vaccine was developed over thirty years ago *(123)*. All vaccines produced worldwide with the exception of Japanese ones contain RA 27/3 strain developed on human diploid fibroblasts *(128)*. RA 27/3 is adopted because of its consistent immunogenicity, induction of resistance to reinfection, and a low rate of side effects *(128)*. Continuous universal use of two-dose vaccination schedule with 98% coverage rate resulted in an excellent control of infection and elimination of CRS *(24, 124)*. In the last decade, with the exception of 2007 when a limited outbreak with 39 cases were recorded, no more than 10 subjects with rubella have been reported to CNIPH *(24)*. In the same period no CRS cases have been reported from Croatia *(124)*.

VACCINATION FOR CHILDREN AT RISK

According to Croatian legislation, the vaccination against influenza, pneumococcal disease and rotavirus infection is recommended for certain groups of children at risk *(129)*.

Vaccination against influenza

Influenza is a serious infectious disease that continues to contribute to significant morbidity and mortality worldwide. Yearly influenza vaccination benefits have been demonstrated and vaccination for high-risk groups is well recognised in Europe and the rest of the world as a means of preventing infection and its complications *(130)*. High risk groups for for influenza complications include elderly (>65 years of age) as well as infants and children with existing health complications and vaccination of these groups is a current practice in many countries *(130)*. Although some countries (US, Canada) recommend universal vaccination of healthy children, this practice is not widespread in Europe despite clear demonstration of the benefits of vaccination in reducing the large health and economic burden of influenza *(130, 131)*. In Europe only six countries (Austria, Estonia, Finland, Latvia, Romania, Slovakia, Slovenia) advise routine vaccination for all children *(130)*. The rest of the Europe, including Croatia recommend vaccination of high-risk populations of children that include patients with chronic pulmonary and cardiovascular diseases, with haematological or metabolic disorders, immunological disorders and chronic renal diseases *(130)*. The two doses of trivalent inactivated influenza vaccine con-

taining one influenza A(H3N2) strain, one influenza A(H1N1) strain and one influenza B strain are administered at least one month apart for children aged 6 months to 8 or 9 years (depending on manufacturer's recommendations) who either receive an influenza vaccine for the first time or have not been exposed to influenza previously. For children under 3 years of age a half dose is required per injection. For older children receiving the seasonal influenza vaccine for the first time, a single dose of the vaccine is appropriate *(130)*.

The efficacy and effectiveness of trivalent inactivated vaccine (TIV) in healthy children (under 19 years of age) has been examined in a number of meta-analyses, and the overall vaccine efficacy estimates were similar for laboratory confirmed influenza (59-63%) and clinical cases (36-45%) *(132, 133)*. For children aged less than 5 years the vaccine efficacy against influenza is broad ranging, from 12 to 83% *(134)*. The inactivated influenza vaccine is safe and well-tolerated in children (130).

Vaccination against pneumococcal disease

Streptococcus pneumoniae (pneumococcus) is the most important bacterial pathogen in children younger than 5 years. Pneumococcus frequently colonizes the nasal mucosa of children and adults, especially during the winter months. Up to 70% of children who attend day care are colonized with pneumococci during the colder part of the year *(135)*. Pneumococcus in humans causes a wide range of different diseases. Non-invasive (mucosal) diseases – acute otitis media (AOM), pneumonia and sinusitis, are more common than invasive. Invasive pneumococcal diseases (IPD) are characterized by the penetration of pneumococci from the respiratory mucosa into the bloodstream or other primary sterile sites (cerebrospinal fluid, pleural, peritoneal, or articular cavity). Of all the IPDs, the most common is occult bacteremia characterised by fever, chills, and leukocytosis, frequently without concomitant other signs of disease *(135, 136)*.

Significant differences in the incidence of IPD in various parts of the world exist. Prior to the introduction of the universal active immunization among children younger than two years the incidence of IPD in US amounted to 188 cases IPB per 100 000 children annually. In Western Europe, the incidence of IPD is significantly lower and amounts to 30-90 patients per 100 000 children younger than 2 years per year. Notwithstanding the significant differences in incidence, IPD is now in all parts of the world the most common invasive bacterial disease in young children. Although more than 90 different pneumococcal serotypes exist, the majority of IPD cases in children occur as a result of infection by the different serotypes. Newly developed pneumococcal conjugate vaccines (PCV), which contain ten (10PCV) or thirteen (13PCV) different capsular antigens, can thus prevent most cases of IPD. Although often affecting healthy children, the incidence of IPD is higher in patients with certain chronic conditions / diseases like those with congenital heart disease, chronic lung, kidney and liver disease, cerebrospinal fluid leaks, children with cochlear implants and children with congenital and acquired immunodeficiencies *(137-141)*.

In Croatia the incidence of IPD is highest among children younger than 2 years – 36.8 / 100 000 children per year (95% CI 27.7 to 48.9). In children aged 2-5 years the incidence of IPD is 16.3 / 100 000 (95% CI 11.5 to 22.9), while in those older than 5 years the incidence of IPD is 2.9 / 100 000 (95% CI 1 0.8 to 4.6) (Table 2.) *(142)*. The most common serotypes are 14, 6B, 18 F and 23F, which account for 67% of the isolates. Analyzing the vaccine coverage of pneumococcal isolates taking into account their serodistribution it could be calculated that the majority of IPD cases in Croatia could be prevented using 10PCV or 13PCV (Table 3.) *(141, 142)*.

