

U. PORTO



INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR
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EARLY CHILDHOOD VACCINATION SCHEDULES IN EUROPEAN UNION COUNTRIES: A REVIEW.

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Artigo de Revisão Bibliográfica

Mestrado Integrado em Medicina

Orientador

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Porto, 2017

Early Childhood Vaccination Schedules in European Union Countries: a review.

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According to: Experts Review of Vaccines

Acknowledgements

I am very grateful to my supervisor Dr^a Laura Marques for her support, assistance and advice during this thesis.

To Sara and Matilde for being my everything.

To Mario for all the support, and May the force continue with us.

To my mom and dad for their love and unconditional support, I owe you everything.

To my adorable sister...she is a very good friend. And to my brother...

To the best boss in the world, thank you for helping and supporting me always, Professor Jorge Mota.

To my best friends Teresa, Vera, Carla, Rita and Isabel...I love you.

To the knights of my heart: Rose, Sandra, Susana, Ana and Inês.

To the friends I have made during this degree, thank you for your support.

To my father in law...I know you would be very very happy and proud of me...lets toast to it.

To Dalva and D. Fernanda for being always available.

To all that in some way helped me in this hard path.

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Abstract

Introduction: Vaccination is the most important medical accomplishment; its effect on mortality reduction is unsurpassed by other medical achievements. Free circulation of people in the European Union (EU) and European Economic Area (EEA) has brought the need to analyze the different childhood immunization schedules among countries that form EU/EEA since; countries do not have a common immunization policy.

Areas covered: The aim of this review is to analyze early childhood immunization programs across EU/EEA at the light of the existing evidence. The immunization programs were accessed throughout the European Centre for Disease Prevention and Control dataset.

Expert commentary: The large variation observed, concerning both vaccines included and immunization schedules, between countries raises some questions and concern as; a child that moves from one country to another might easily miss a vaccine dose. Therefore, we present a recommendation, based on the available knowledge, for early childhood immunization schedule of the EU/EEA. To the best of our knowledge, this is the first review presenting a suggestion for early childhood immunization schedule in the EU/EEA.

Key Words: childhood, immunization, schedule, European Economic Area, vaccines.

1. Introduction

Vaccination is one of the most valuable medical accomplishments. Its effect on mortality reduction is unsurpassed by other medical achievements [1].

Free circulation of people in the European Union (EU) and European Economic Area (EEA) has brought the need to analyse the different childhood immunisation schedules among the 28 countries that form the EU/EEA. Considerable variations were observed, concerning both vaccines included and immunisation schedules, among countries, which raise several questions and concerns. As an example, a child moving from one country to another can easily miss an administration and, thus, decrease immunisation compliance. Nevertheless, all schedules work and a child who is protected in accordance with a programme is well-protected.

In this paper, we analyse the immunisation schedules of early childhood (until two years old), in 30 EU/EEA countries (28 EU countries plus Norway, Liechtenstein and Iceland) to highlight their similarities and differences considering the current evidence. Vaccination schedules of different countries were accessed through the European Centre for Disease Prevention and Control website (<http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx>) between May 2016 and December 2016.

2. Vaccines included in childhood vaccination schedules across European Economic Area

2.1. Tuberculosis vaccine

The bacilli Calmette-Guérin (BCG) vaccines are the oldest of the vaccines currently used [2]. BCG vaccinations aim to provide protection against severe forms of childhood tuberculosis (TB), such as TB meningitis and miliary TB [3]. BCG vaccinations do not prevent infection by *Mycobacterium tuberculosis* (*M. Tuberculosis*) [4]. They also do not prevent reactivation of latent pulmonary infections [4], but rather help the host to delay the growth of organisms in the lungs and lymph nodes, as this prevents their dissemination [2]. However, this is a very controversial vaccine, since its effects in randomised controlled trials and case studies have been inconsistent; some have demonstrated a great degree of protection, and others have not displayed any benefits [2,5].

This is a live attenuated vaccine that is dispensed using an intradermal route of administration with a syringe and needle, as the dosage can be measured precisely and the administration can be controlled; the deltoid is the most common site for administration [2]. As a live attenuated vaccine, it is expected to induce a T-dependent response; BCG-induced T-cells contribute to macrophage activation, and antibody production is inefficient [2].

The recommended dosage depends on the vaccine strain and the age of the receiver [2]. Some BCG strains are considered strong and others weak. Strong strains are more immunogenic, and provide better protection than weaker strains [2]. However, there is no agreement about which strain of BCG is optimal for universal use [2,5].

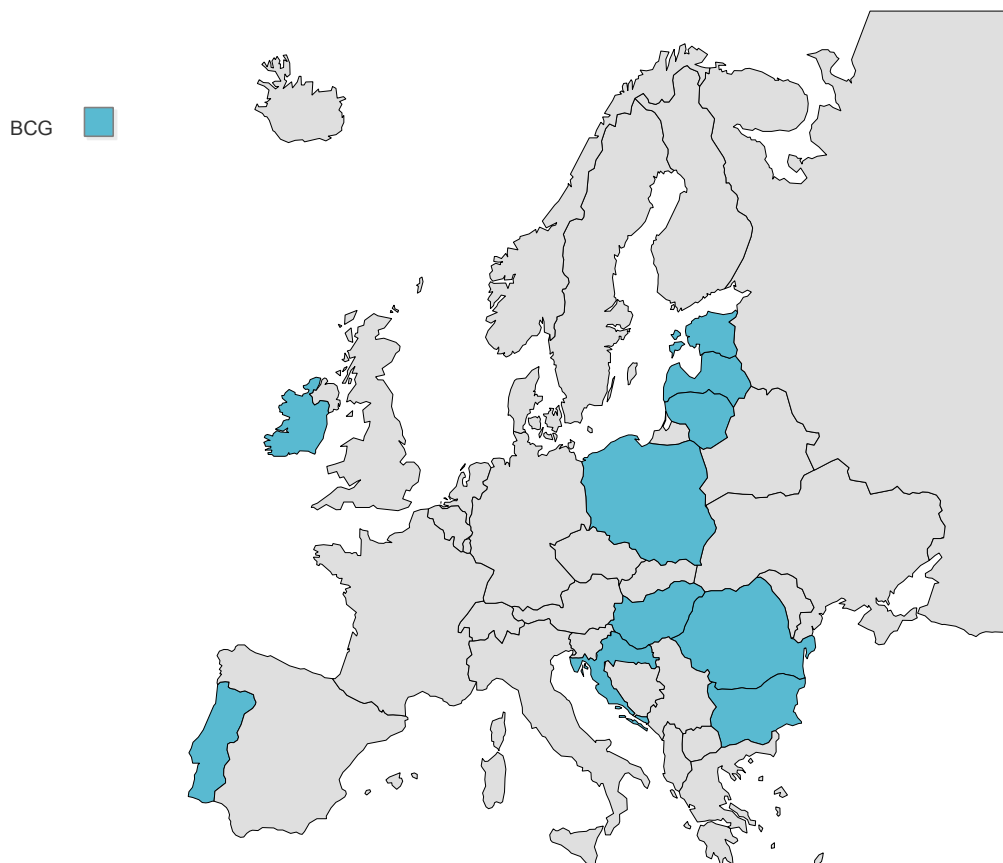
Nowadays, in the EU/EEA, only 10 countries (Figure 1) utilise BCG vaccines in their childhood immunisation schedules. All of them administer these vaccines once, from birth until the seventh day of life. However, the literature is not consensual concerning the age of administration. A clinical trial has demonstrated that delaying BCG vaccinations from birth to 10 weeks of age results in an enhanced memory T-cell response [6]. Furthermore, delaying the vaccinations until nine months of age was demonstrated to result in higher rates of immunogenic sensitivity in children [2]. Conversely, another clinical trial demonstrated that delaying BCG vaccinations from birth until 18 weeks of age lead to a decrease in Th1 responses [7]. However, delaying tuberculosis vaccinations would mean that the child would be unprotected for several months.

Studies have indicated that BCG might reduce childhood mortality from other causes, presumably because BCG promotes a T-helper-1 immune response [3]. Newborns vaccinated with BCG in the first days of life seem to develop a Th1 response, while non-vaccinated infants develop a Th2 response [8]. Therefore, BCG vaccines might stimulate the immune system and prevent anergy. Furthermore, children vaccinated after the first month of life were less anergic than children vaccinated during the first month [8]. BCG may have a more general effect on the immune system beyond protection against severe forms of childhood tuberculosis [8]. Authors have reported that BCG vaccines might be associated with a lower prevalence of anergy and might impact cellular immunity to other antigens, apart from tuberculin [8]. Furthermore, it seems that BCG vaccines induce nonspecific protection against distinct pathogens via epigenetic reprogramming of monocytes [9].

Kleinnijenhuis et al. [10] have documented that BCG vaccines can induce two distinct types of immune responses: a specific immune response involving antigen-specific T cells and memory conducting to protection against TB, and the induction of adaptive-trained immunity based on functional reprogramming of mononuclear phagocytes that leads to protective effects not only against TB, but also against other infections. Moreover, a recent study conducted in Spain indicated that BCG vaccinations might have additional protective effects against moderate to severe forms of respiratory infections sepsis requiring hospitalisation [11]. Therefore, studies have indicated that BCG vaccines might have nonspecific benefits [9,12]. However, it is difficult to conclude that BCG vaccines might have nonspecific benefits, since several confounding factors exist.

The World Health Organisation (WHO) recommends vaccination as soon as possible after birth in high-burden countries [4]. Countries with a low burden may choose to limit BCG vaccinations to neonates and infants of recognised high-risk groups [4].

Figure 1: BCG primary immunisation schedules across European Union/European Economic Area.



2.2. Rotavirus vaccines

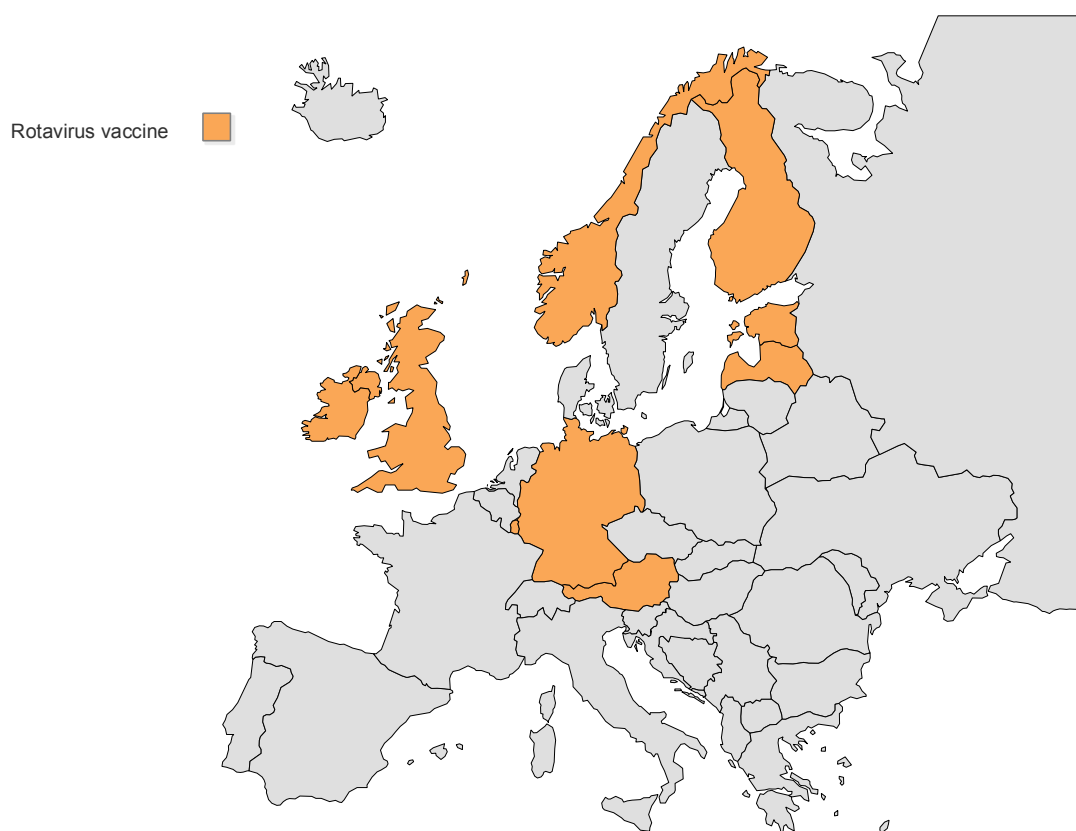
Rotavirus infection is the main cause of severe dehydrating diarrhoea disease in infants and toddlers worldwide [13]. Rotavirus is mainly transmitted by faecal-oral route [13], and between ages two and three, virtually all children are infected [2].

Even though WHO recommends that rotavirus vaccines should be included in all national immunisation programmes and considered a priority [14], only nine EU/EEA countries presently include it in their immunisation plans (Figure 2). Each country has its own immunisation schedule: seven countries administer the first dose at age two months, while two countries administer it at age six weeks; four countries dispense two doses, while five countries dispense two or more, depending on the chosen brand.