However, use of PCV only for children at increased risk for developing IPD will not significantly affect the incidence of disease in the entire Croatian population. Experience from countries that have introduced universal immunization with PCV has showed, however, that the PCV significantly reduces the incidence of IPV not only among vaccine recipients, but also in the non-immunized rest of the population *(141, 143, 144)*. Another interesting observation from nations who implemented vaccination for the whole population of infants is that the universal usage of PCV significantly reduced the number of non-invasive forms of pneumococcal disease such as AOM and pneumonia *(141, 145)*. Although the favourable effect of PCV on the epidemiology of IPD is not questionable, bearing in mind the differences in the composition of available vaccines, before a final decision on the introduction of certain PCV in the national immunization program is made, careful seroepidemiological study in each population should be done, as a prerequisite for the selection of an optimal vaccine *(146)*.

Vaccination against rotavirus infection

Rotavirus (RV) is a major cause of infectious diarrhoea in children less than 5 years of age worldwide, responsible for more than 140 million episodes annually *(147)*. In developed nations community-acquired RV (CARV) infection is an important cause of morbidity in primary healthy children, with a significant impact on the total medical costs. Concurrently, nosocomial RV (NRV) infections represent a major component of hospital-acquired infections in children (31–87% of all nosocomial enteric infections) causing significant prolongation of hospitalization and increasing the amount of medical costs *(147–151)*. Both CARV and NRV are characterised by seasonality which is especially expressed in temperate climate zones and peak of incidence coincides with cooler part of the year (from November to May) *(147, 152, 153)*. Although numerous different types of RV exist, the majority of human infections are caused by a limited number of strains belonging to group A geno/ serotypes (154). No more than 4 RV serotypes: (G1P[8], G2P[4], G3P[8] and G4P[8]), have been linked to 90–95% of all hospitalized RV cases in Europe *(154-156)*.

TABLE 2

The incidence of invasive pneumococcal disease (IPD) in Croatian children younger than 14 years in years 2001, 2005 and 2006*

Year	Age (year)	Estimated population	Incidence of IPD	95% CI
2001	<2	38 948	33.9	19.7-58.4
	2-5	69 045	11.8	5.9-25.5
	5-14	232 347	2.2	0.9-5.2
2005	<2	40 066	34.7	20.9-57.7
	2-5	66 410	14.3	7.4-27.5
	5-14	241 117	3.1	1.4-6.5
2006	<2	47 056	41.0	26.4-63.5
	2-5	66 609	22.2	13.6-36.3
	5-14	240 158	3.5	1.7-7.4
Total	**<2**		**36.8**	**27.7-48.9**
	2-5		**16.3**	**11.5-22.9**
	5-14		**2.9**	**1.8-4.6**

*According to:
142. GUŽVINEC M, TEŠOVIĆ G, TAMBIĆ-ANDRAŠEVIĆ A, ŽIDOVEC-LEPEJ S, TROŠELJ VUKIĆ, B, BEGOVAC J 2008 Epidemiology of invasive *Streptococcus pneumoniae* disease in Croatian children. *Med Sci Monit 14:* PH59-64

TABLE 3

Analysis of vaccinal coverage of pneumococcal serotypes that caused invasive pneumococcal disease by conjugate vaccines registerred in Croatia**

	2001/06	2007/09	
vaccine	**Number of isolates (%)**	**Number of isolates (%)**	**p-value***
10 PCV	83 (83,0)	95 (74,2)	0,32 (NS)
13 PCV	90 (90,0)	112 (87,5)	0,77 (NS)

*Fisher- exact test
NS – statistically nonsignificant
**Acording to: 141. TEŠOVIĆ G, GUŽVINEC M, TAMBIĆ-ANDRAŠEVIĆ A 2011 Invazivna pneumokokna bolest u djece. *Pediatr Croat 55:* 75-80

RV gastroenteritis is a highly contagious disease transmitted by fecal-oral route. The usual precaution measures are ineffective in transmission control. Breastfeeding also does not contribute to the protection against infection / disease caused by RV. Vaccination, thus remains the only effective preventive measure in reducing the burden of RV infection *(147)*. The development of both oral RV live-attenuated vaccines currently available on the market – pentavalent human-bovine reassortant vaccine (RV5) and monovalent human vaccine (RV1) was based on the observation that natural infection could serve as an immunizing process. Namely, after primary infection with wild RV strains, the probability for reinfection with any genotype of the virus is reduced by 40%. Previous RV infection reduces the likelihood for symptomatic reinfection by 75% and the probability that the next RV infections would cause severe gastroenteritis for 88% *(157)*. Immunogenicity, reatogenicity and efficacy of both vaccines were tested in numerous prelicensure studies that included more than 100 000 vaccinees. All studies showed good immunogenicity and low reactogenicity *(156)*. Later experiences from nations that included RV vaccines into their national immunization programs further confirmed the previous observations *(158, 159, 160)*. Universal immunization against RV infection significantly reduced the number of diarrheal episodes as well as the number of hospitalizations *(158, 159)*. Although regional differences in the distribution of serotypes exist it seems that both vaccines give excellent protection against RV disease, especially against severe forms *(154, 158, 159)*.

In Croatia, the active immunization against RV infection was introduced in 2011 just for certain risk populations: 1. prematures born before the end of the 33rd week of gestation; 2. infants with congenital heart defects; 3. infants with congenital metabolic disorders; 4. infants with chronic liver and kidney diseases and 5. infants with serious neurological damage *(129)*. Infants should be vaccinated within the first six months of age with two (for RV1) or three (for RV5) doses of vaccine. First dose of vaccine should be applied after the age of six weeks *(129)*.

CONCLUSION

The immunization program in Croatia has been based on mandatory vaccinations for more than 60 years. Since the introduction of the first vaccines against diphtheria and tuberculosis in 1948, the program has undergone many changes and improvements, particularly in the last decade. The favourable impact of vaccination on the epidemiology of infectious diseases in Croatia is immense. Thanks to the continuous vaccination with high coverage rates some diseases, like poliomyelitis, measles, rubella, CRS and invasive Hib disease have disappeared or are about to disappear from Croatian population. Although the CVP in Croatia is undoubtedly effective and based on modern vaccines, further adjustments and the introduction of new vaccines, particularly vaccines against IPD and RV infection is highly desirable.

Acknowledgement: The author wishes to thank Ms. Arijana Pavelic for her help during the preparation of this manuscript.

REFERENCES

1. INTERNATIONAL BANK FOR RECONSTRUCTION AND DEVELOPMENT 1993 World Development Report: Investing in Health. Available from: http://files.dcp2.org/pdf/ World Development Report 1993.
2. WORLD HEALTH ORGANIZATION 2005 Immunization against disease of public health importance: fact sheet no. 288. Available from: http://www.who.int/mediacentre/factsheets/fs288/en/index.html.
3. PLOTKIN S L, PLOTKIN S A 2004 A short history of vaccination. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 1-15
4. MULLANY L 2002 Considerations for implementing a new combination vacine into managed care. *Am J Manag Care 9:* S23-S29
5. SALISBURY D M, OLIVÉ J M 2004 Immunization in Europe. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 1387-1406
6. ADA G 2004 The immunology of vaccination. *In*: Plotkin S A, Orenstein W A, Offit P A, *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 31-45
7. EBBERT G E, MASCOLO E D 2004 Vaccine manufacturing. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 53-68
8. ZEPP F 2010 Priniciples of vaccine design – Lessons from the nature. *Vaccine 28S:* C14-C24
9. SIEGRIEST CA 2008 Vaccine immunology. *In*: Plotkin SA, Orenstein WA, Offit PA *(eds)* Vaccines. 5th edition. Elsevie, New York, p 17-36
10. KAIĆ B, GJENERO-MARGAN I, BRZOVIĆ M *et al* 2007 Vaccine regulations in Croatia. *Coll Antropol 31:* 117-20
11. WAISBORD S, LARSON HJ 2005 Why invest in communication for immunization? Evidence and lessons learned. Baltimore: Johns Hopkins Bloomberg School of Public Health, Center for Communication Programs, Health Communication Partnership.
12. ORENSTEIN WA, RODEWALD LE, HINMAN AR 2004 Immunization in the United States. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 1357-86
13. CENTERS FOR DISEASE CONTROL AND PREVENTION 1999 Intussusception among recipients of rotavirus vaccine – United States, 1998-1999. *MMWR 48:* 577-81
14. MILLER E, GOLDACRE M, PUGH S *et al* 1993 Risk of aseptic meningitis after measles, mumps and rubella vaccine in UK children. *Lancet 341:* 979–82
15. CENTERS FOR DISEASE CONTROL AND PREVENTION 1999 Impact of vaccines universally recommended for children – United States, 1900-1999. *MMWR 48:* 243-8
16. LOPALCO P L, DE CARVALHO H G, KREIDL P, LEITMEYER K, GIESECKE J 2009 Childhood vaccination schedules in Europe vary widely. Is this a problem? *Bundesgesundheitsbl 52:* 1095–8
17. CENTRAL EUROPEAN VACCINATION AWARENESS GROUP. Available from: http://www.cevag.org/index.php
18. VACCINE EUROPEAN NEW INTEGRATED COLLABORATION EFFORT (VENICE) 2007 Report on First survey of Immunisation Programs in Europe. Available from: http://venice.cineca.org/Report_II_WP3.pdf
19. The Treaty of the European Union, consolidated version 2002 Available from: http://eur-lex.europa.eu/en/treaties/dat/12002E/htm/C_2002325EN.003301.html)
20. Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control. Available from: http://eur- lex.europa.eu/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=EN&numdoc=32004R0851&model=guicheti
21. HRVATSKI ZAVOD ZA JAVNO ZDRAVSTVO 2001 Kalendar kontinuiranog cijepljenja u Hrvatskoj u 2011. godini. Available from: http://www.hzjz.hr/epidemiologija/cijepljenje.htm
22. STARKE J R 2009 Tuberculosis. *In*: Feigin R D, Cherry J D, Demmler-Harrison G J, Kaplan S L J *(eds)*. Feigin and Cherry's Textbook of Pediatric Infectious Diseases. 6th edition. W.B. Saunders Company, Philadelphia, p 1426–68
23. JURČEV-SAVIĆEVIĆ A, KATALINIĆ-JANKOVIĆ V, GJENERO-MARGAN I *et al*. 2011 Epidemiological patterns of tuberculosis in Croatia in the period 1996-2005. *Coll Antropl 35:* 523–8
24. HRVATSKI ZAVOD ZA JAVNO ZDRAVSTVO 2010 Hrvatski zdravstveno-statistički ljetopis za 2010. godinu. Available from: http://www.hzjz.hr/publikacije/hzs_ljetopis/index.htm
25. SMITH K C, STARKE JR. BACILLE CALMETTE-GUERIN VACCINE 2004 *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)*. Vaccines. 4th edition. Saunders, Philadelphia, p 179-209
26. WORLD HEALTH ORGANIZATION 1986 Tuberculosis Control Program and Expanded Program on Immunization. Efficacy of infant BCG immunization. *Wkly Epidemiol Rec 28:* 216-8
27. LAVANCHY D 2004 Hepatitis B virus epidemiology, disease burden, treatmenst and current and emerging prevention and control measures. *J Viral Hepatol 11:* 97-107
28. WORLD HEALTH ORGANIZATION 2002 Global Prevalence of Hepatitis A, B and C. *Wkly Epidemiol Rec 77:* 6
29. MAHONEY F J 1999 Update on diagnosis, management and prevention of hepatitis B virus infection. *Clin Microbiol Rev 12:* 1682-3
30. LESNIKAR V 2005 Epidemiologija hepatitisa B i hepatitisa C u Hrvatskoj. *Acta Med Croat 59:* 377-81
31. MULIĆ R, UGLEŠIĆ L, KLIŠMANIĆ Z *et al*. 2006 Epidemiološke karakteristike hepatitisa B u Splitsko-Dalmatinskoj županiji. *Liječ Vjesn 128:* 65–71
32. LESNIKAR V 2005 Prevencija hepatitisa B i hepatitisa C. *Acta Med Croat 59:* 383-8
33. BONANNI P 1998 Universal hepatitis B immunization: infant and infant plus adolescent immunization. *Vaccine 16:* 17-22
34. TEŠOVIĆ G 2007 Akutni tijek bolesti u djece – analiza liječenih od akutnog hepatitisa B na pedijatrijskom odjelu Klinike za infektivne bolesti »Dr. Fran Mihaljević« u Zagrebu od 1996. do 2005. godine. *Medix 13:* 89-92
35. KAIĆ B, GJENERO-MARGAN I, ALERAJ B *et al* 2008 Promjene u Programu obveznih cijepljenja u Republici Hrvatskoj u 2008. godini. Hrvatski časopis za javno zdravstvo 7. Available from: http://www.hcjz.hr/old/clanak.php?id=13742
36. MAST E, MAHONEY F, KANE M, MARGOLIS H 2004 Hepatitis B Vaccine. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 299-337
37. WORLD HEALTH ORGANIZATION 2000 Fact sheet no. 204: Hepatitis B. Available from : http://www.who.int/mediacentre/factsheets/fs204/en/index.html
38. KEATING G M, NOBLE S 2003 Recombinant Hepatitis B Vaccine (Engerix-B®). *Drugs 63:* 1021-51
39. BORČIĆ B, SINDIK MILOŠEVIĆ T, DŽEPINA MLINARIĆ A, DOBROVŠAK-ŠOUREK V, LJUBIČIĆ M, GOTOVAC P 1996 Imunost hrvatskog pučanstva na difteriju 1994. godine. *Liječ Vjesn 118:* 227-9
40. WHARTON M, VITEK C R 2004 Diphteria toxoid. *In*: Plotkin SA, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 211-228