There are two available oral vaccines in the European market: Rotateq®, a pentavalent vaccine (RV5); and Rotarix®, a monovalent vaccine (RV1). The two vaccines differ in strain composition and administration schedules. Given the huge diversity and capability of the human rotavirus to change, vaccines need to efficiently protect against major circulation strains [13]. Rotarix® is an oral, live-attenuated human rotavirus of entirely human origin, vaccine-derived from the most common circulating wild-type strain G1P [13]. Its vaccination course consists of two doses, and the first dose may be administered at age six weeks. There should be an interval of at least four weeks between doses [15]. The vaccination course should preferably be administered before 16 weeks of age and should be completed by age 24 weeks. Rotateq® is an oral, live, human-bovine, reassortant, multivalent, rotavirus vaccine. It contains five live, human-bovine, reassortant rotavirus strains: VP7 (G) types, including G1, G2, G3, and G4, and VP4 (P) type [13]. Its vaccination course requires three doses [16]. According to the manufacturer, the first dose may be administered from the age of six weeks and no later than 12 weeks. There should be at least a 4-week interval between doses. The vaccination course should be completed by age 20 to 22 weeks. If necessary, the last dose may be given up to the age of 32 weeks. Both vaccines can diminish the number of rotavirus gastroenteritis cases [13].

Rotavirus vaccines have been associated with a short-term risk of intussusception, especially after the first dose and between one to seven days after vaccination [17]. A recent study has indicated that there is an increased risk for intussusception one to seven days after the first dose, for both vaccines, when the infant is three to five months of age [18].

Figure 2: Rotavirus primary immunisation schedules across European Union/European Economic Area.



2.3. Diphtheria

Diphtheria is an acute communicable respiratory disease [2] that occurs mainly in tropical countries and is rare in industrialised countries [19]. Diphtheria was a major cause of childhood mortality before the implementation of specific immunisations [2].

The bacterial exotoxin and its cell-wall components, as O- and K- antigens, are important in the disease pathogenesis [20]. However, the most important virulence factor of *C.dipheteriae* is the exotoxin, which can cause local and systemic destruction [20]. Diphtheria vaccine is a toxoid and is adsorbed onto an adjuvant [2]. Presently, it is almost exclusively administered in combination with tetanus toxoid or with tetanus and pertussis vaccines in order to reduce the number of injections [20]. It can also be combined with hepatitis B and *Haemophilus Influenza type b*. Intriguingly, no controlled clinical trial of the efficacy of the toxoid in preventing diphtheria has been conducted [2]. Solid evidence supporting the effectiveness of the vaccine has arisen from observational studies [2].

Severe forms of local and systemic disease demand an immune response that is dependent on the antitoxin antibodies of IgG type, while type-specific protection against carriage and mild forms of local disease is induced by antibodies to the variable K-antigens of the bacterial cell wall [20]. However, cell-mediated immunity might also play a role [20]. Antibody levels of 1.0 IU/mL are associated with long-term protective immunity, whereas circulating anti-toxin levels below 0.01 IU/mL do not provide protection against the disease [20]. Therefore, suffering from the infection might not confer protective immunity [20].

In the EU/EEA, diphtheria is included in all vaccination schedules [21]. However the vaccination schedule varies considerably among countries. Presently, the first dose is given at age two months in 22 countries, and at age three months in eight countries (Figure 3). Certain countries, such as Austria, Denmark, Iceland, Norway, Sweden and Finland, use identical schedules at three, five, and 12 months. Others, such as Portugal, Spain, and Lithuania, use two, four, six, and 18 months. The United Kingdom uses two, three, and four months. (Figure 3) Throughout the EU/EEA, variability is observed not only concerning primary series, but also regarding the age for boosters (Table 1). For example, in Germany, the primary series is two, three, and four months, and the first booster is between age 11 and 14 months; in Belgium, Bulgaria, the Czech Republic, Hungary, Luxembourg, Malta, and the Netherlands, the primary series is two, three, and four months for all countries, but the first booster is administered at the age of 15, 16, 10, 18, 13, 18, and 11 months, respectively.

WHO recommends that the first dose should be administered as early as six weeks of age, followed by second dose in the 10th week and the third in the 14th week of life [20]. Maternal antitoxin levels affect the immune response of infants, even though high maternal antibody titres suppress, but do not prevent, adequate responses of infants to two doses of vaccines; after the third dose, the suppressive effect is gone [2]. Therefore, some interfering is likely between maternal antibodies and the diphtheria vaccine [22]. However, after three doses, practically all infants develop levels of antibodies greater than 0.01 IU/mL [2].

2.4. Tetanus

The pathogen responsible for tetanus, an acute fatal disease that leads to generalised rigidity and convulsive spasms of skeletal muscles, is found in the environment [2,19]. Many animals can harbour and excrete the organism and its spores [2,19].

Tetanus vaccine is a toxoid and is acquired through a *C. tetani* strain [2]. The protection is achieved through an antibody-dependent path and depends upon the ability of antitoxins to neutralise tetanospasmin, the most important toxin of *C. tetani* that blocks inhibitory neurotransmitters in the central nervous system and causes muscular stiffness and spasms [23]. However, the primary immunisation with tetanus toxoid also induces cellular immune responses in a high percentage of subjects [2]. Moreover, protection to tetanus can be only achieved by active and passive immunisation, recovering from the disease does not result in protection against it [23]. This vaccine, as other inactivated and toxoid vaccines, requires more than one dose to confer protection [2]. Normally, protective concentrations of antitoxins are achieved with two doses, and the third allows immunity in 100% of immunised subjects [23].

Studies have indicated that maternal-transmitted antibodies for tetanus toxoid do not interfere with neonatal responses when the vaccine is given soon after birth [22]. For the tetanus vaccine, the age at first dose does not seem critical, owing to its great immunogenicity [22]. Therefore, no matter what age the first dose is provided, an equally beneficial immune response will be achieved [22]. Consequently, protective levels can be obtained with schedules starting in the newborn period [2]. WHO suggests that the primary series of three doses should be administered before the age of one year [23]. Additionally, an interval of at least four weeks between doses is recommended [23]. WHO recommends that the first dose, since it is dispensed in combination with diphtheria and pertussis, should be administered as early as six weeks of age, since pertussis is of particular risk for young infants [23]. However, the vaccination schedule among EU/EEA countries is heterogeneous, and no country administers the vaccine at age six weeks; the first dose is given at age two months in 22 countries, and at age three months in eight countries (Figure 3). Some countries, such as Austria, France, Denmark, Iceland, Norway, Sweden, and Finland, use identical schedules at three, five, and 12 months; others, such as Portugal, Spain, and Lithuania, use two, four, six, and 18 months; the United Kingdom uses two, three, and four months (Figure 3).

2.5. Pertussis

Pertussis, or whooping cough, is an acute respiratory infection that results in a protracted intense cough that last for weeks, and is more severe in children [2,19]. This infection was one of the most common childhood diseases in the United States of America during the 20th century [19]. Despite the efficiency of the pertussis vaccine in preventing the disease, it has little impact on the circulation of *B. Pertussis*, even in countries with elevated vaccination coverage [24]. This makes unvaccinated children and susceptible individuals (adolescents, adults, and older subjects) reservoirs that are able to transmit it, allowing the occurrence of pertussis outbreaks [24]. It is during the first three months of life that hospitalisation and mortality rates are higher for pertussis [25]. It seems that pertussis is far from being controlled in the EU/EEA, and is still a pertinent public health concern [26,27]. Therefore, pertussis is the least controlled of the vaccine-preventable diseases in the EU/EEA [28]. In 2014, 40,727 cases were reported from EU/EEA countries, a higher number than in 2013 [28]. The highest rates were in children below one year of age (51.6 cases per 100,000 population), and 83% of these cases were under six months of age [28]. Since 2013, significant increases in the notification rate were observed in the Netherlands, the Czech Republic, Slovenia, Denmark, Belgium, Sweden, and Lithuania [28].