41. WASSILAK S G F, ROPER M H, MURPHY T V, ORENSTEIN W A 2004 Tetanus toxoid. *In:* Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 745-81
42. BUKOVSKI-SIMONOSKI S 2007 Istraživanje imunološkog odgovora osoba srednje i starije dobi nakon provedenog cijepljenja protiv tetanusa primjenom dvije doze cjepiva / doktorska disertacija. Medicinski fakultet Sveučilišta u Osijeku, Osijek, p 1-125
43. GUILFOILE P 2008 Tetanus. 1st edition. Chelsea House, New York, p 1- 100
44. BAĆE A 2005 Hripavac – klinika, dijagnostika, liječenje i profilaksa. *Medicus 14:* 127-35
45. TEŠOVIĆ G 2004 Potreba za arteficijelnom ventilacijom u bolesnika s teškim oblikom hripaca / magistarski rad. Medicinski fakultet Sveučilišta u Zagrebu, Zagreb, p 1-109
46. DE MELKER H E, SCHELLEKENS I F, NEPPELENBROEK S E, MOOL F R, RUMKE H C, CONYN-VAN SPAENDONCK M A 2000 Reemergence of pertussis in the highly vaccinated population of the Netherlands: observation of the surveillance data. *Emerg Infect Dis 6:* 348-357
47. CHERRY J D, BRUNELL P A, GOLDEN G S, KARZON D T 1988 Report of the task force on pertussis and pertussis immunization. *Pediatrics 81:* 939-84
48. BLACK S 1997 Epidemiology of pertussis. *Pediatr Infect Dis J 16:* S85-9
49. CHERRY J D, HEININGER U 1998 Pertussis and other *Bordetella* infections. *In*: Feigin R D, Cherry J D *(eds)* Textbook of pediatric infectious diseases, 4th edition. WB Saunders, philadelphia, p 1423-40
50. BORČIĆ V, DOBROVŠAK-ŠOUREK V 1995 Utjecaj obveznih cijepljenja na pobol od određenih zaraznih bolesti. *In:* Hajsig D *(ed)* Vakcinologija danas i sutra. Hrvatsko mikrobiološko društvo, Zagreb, p 42-7
51. KÖHLER-KUBELKA N 1957 Rezultati seroloških ispitivanja kod djece cijepljene našom vakcinom Di-te-Per. *Lij Vjes 11-12:* 745-8
52. IKIĆ D, KÖHLER-KUBELKA N, HRABAR A, HIGY-MANDIĆ L J, MRAVUNAC B, PREJAC N 1971 Efficiency of pertussis vaccine. Proc. Symposium on Bacterial Vaccines. Yug Acad Sci Arts. Zagreb, 27&28 October, p 321-4
53. HIGY-MANDIĆ LJ 1966 Standardization of the pertussis component in DTP (diphteria-tetanus-pertussis) combined prophylaxis. I. Relationship between the results of parallel assays of plain and adsorbed preparations. *Radovi Imunol zavoda 5-6*: 85-102
54. GUISO N 2003 An overview of pertussis in the population: a global approach. *In*: Book of abstracts. Giardini Naxos-Taormina: 21st Annual meeting of the European Society for Pediatric Infectious Disease, p 6
55. CHRISTIE C D C, PEDS D M, BALTIMORE R S 1989 Pertussis in neonates. *Am J Dis Child 143:* 1199-1202
56. AOYAMA T, TAKEUCHI Y, GOTO A, IWAI H, MURASE Y, IWATA T 1992 Pertussis in adults. *Am J Dis Child 146:* 163-6
57. SMITH C, VYAS H 2000 Early infantile pertussis; increasingly prevalent and potentially fatal. *Eur J Pediatr 159:* 898-900
58. VON KONIG C H W, HALPERIN S, RIFFELMANN M, GUISO N 2002 Pertussis of adults and infants. *Lancet Infect Dis 2:* 744-50
59. HOFFMAN H J, HUNTER J C, DAMUS K *et al* 1987 Diphteria-tetanus-pertussis immunization and sudden infant death: Results of the National Institute of Child Health and Human Development Cooperative Epidemiological Study of Sudden Infant Death Syndrome Risk Factors. *Pediatrics 79:* 598-611
60. WALER A M, JICK H, PERERA D R *et al* 1987 Diphteria-tetanus-pertussis immunization and sudden infant death syndrome. *Am J Public Health 77:* 945-51
61. ROMANUS V, JONSELL R, BERGQUIST S O 1987 Pertussis in Sweden after cessation of general immunization in 1979. *Pediatr Infect Dis J 6:* 364-71
62. POLLOCK T M, MILLER E, LOBB J 1984 Severity of whooping cough in England before and after the decline in pertussis immunization. *Arch Dis Child 59:* 162-5
63. PLOTKIN S A, CADOZ M 1997 The acellular pertussis vaccine trials: an interpretation. *Pediatr Infect Dis J 16:* 508-17
64. KIMURA M, KUNO-SAKAI H 1990 Developments in pertussis immunization in Japan. *Lancet 39:* 57-9
65. OLIN P 1997 The best acellular pertussis vaccines are multicomponent. *Pediatr Infect Dis J 16:* 517-9
66. SCHMITT H J, BOOY R, WEIL-OLIVIER C, VAN DAMME P, COHEN R, PELTOLA H 2003 Child vaccination policies in Europe: a report from the Summits of Independent European Vaccination Experts. *Lancet Infect Dis 3:* 103-8
67. SUTTER R W, KEW O M, COCHI S L 2004 Poliovirus vaccine – Live. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 651-705
68. PLOTKIN S A, VIDOR E 2004 Poliovirus vaccine-Inactivated. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 625-649
69. BORČIĆ B, KRUŽIĆ V, KAIĆ B, LJUBIN-STERNAK S, LJUBIČIĆ M, DOBROVŠAK-ŠOUREK V 1998 Immunity of the Croatian population to poliomyelitis – a serosurvey. *Acta Med Croat 52:* 229-33
70. IKIĆ D, JANČIKIĆ B, LULIĆ D *et al* 1964 Konačni izvještaj o masovnom cijepljenju protiv poliomijelitisa živom oralnom vakcinom Koprowski u Hrvatskoj. *Lijekovi 2:* 56-65
71. TEŠOVIĆ G, BOŽINOVIĆ D, VOKIĆ Ž, KNEZOVIĆ I, VUKELIĆ D, KRUŽIĆ V. 1996 Vaccine Associated Paralytic Poliomyelitis in a Hypogammaglobulinemic Child. *Neurol Croat 45:* 23-8
72. KEW O M, SUTTER R W, AYLWARD R B 2005 Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annu Rev Microbiol 59:* 587-635
73. WHO 2006 Vaccine-derived polioviruses – update. *Wkly Epidemiol Rec 81:* 398-404
74. WHO 2003 Introduction of inactiveted poliovirus vaccine into oral poliovirus vaccine-using countries. *Wkly Epidemiol Rec 78:* 241-52
75. WENGER J D, WALD J I 2004 Haemophilus influenzae vaccine. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 229-68
76. KNEZOVIĆ I, ČELJUSKA-TOŠEV E, KOVAČEVIĆ G, TEŠOVIĆ G 2011 Invazivne bakterijske bolesti prije i nakon uvođenja cijepljenja protiv *Haemophilusa influenzae* tipa b. *Pediatr Croat 55:* 91-7
77. PICHICHERO M E 2000 New combination vaccines. *Pediatr Clin North Am 47:* 407-26
78. OBARO S K, PALMER A 2003 Vaccines for children: policies, politics and poverty. *Vaccine 21:* 1423-31
79. PLOTKIN S A, LIESE J, MADHI S A, ORTIZ E 2011 A DTaP-IPV/PRP-T vaccine (Pentaxim™): a review of 16 years' clinical experience. *Expert Rev Vacc 10:* 981-1005
80. DHILLON S 2010 DTPa-HBV-IPV/Hib vaccine (Infanrix hexa™). A review of its use as primary and booster vaccination. *Drugs 70:* 1021-58
81. TREGNAGHI M, ZAMBRANO B, SANTOS-LIMA E 2012 Antibody Persistence After a Primary Series of a New DTaP-IPVHepB--PRP-T Combined Vaccine or Separate DTaP-IPV//PRP-T and Hepatitis B Vaccines at 2, 4, and 6 Months of Age and the Effect of a Subsequent DTaP-IPV//PRP-T Booster Vaccination at 18 Months of Age in Healthy Argentinean Infants. *Pediatr Infect Dis J 31:* e24-e31
82. STREBEL P M, PAPANIA M J, HALSEY N A 2004 Measles vaccine. *In:* Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 389-440
83. BORČIĆ B, SMERDEL S, ELDAN J A *et al* 1989 Stvaranje i trajanje imunosti u djece cijepljene protiv ospica vakcinom Edmonston-Zagreb. *Liječ Vjesn 111:* 131-4
84. ANONIMOUS 1975 Pravilnik o uvjetima i načinu obavljanja obvezatne imunizacije, seroprofilakse i kemioprofilakse protiv zaraznih bolesti. *Služ list SFRJ 19:* 602
85. ANONIMOUS 1980 Pravilnik o uvjetima i načinu obavljanja obvezatne imunizacije, seroprofilakse i kemioprofilakse protiv zaraznih bolesti. *Služ list SFRJ 8:* 235
86. BORČIĆ B, MAŽURAN R, KAIĆ B 2003 Immunity to measles in the Croatian population. *Eur J Epidemiol 18:* 1079-83
87. KAIĆ B, GJENERO-MARGAN I, KUREČIĆ-FILIPOVIĆ S, MUSCAT M 2009 A measles outbreak in Croatia, 2008. Euro Surveill 14(1):pii=19083. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19083
88. MUSCAT M, BANG H, WOHLFAHRT J, GLISMANN S, MØLBAK K 2009 Measles in Europe: an epidemiological assessment. *Lancet 373:* 383-9
89. FILIA A, DE CRESCENZO M, SEYLER T, BELLA A, CIOFI DEGLI ATTI M L, NICOLETTI L, MAGURANO F, SALMASO S 2008 Measles resurges in Italy: preliminary data from September