The objective of the pertussis vaccination is to reduce the incidence and the likelihood of severe pertussis in childhood [24]. Two vaccines are available: the whole-cell vaccine, and the acellular pertussis vaccine [2]. Whole-cell vaccines are suspensions of inactivated *B. Pertussis*, while acellular pertussis vaccines are subunit vaccines that contain purified inactivated components of *B. Pertussis* [2,19]. The acellular pertussis vaccine was formulated due to the common occurrence of minor, but undesirable, adverse local reactions; and some, less frequent, systemic reactions that have been associated with whole-cell vaccine administration, raising concerns about its safety [2]. Furthermore, adverse reactions of the whole-cell pertussis vaccine tend to increase with age and number of doses; thus, it is not recommended for immunisation of adolescents and adults [24]. The acellular vaccine is less reactogenic, and adverse reactions are minor and less frequent [2]. Acellular vaccines and whole-cell vaccines have similar efficacy rates, more than 85% [24]. However, it seems that the duration of protection is shorter with the acellular vaccine [29]. Pertussis incidence is also increasing in countries where the whole-cell vaccine is in use [29]. Therefore, the fading immunity induced by the acellular vaccine is not the main

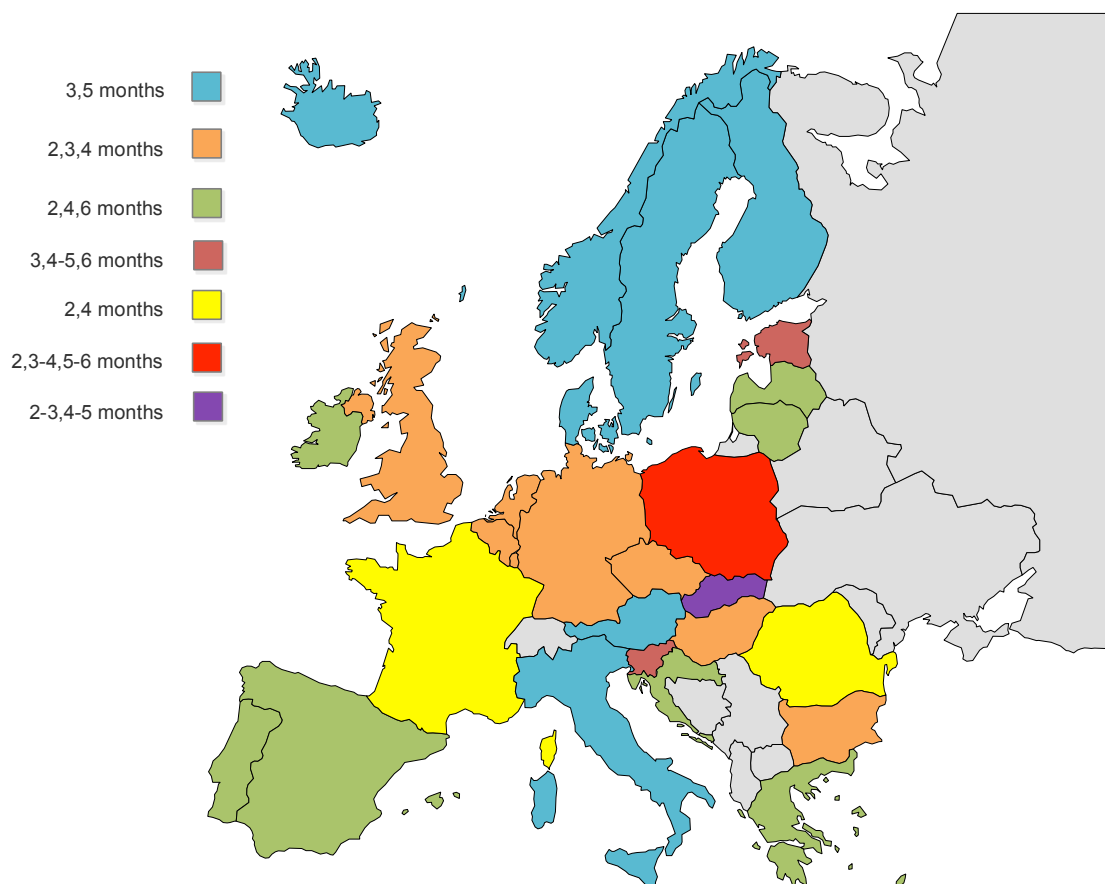
reason for the resurgence of pertussis [29]. One possible explanation for pertussis resurgence is that the acellular vaccine does not prevent transmission of *B. pertussis*, although it protects against the disease, and none of the vaccines prevent disease from virulent *B. pertussis* [29].

The majority of EU/EEA countries now utilise the acellular vaccine in their immunisation schedules [22]. Various acellular pertussis vaccines are available for specific age groups, and they are different in terms of component concentrations [19]. Acellular vaccines elicit the production of antibodies as well as T-cell specific responses to vaccine antigens, and Th1 cells seem to be involved in the immune responses [2]. Maternal antibodies do not seem to affect responses to vaccination [22]. . Thus, it seems improbable that transplacentally-acquired antibodies restrain vaccine-induced immune responses [22].

WHO recommends that the first dose of the pertussis vaccine should be administered as early as six weeks of age, since pertussis is of particular risk for young infants, followed by a second dose at 10 weeks and a third at week 14 of life [24]. In addition, according to clinical trials, it seems that whole-cell and acellular vaccines that are given in combination with diphtheria and tetanus can be safely administered very early in life [24]. However, evidence is still not sufficient to support the implementation of pertussis vaccines in newborns [24]. Nevertheless, vaccination schedules among EU/EEA countries are heterogeneous, and no country administers the vaccine at age six weeks, as recommended by WHO. The first dose is given at age two months in 22 countries, and at age three months in eight countries (Figure 3). Countries such as Austria, France, Denmark, Iceland, Norway, Sweden, and Finland use identical schedules at three, five, and 12 months; others, such as Portugal, Spain, and Lithuania, use two, four, six, and 18 months; the United Kingdom uses two, three, and four months (Figure 3). Therefore, the primary schedules in EU countries can be grouped as follows: three doses at two, three, and four months; three doses at two, four, and six months; and two doses at three and five months. The observed discrepancies between countries for the primary schedule of the pertussis vaccine is an issue, since an early protection for pertussis is an important goal to achieve [30]. The schedule of two, three, and four months results in an early protection [22]. And, it is not known, due to lack of studies, if the schedule of two, four, and six months already induces immunological memory with the first two doses [22]. Additionally, in the schedule of two, three, and four months, protection is

achieved one month earlier than with the schedule of three and five months, but it requires an additional dose [22]. All the existing schedules induce priming with success; the benefits of an early protection favour early implementation schedules. A possible and apparently effective strategy to ensure early protection is maternal immunisation between 28 and 38 weeks of gestation [29]. Another future strategy may consist of a new vaccine that is currently under clinical development [29].

Figure 3: Diphtheria, tetanus and pertussis primary immunisation schedules across European Union/European Economic Area.



2.6. Poliomyelitis

Poliomyelitis is an acute communicable disease and can be caused by any of three serotypes of poliovirus: type 1, type 2, and type 3 [31]. In 2012, polio eradication was considered by the World Health Assembly a global public health emergency, consequently the Polio Eradication and Endgame Strategic Plan 2013-2018 was developed [31].

With the widespread of poliovirus vaccination, type 2 poliovirus has not been detected since 1999 [32]. And, type 3 has not been detected since November 2013 [32]. However, type 1 remains in circulation [32]. Poliovirus was, in the pre-vaccination period, the primary cause of permanent disability in children [2]. The viruses are spread by faecal-to-oral and oral-to-oral transmissions [31]. The poliovirus initially replicates in the gastrointestinal tract, but it can affect the central nervous system, leading to flaccid paralysis of the muscles [2].

There are two types of poliovirus vaccines: the inactivated poliovirus vaccine (IPV), and the live oral poliovirus vaccine (OPV). IPV is the vaccine of choice in areas free of poliovirus or with very low levels. Therefore, it is the vaccine used in the EU/EEA schedules with the exception of Poland, which uses a booster of OPV at the age of six years.

IPV contains all three serotypes of poliovirus, and is an inactive antigen vaccine [2]. This vaccine development is based on the inactivation of cell-culture-derived polioviruses using formaldehyde [31]. It is available as a stand-alone vaccine or in combination with DTP, hepatitis B, and haemophilus influenza type B infection vaccines [31]. It is considered a very safe vaccine with no major side effects [31].

IPV immunogenicity depends of a number of factors, such as number of doses, interval between doses, level of maternally-acquired poliovirus antibodies present at the time of vaccination that can suppress the immune response, and IPV type [2]. In the EU/EEA, primary vaccine schedules vary considerably among countries. Some countries have a two-dose schedule at three and five months, such as Austria, Denmark, Finland, and Iceland; others have a three-dose schedule that can either be administered at two, three, and four months, as in Belgium and the Czech Republic, or at two, four, and six months, as in Portugal, Ireland, and Spain. The first booster after the primary schedule also varies considerably. For example, in Austria, Denmark, Finland, Iceland, it is administered at age 12 months, Belgium provides it at age 15 months, the Czech Republic dispenses it at age 10 months, and Portugal administers it at age 18 months. Concerning primary immunisations, studies have indicated that seroprotection rates are clearly superior when three doses are administered, particularly when the schedule of two, four, and six months is utilised [2]. Schedules of three, four, and five months, and two, three, and four months also provide good protection rates; however, they are not as effective as the schedule of two, four, and six months [2]. WHO recommends a primary series of three doses of

IPV, beginning at the age of two months [31]. If an early schedule as, for example, six, 10 and 14 weeks of age is adopted, a booster should be dispensed after an interval of at least six months [31]. In the EU/EEA, the poliovirus vaccine is provided in combination with diphtheria, tetanus, and pertussis; thus, the primary schedule is coincidental (Figure 3).

2.7. Haemophilus Influenza Type B Infection

Haemophilus influenzae, a pathogen that spreads from person to person via respiratory droplets, is responsible for considerable morbidity and mortality in children under the age of five years [2]. Prior to the pre-vaccine era, *H. influenzae* serotype b (Hib) was responsible for 95% of invasive diseases [2]. The occurrence of this disease is relatively infrequent in the first two months of age, probably due to protection from maternal antibodies acquired through the placenta [33]. The greatest disease burden is observed between four and 18 months of age [33]. The widespread of the *H. influenzae b* vaccine led to large reductions in the incidence of invasive Hib diseases (decrease of 90%) [33]. Invasive diseases include meningitis, pneumonia, septic arthritis, osteomyelitis, pericarditis, cellulitis, and epiglottitis [33]. Even with adequate medical treatment, 5% of children with *H. influenzae b* meningitis die, and 20 to 40% of survivors suffer from severe sequela [33].

Haemophilus influenzae is a gram-negative coccobacillus, and its polysaccharide capsule is the major virulence factor for severe disease [2]. The serotype b capsule as well as other aspects makes it particularly virulent [2]. Since the pure polysaccharide vaccine was not effective in children under 18 months of age, and the greatest disease burden is observed in children younger than two years of age, a conjugated vaccine (bound of the polysaccharide with a carrier protein, i.e. diphtheria protein, tetanus toxoid, or meningococcal membrane protein) was created in order to improve its immunogenicity in young children [2,19]. If the immune response for the first vaccine was a typical, T-independent antigen response, which is age-dependent, so it fail to induce immunological memory in the youngest children. [2,19,33]. The conjugated vaccine turns the immune response into a T-dependent B-cell immune response that produces longer immunity, and immunological memory is induced [2,19,33].

In the EU/EEA, primary vaccine schedules vary considerably. Some countries have a two-dose primary schedule at three and five months, as in Austria, Denmark,

Finland, and Iceland. Others have a three-dose schedule that can either be administered at two, three, and four months, as in Belgium, the United Kingdom, and the Czech Republic, or two, four, and six months, as in Portugal and Spain. The first booster after the primary schedule exists in all schedules, but it also varies considerably: in Austria, Denmark, Finland, and Iceland, it is administered at age 12 months, the United Kingdom dispenses it between age 12 to 13 months, Belgium provides it at age 15 months, Netherlands dispenses it at 11 months of age, the Czech Republic administers it until age 18 months, and Portugal, Hungary, Spain, and Malta provide it at age 18 months. In the EU/EEA, the Hib primary vaccination is administered in combination with diphtheria, tetanus, pertussis; thus, the primary schedule is coincidental (Figure 3). Evidence has indicated that at least three doses are needed to achieve vaccine efficacy and effectiveness [33]. These can be achieved with three primary doses with or without a booster (3p+0 or 3p), or with two primary doses and a booster (2p+1) [33]. However, all countries of the EU/EEA provide a booster dose around the second year of life, as the vaccine-induced antibody wanes over time, leading to an increase susceptibility to disease [34]. A recent systematic review indicated that 3p+1 and 2p+1 schedules achieve similar immunological responses [35]. Therefore, it seems that the primary schedule of two or three doses confer identical short-term levels of protection [33]. Since serious Hib disease occurs most frequently in children aged between four and 18 months, WHO recommends that immunisation should start from six weeks of age, or as early as possible thereafter [33].