2007 to May 2008. Euro Surveill 13(29):pii=18928. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18928

90. IKIĆ D, JUZBAŠIĆ M, BECK M, *et al* 1972 Attenuation and characterization of Edmonston-Zagreb measles virus. *Ann Immunol Hing 16:* 175-81
91. ABU ELDAN J, BORČIĆ B, SMERDEL S 1991 Persistence of immunity against measles in persons immunized with Edmonston-Zagreb vaccine. *Acta Med Croat 45:* 297-304
92. KAIC B, GJENERO-MARGAN I, ALERAJ B, VILIBIC-CAVLEK T, SANTAK M, CVITKOVIĆ A, NEMETH-BLAZIC T, IVIC HOFMAN I 2010 Spotlight on measles 2010: Excretion of vaccine strain measles virus in urine and pharyngeal secretions of a child with vaccine associated febrile rash illness, Croatia, March 2010. Euro Surveill 15(35): pii=19652
Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19652
93. LITMAN N, BAUM S G, MUMPS VIRUS 2005 *In*: Mandell G L, Bennett J E, Dolin R, *(eds)* Principles and Practice of Infectious Diseases. 6th edition. Elsevier Inc, Philadelphia, p 2003-7
94. AMERICAN ACADEMY OF PEDIATRICS. Mumps. *In*: Pickering L K *(ed)* Red Book: 2003 Report of the Comittee on infectious Diseases. 26. edition. American Academy of Pediatrics, Elk Grove Village, IL, p 439-43
95. PELTOLA H, HEINONEN OP, VALLE M, *et al* 1994 The elimination of indigenous measles, mumps and rubella from Finland by a 12-year, two-dose vaccination program. *N Eng J Med 331:*1397--402
96. WINTERHALTER-MATAS A 1986 Istraživanje genetskih markera virusa parotitisa / doktorska disertacija. Medicinski fakultet Sveučilišta u Zagrebu, Zagreb, p 1 – 80
97. KRUGMAN S, WARD R, KATZ S L 1977 Mumps (epidemic parotitis). *In*: Krugman S, Ward R, Katz S L *(eds)* Infectious Diseases of Children. 6th edition. CV Mosby Comp, St. Louis, p 181-93
98. BALRAJ V, MILLER E 1995 Complications of mumps vaccines. *Rev Med Virol 5:* 219-27
99. GALAZKA A M, ROBERTSON S E, KRAIGHER A 1999 Mumps and mumps vaccine: a global review. *Bull World Health Org 77:* 3-14
100. BECK M, WEISZ-MALEČEK R, MEŠKO-PREJAC M *et al* 1989 Mumps vaccine L-Zagreb, prepared in chick fibroblasts: I. Production and field trials. *J Biol Stand 17:* 85-90
101. PERIĆ D 1999 Rizik od ospica i zaušnjaka kod novaka hrvatske vojske/magistarski rad. Medicinski fakultet Sveučilišta u zagrebu, Zagreb, p 1-67
102. TEŠOVIĆ G 2005 Virusna cjepiva – stanje u Hrvatskoj početkom 2005. godine. *Paediatr Croat 49:* 171-8
103. PLOTKIN S A 2004 Mumps vaccine. *In*: Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 441-62
104. KOŠUTIĆ GULIJA T 2002 Mutacije gena za hemaglutinin-neuraminidazu pri atenuaciji virusa mumpsa/magistarski rad. Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, Zagreb, p 1 – 60
105. JANČIKIĆ B, ŠVEL I, SMERDEL S *et al* 1976 Application of combined live vaccine against measles, rubella and mumps. Clinical-epidemiological and laboratory results. *Arhiv za zaštitu majke i djeteta 11:* 151-9
106. TEŠOVIĆ G 2006 Neurovirulencija cjepnog soja virusa mumpsa L-Zagreb/doktorska disertacija. Medicinski fakultet Sveučilišta u Zagrebu, Zagreb, p 1-137
107. WAKEFIELD A J, MURCH S H, ANTHONY A *et al* 1998 Ileal lymphoid nodular hyperplasia, non-specific colitis and pervasive developmental disorder in children. *Lancet 351:* 637-41
108. MILNE L M 2001 Difficulties in assessing the relationship, if any, between mumps vaccination and diabetes mellitus in childhood. *Vaccine 19:* 1018-25
109. FARRINGTON P, PUGH S, COLVILLE A *et al* 1995 A new method for active surveillance of adverse effects from diphteria/tetanus/pertussis and measles/mumps/rubella vaccines. *Lancet 345:* 567-9
110. PATJA A M, DAVIDKIN I, KURKI T, KALLIO M J, VALLE M, PELTOLA H 2000 Serious adverse events after measles-mumps-rubella vaccination during a fourteen-year prospective follow-up. *Pediatr Infect Dis J 19:* 1127-34
111. PLESNER A M, HANSEN F J, TAUDORF K, NIELSEN L H, LARSEN C B, PEDERSEN E 2000 Gait disturbances interpeted as cerebellar ataxia after MMR vaccination at 15 months of age: a follow-up study. *Acta Paediatr 89:* 58-63