2.8. Hepatitis B

Hepatitis B virus (HBV) infection is a highly prevalent infection around the globe, and approximately 30% of the world's population has serologic evidence of HBV infection [2]. HBV is a leading cause of chronic hepatitis and cirrhosis, causing significant morbidity and mortality worldwide [2]. It is also the leading cause of hepatocellular carcinoma [2]. Transmission is believed to be through percutaneous or mucosal exposure with body fluids from persons who have acute or chronic disease [19]. The disease outcome is age-dependent [36]. For infants that acquire the disease from their mother or in infancy, the odds of being chronically infected are 90% [19].

Children that become infected between age one year and five years of age have a 30% to 50% chance of chronic infection [19].

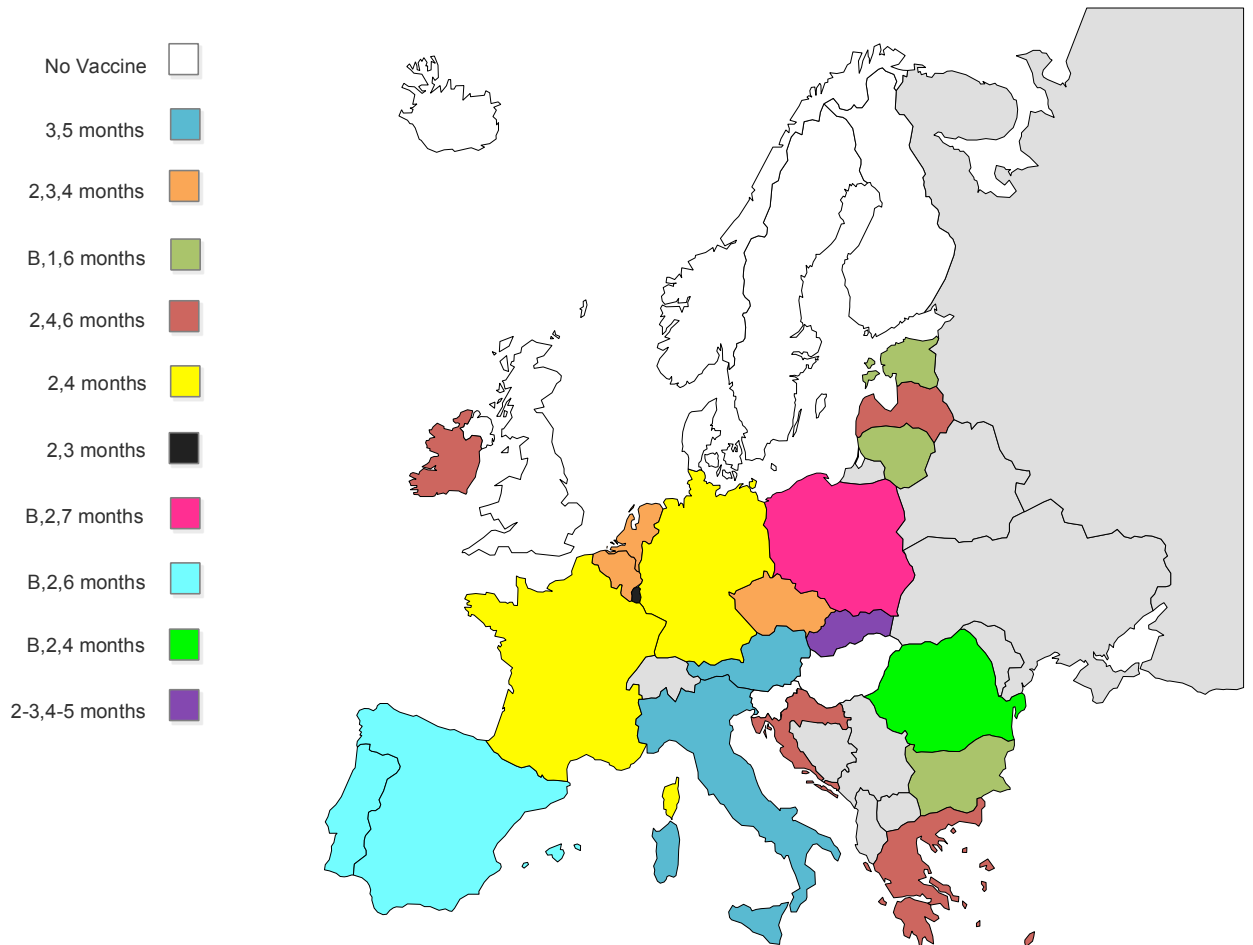
Hepatitis B vaccine is available as a monovalente formulation or in combination with diphtheria-tetanus-pertussis, Hib, hepatitis A, and inactivated polio [36]. Nowadays, hepatitis B vaccine is a recombinant vaccine [2]. The active substance in recombinant hepatitis B vaccine is a hepatitis B surface antigen that is produced in yeast or mammalian cells [36].

WHO considers that perinatal and early postnatal transmission is a significant cause of chronic infections worldwide [36]. Thus, WHO recommends the administration of hepatitis B vaccine as soon as possible after birth, preferably in the first 24 hours, even in low-endemicity countries [36]. This dose should be followed by two or three doses with a minimum interval of four weeks to complete the primary series [36]. According to WHO the following two schemes to complete the primary series are appropriate: a three-dose schedule where the first dose (monovalent) is administered at birth, and the second and third dispensed simultaneously as the first and third doses of the DTP vaccine; or a four-dose schedule, in which a monovalent birth dose is followed by three monovalent or combined vaccine doses [36]. Previous evidence does not support the need of a booster [36].

Twenty-three member states of the EU/EEA included hepatitis B in their immunisation schedules: eight targeted newborn babies, 12 targeted infants with two months of age, two targeted children with three months of age, and one targeted children with 12 months of age. Eight countries (Denmark, Finland, Hungary, Iceland, Liechtenstein, Norway, Slovenia, Sweden, and the United Kingdom) do not have hepatitis B vaccine in their routine childhood immunisation programme (Figure 4); it is only provided to certain well-defined risk groups. In the rest of the countries, the primary schedules vary significantly. For example, in Bulgaria, Estonia, and Lithuania, it is administered at birth, one month of age, and six months of age; in Portugal and Spain, it is administered at birth, two months, and six months of age; and in Croatia and Ireland, it is dispensed at age two, four, and six months (Figure 4).

According to WHO, the timely delivery of a birth dose of hepatitis B vaccine should be a performance measure for all immunisation programmes [36]. In addition, WHO recommends that all infants should receive the first dose as soon as possible after birth, that is, within 24 hours of birth, even in countries with low and intermediate endemicity [36].

Figure 4: Hepatitis B primary immunisation schedules across European Union/European Economic Area.



2.9. Pneumococcal Disease

Streptococcus pneumoniae is an asymptomatic coloniser of the nasopharynx, and can cause serious diseases as pneumonia, meningitis, bacteraemia, as well as milder and more common illnesses, such as sinusitis and otitis media [2,37]. This agent is mostly transmitted through respiratory droplets, and the main reservoir is believed to be infants and young children [37]. More than 90 pneumococcal serotypes have been identified [2], and the distribution of serotypes that cause disease varies by age, disease syndrome, disease severity, geographic region, and over time [37]. In the EU/EEA, the highest levels of infection, before the introduction of a vaccine, were in children with two years of age or younger [2]. *Streptococcus pneumoniae* antimicrobial resistance is a matter of major concern, and as pneumococcal infections become increasingly more difficult to treat, the focus should be placed on preventing the disease through vaccination [38].

Streptococcus pneumoniae is a gram-positive, encapsulated diplococcus, and its polysaccharide capsule is one of the primary factors responsible for the virulence of this bacterium [2,37]. Two types of vaccines are available: the pneumococcal polysaccharide vaccine (PPV23), and the pneumococcal conjugate vaccines (PCV7, PCV10 and PCV13) [39]

Pneumococcal polysaccharide vaccine is associated with poor or absent immunogenicity in children below two years of age [37]. These vaccines fail to elicit a protective immune system response among infants and very young children since children respond poorly to T-independent antigens [40]. Conversely, the pneumococcal conjugate vaccine enhances the antibody response and induces effective immune memory in children below two years of age, by changing the nature of the antipolysaccharide response from T-independent to T-dependent.

[37]. Additionally, conjugate vaccines can reduce nasopharyngeal carriage of vaccine type pneumococci, and has effectiveness against serotypes currently causing most invasive diseases, and evidence for effectiveness against non-invasive syndromes, including non-bacteraemic pneumonia and otitis media [40,41].

Pneumococcal immunisation schedules across the EU/EEA vary greatly among countries in terms of age groups and vaccines administered [39]. Currently, the pneumococcal vaccine is dispensed in routine immunisation programmes either in a 2p +1 or 3p+1 schedule (Figure 5). For example, in Belgium, Hungary, Luxembourg, Portugal, Spain, and the United Kingdom, primary immunisation is at two and four months, and a booster is given at age 12 months; in Denmark, Finland, Iceland, and Sweden, the primary immunisation is administered at three and five months of age, and a booster is given at age 12 months; in Bulgaria, the Czech Republic, Greece, Italy, and Slovenia, the primary immunisation schedule is at age two, three, and four months, and a booster is given around age 11 months. WHO recommends three primary doses, or as an alternative, two primary doses plus a booster [37]. According to WHO, when choosing between a 3p or a 2p+1 schedule, countries should consider particularities such as epidemiology of the disease, local factors, and timelessness of doses [37]. For example, if the peak of the disease is observed in young infants with less than 32 weeks of age, a 2p+1 schedule might not offer the optimal protection for certain serotypes compared to the 3p+0 schedule [37], thus, the second scheme should be favoured. However, the third dose, in the 2p + 1 scheme, induces higher antibody levels compared to the 3p+0 schedule, and this may be important for the duration of

protection or effectiveness against some serotypes [37]. According to WHO, if a 3p+0 schedule is the option, vaccination can be initiated as early as six weeks of age with doses given at six, 10 and 14 weeks, or at two, four, and six months, depending on convenience [37]. However, if a 2p+1 schedule is the choice, WHO recommends that the first dose be administered as early as six weeks of age, with the second at an interval of 8 weeks or more, and the booster should be dispensed between nine to 15 months of age [37].

Figure 5: Pneumococcal primary immunisation schedules across European Union/European Economic Area.

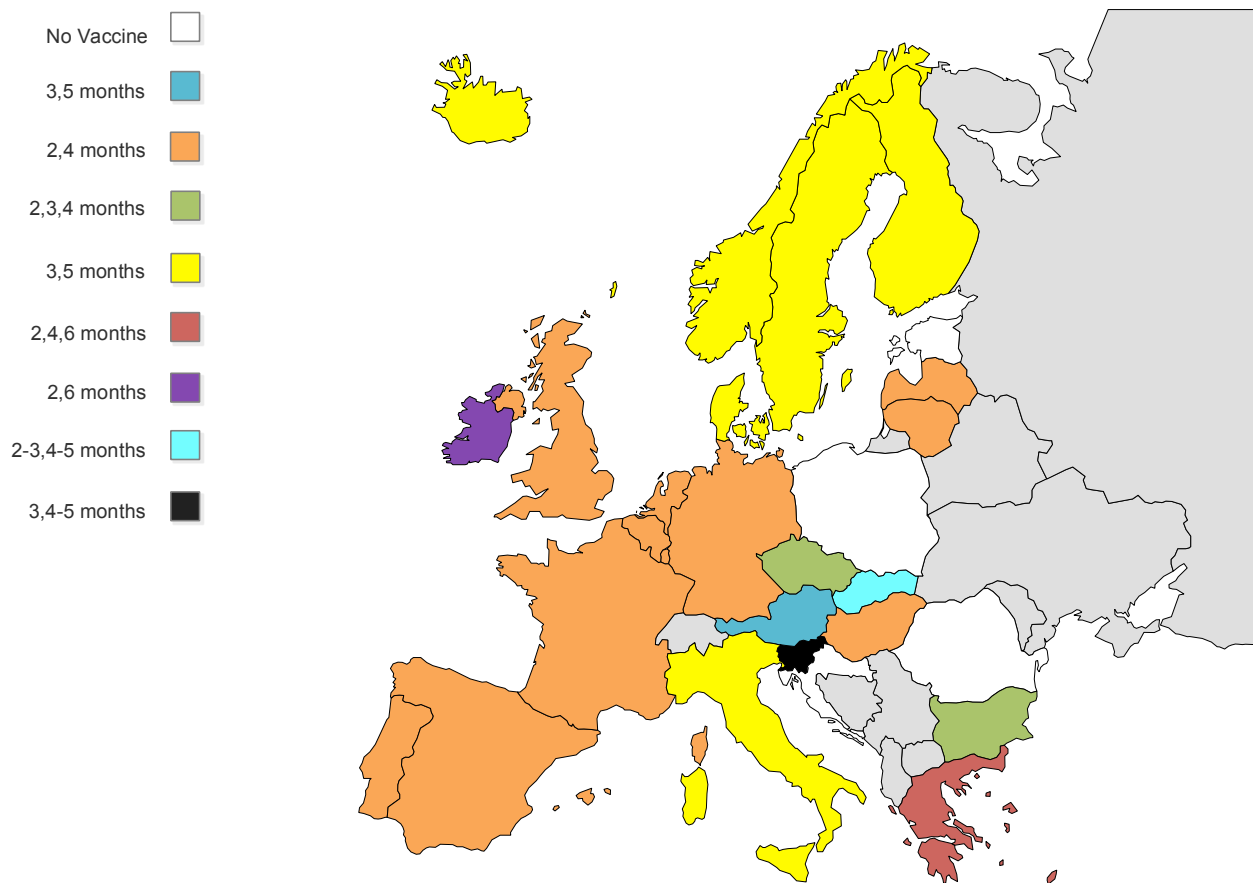


Table 1. Primary immunisation and first booster doses across European Union/European Economic Area.