112. MADSEN K M, HVIID A, VESTERGAARD M *et al* 2002 A population-based study of measles, mumps and rubella vaccination and autism. *N Eng J Med 347:* 1477-82
113. ANDREWS N, MILLER E, TAYLOR B *et al* 2002 Recall bias, MMR and autism. *Arch Dis Child 87:* 493-4
114. JEFFERSON T, PRICE D, DEMICHELI V, BIANCO E 2003 Unintended events following immunization with MMR: a systematic review. *Vaccine 21:* 3954-60
115. SUGIURA A, YAMADA A 1991 Aseptic meningitis as a complication of mumps vaccination. *Pediatr Infect Dis J 10:* 209-13
116. KIMURA M, KUNOSAKAI H, YAMAZAKI S *et al* 1996 Adverse events associated with MMR vaccines in Japan. *Acta Paediatr Jpn 38:* 205-11
117. BLACK S, SHINEFIELD H, RAY P *et al* 1997 Risk of hospitalization becuase of aseptic meningitis after measles-mumps-rubella vaccination in one- to two-year-old children:an analysis of the Vaccine Safety Datalink (VSD) Project. *Pediatr Infect Dis J 16:* 500-3
118. ČIŽMAN M, MOZETIČ M, RADEŠČEK-RAKAR R, PLETERSKI-RIEGLER D, SUŠEC-MICHIELI M 1989 Aseptic meningitis after vaccination against measles and mumps. *Ped Infect Dis J 8:* 302-8
119. TEŠOVIĆ G, LESNIKAR V 2006 Aseptic meningitis after vaccination with L-Zagreb mumps strain – virologically confirmed cases. *Vaccine 24:* 6371-3
120. DAYAN G H, RUBIN S 2008 Mumps outbreaks in vaccinated populations: are available mumps vaccines effective enough to prevent outbreaks? *Clin Infect Dis 47:* 1458-67
121. DAYAN G H, QUINLISK M P, PARKER A A *et al* 2008 Recent resurgense of mumos in the United States. *N Engl J Med 358:* 1580-9
122. CARR M J, MOSS E, WATERS A *et al* 2010 Molecular epidemiologicam evaluation of the recent resurgence in mumps virus infections in Ireland. *J Clin Microbiol 48:* 3288-94
123. PLOTKIN S A 2006 The history of rubella and rubella vaccination leading to elimination. *Clin Infect Dis 43:* S164–8
124. USONIS V, ANCA I, ANDRÉ F *et al* 2011 Rubella revisited:where are we on the road to disease elimination in Central Europe? *Vaccine 29:* 9141-7
125. WHO 2003 Strategic plan for measles and congenital rubella infection in the European Region of WHO. Copenhagen, WHO Regional Office for Europe; 2003. Available from: http://www.euro.who.int/document/e81567.pdf
126. WHO 2005 Eliminating measles and rubella and preventing congenital rubella infection. WHO European Region strategic plan; 2005–2010. Available from: www.euro.who.int/ data/assets/pdf file/0008/79028/E87772.pdf
127. WHO 2010 Renewed commitment to measles and rubella elimination and prevention of congenital rubella syndrome in the WHO European Region by 2015; Available from: http://www.euro.who.int/data/ assets/pdf file/0008/119546/RC60 edoc15.pdf
128. PLOTKIN S A, REEF S 2004 Rubella vaccine. *In:* Plotkin S A, Orenstein W A, Offit P A *(eds)* Vaccines. 4th edition. Saunders, Philadelphia, p 707-43
129. MINISTARSTVO ZDRAVLJA R H 2012 Program imunizacije, seroprofilakse i kemoprofilakse za posebne skupine stanovništva i pojedince pod posebnim rizikom. Official document.
130. USONIS V, ANCA I, ANDRÈ F *et al* 2010 Central European Vaccination Advisory Group (CEVAG) guidance statement on recommendations for influenza vaccination in children. *BMC Infect Dis 10:* 168
131. MERECKIENE J, COTTER S, NICOLL A, LEVY-BRUHL D, FERRO A, TRIDENTE G, ZANONI G, BERRA P, SALMASO S, O'FLANAGAN D, VENICE PROJECT GATEKEEPERS 2008 National seasonal influenza vaccination survey in Europe, 2008**.** Euro Surveill 13(43pii): 19017 Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19017
132. JEFFERSON T, RIVETTI A, HARNDEN A, DI PIETRANTONJ C, DEMICHELI V 2008 Vaccines for preventing influenza in healthy children**.** Cochrane Database Syst Rev CD004879
133. MANZOLI L, SCHIOPPA F, BOCCIA A, VILLARI P 2007 The efficacy of influenza vaccine for healthy children: a meta-analysis evaluating potential sources of variation in efficacy estimates including study quality**.** *Pediatr Infect Dis J 26:* 97-106

134. ZANGWILL K M, BELSHE R B 2004 Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination**.** *Pediatr Infect Dis J 23:* 189-97

135. KUZMAN I 2006 *Streptococcus pneumoniae*. *In*: Begovac J, Božinović D, Lisić M, Baršić B, Schönwald S *(eds)* Infektologija. 1st edition. Profil, Zagreb, p 579-88

136. BOŽINOVIĆ D 2006 Otitis media i mastoiditis. *In:* Begovac J, Božinović D, Lisić M, Baršić B, Schönwald S *(eds)*. Infektologija. 1st edition. Profil, Zagreb, p 149-53

137. PINEDA V, FONTANALS D, LARRAMONA H, DOMINGO M, ANTON J, SEGURA F 2002 Epidemiology of invasive *Streptococcus pneumoniae* infections in children in an area of Barcelona, Spain. *Acta Paediatr 91:* 1251-6

138. JEFFERSON T, FERRONI E, CURTALE F, ROSSI P G, BORGIA P 2006 Streptococcus pneumoniae in Western Europe: serotype distribution and incidence in children less than 2 years of life. *Lancet Infect Dis 6:* 405-10

139. REINERT R R 2004 Pneumococcal conjugate vaccines – a European perspective. *Int J Med Microbiol 294:* 277-94

140. ROBINSON K A, BAUGHMAN W, ROTHROCK G, BARRETT N L, PASS M, LEXAU C *et al* 2001 Epidemiology of invasive Streptococcus pneumoniae infections in the United States, 1995--1998: oportunities for prevention in the conjugate vaccine era. *JAMA 285:* 1729-35

141. TEŠOVIĆ G, GUŽVINEC M, TAMBIĆ-ANDRAŠEVIĆ A 2011 Invazivna pneumokokna bolest u djece. *Pediatr Croat 55:* 75-80

142. GUŽVINEC M, TEŠOVIĆ G, TAMBIĆ-ANDRAŠEVIĆ A, ŽIDOVEC-LEPEJ S, TROŠELJ VUKIĆ, B, BEGOVAC J 2008 Epidemiology of invasive *Streptococcus pneumoniae* disease in Croatian children. *Med Sci Monit 14:* PH59-64

143. WHITNEY C G, FARLEY M M, HADLER J *et al* 2003 Decline in invasive pneumococcal disease after the introduction of protein--polysaccharide conjugate vaccine. *N Eng J Med 348:* 1737-46

144. SHAFINOORI S, GINOCCHIO C C, GREENBERG A J, YEOMAN E, CHEDDIE M, RUBIN L G 2005 Impact of pneumococcal conjugate vaccine and the severity of winter influenza like-illness on invasive pneumococcal infections in children and adults. *Ped Infect Dis J 24:* 10-16

145. GRIJALVA C G, NUORTI J P, ARBOGAST P G, MARTIN S W, EDWARDS K M, GRIFFIN M R 2007 Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. *Lancet 369:* 1179-86

146. PRYMULA R, CHLIBEK R, IVASKIEVICIENE I, ET AL 2011 Pediatric pneumococcal disease in Central Europe. Eur J Clin Microbiol Infect Dis 30: 1311-20

147. PARASHAR U D, HUMMELMAN E G, BRESEE J S, MILLER M A, GLASS R I 2003 Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis 9:* 565–72

148. GLEIZES O, DESSELBERGER U, TATOCHENKO V *et al* 2006 Nosocomial rotavirus infection in European countries: A review of the epidemiology, severity and economic burden of hospital-acquired rotavirus disease. *Pediatr Infect Dis J 25:* S12-S21

149. GIANINO P, MASTRETTA E, LONGO P *et al* 2002 Incidence of nosocomial rotavirus infections, symptomatic and asymptomatic, in breast-fed and non-breast-fed infants. *J Hosp Infect 50:* 13–17

150. LE ROUX P, MARSHALL B, TOUTAIN F *et al* 2004 Nosocomial viral infections in a pediatric service: example of rotaviral gastroenteritis and respiratory syncytial viral bronchiolitis. *Arch Pediatr 11:* 908–915

151. MRUKOWICZ J Z, KROBICKA B, DUPLAGA M *et al* 1999 Epidemiology and impact of rotavirus diarrhoea in Poland. *Acta Paediatr 88:* 53–60

152. KOOPMANS M, BROWN D 1999 Seasonality and diversity of group A rotaviruses in Europe. *Acta Paediatr 88:* 14–9

153. BOKOVOI A G 2002 Nosocomial rotavirus infection among children. *Detskie Infectii 1:* 28–32

154. DESSELBERGER U, WOLLESWINKEL-VAN DEN BOSCH J, MRUKOWICZ J, RODRIGO C, GIAQUINTO C, VESIKARI T 2006 Rotavirus types in Europe and their significance for vaccination. *Pediatr Infect Dis J 25:* S30–S41

155. GENTSCH J R, WOODS P A, RAMACHANDRAN M *et al* 1996 Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. *J Infect Dis 174:* S30–S36

156. SANTOS N, HOSHINO Y 2005 Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol 15:* 29–56

157. DENNEHY P H 2006 Rotavirus vaccines: An update. *Pediatr Infect Dis J 25:* 839-40

158. RAES M, STRENS D, VERGISON A, VERGHOTE M, STANDAERT B 2011 Reduction in pediatric rotavirus-related hospitalizations after universal rotavirus vaccination in Belgium. *Pediatr Infect Dis J 30:* e120-e125

159. BUTTERY J P, LAMBERT S B, GRIMWOOD K *et al* 2011 Reduction in rotavirus-associated acute gastroenteritis following introduction of rotavirus vaccine into Australia's national childhood vaccine schedule. *Pediatr Infect Dis J 30:* S25-S29

160. PATEL M M, LOPEZ-COLLADA V R, BULHOES M M *et al* 2011 Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *New Eng J Med 364:* 2283-92