	Tuberculosis	Rotavirus	Diphtheria	Tetanus	Pertussis	Poliomyelitis	Hib	Hepatitis B	Pneumococcal
Austria		3		2p+1		2p+1	2p+1	2p+1	2p+1
Belgium		3		3p+1		3p+1	3p+1	3p+1	2p+1
Bulgaria	B			3p+1		3p+1	3p+1	3p+0	3p+1
Croatia	B			3p+1		3p+1	3p+1	3p+0	
Cyprus				3p+1		3p+1	3p+1	3p+1	2p+1
Czech Republic				3p+1		3p+1	3p+1	3p+1	3p+1
Denmark				2p+1		2p+1	2p+1		2p+1
Estonia	Ba	2		4p+1		3p+1	3p+1	3p+0	
Finland		3		2p+1		2p+1	2p+1		2p+1
France				2p+1		2p+1	2p+1	2p+1	2p+1
Germany		2		3p+1		3p+1	3p+1	2p+1	2p+1
Greece				3p+1		3p+1	3p+1	2p+1	3p+1
Hungary	B			3p+1		3p+1	3p+1		2p+1
Iceland				2p+1		2p+1	2p+1	B	2p+1
Ireland	B			3p+0		3p+0	3p+1	3p+0	2p+1
Italy				3p+1		3p+1	3p+1	3p+1	3p+1
Latvia	B	3		3p+1		3p+1	3p+1	3p+1	2p+1
Liechtenstein				3p+1		3p+1	3p+1		
Lithuania	B			3p+1		3p+1	3p+1	3p+0	2p+1
Luxembourg		2		3p+1		3p+1	3p+1	2p+0	2p+1
Malta				3p+1		3p+1	3p+1	2p+1	
Netherlands				3p+1		3p+1	3p+1	3p+1	2p+1
Norway		2		2p+1		2p+1	2p+1		2p+1
Poland	B			5p+1		2p+1	2p+1	3p+0	
Portugal	B			3p+1		3p+1	3p+1	3p+0	2p+1
Romania	Bb			2p+1		2p+1	2p+1	3p+1	
Slovakia				4p+1		2p+1	2p+1	4p+1	4p+1
Slovenia				4p+1		3p+1	3p+1		3p+1
Spain				3p+1		3p+1	3p+1	3p+0	2p+1
Sweden				2p+1		2p+1	2p+1		2p+1
United Kingdom		2		3p+0		3p+0	3p+1		2p+1

B= Birth; Ba=1-5 days after birth; Bb= 2-7 days after birth; Hib= Haemophilus influenzae type B.

2.10. Meningococcal Disease

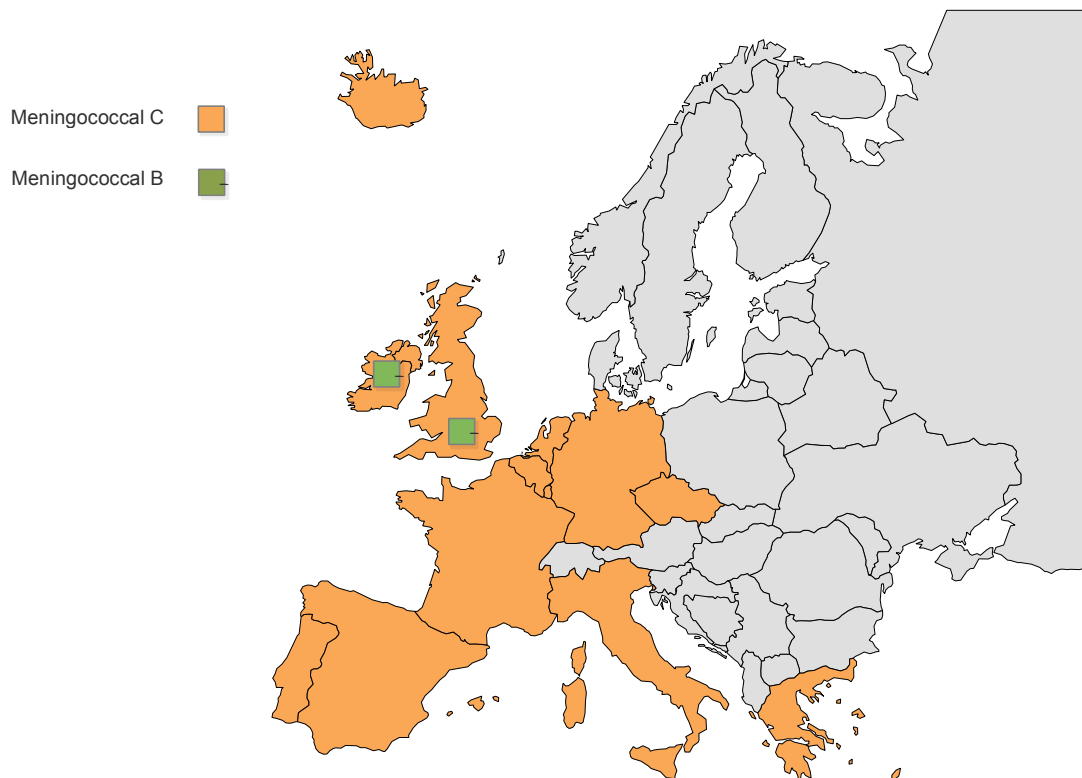
Neisseria meningitides is recognised as a leading cause of meningitis and fulminant septicaemia in several countries, posing a significant public health problem [42]. *N. meningitides* is a fastidious, gram-negative, endotoxin-producing organism that usually resides asymptotically in the human nasopharynx and is easily transmitted by respiratory droplets [2,42]. The serogroups A, B, C, X, W-135, and Y are medically significant, and the main cause for invasive meningococcal infections [42,43]. These serogroups vary considerably in time and with geographic location [42]. In Europe, the majority of cases are caused by serogroup B, particularly in countries that have introduced serogroup C meningococcal conjugate vaccines [42]. However, in England and Wales, an increase in meningococcal group W has been observed [44]. In addition, in some EU/EEA countries, particularly Scandinavian countries, an increase of meningococcal Y has been reported [43]. The disease mainly affects children and adolescents, as the highest rates occur in infants aged three to 12 months [42]. Invasive diseases can cause, aside from meningitis and septicaemia, arthritis, myocarditis, pericarditis, and endophthalmitis [42]. Meningococcal disease can advance rapidly and death can occur, even with medical attention [2]. Devastating long-term sequelae may lead to permanent disability [45].

Presently, polysaccharide and conjugated polysaccharide meningococcal vaccines are accessible [46]. Conjugate vaccines reduce nasopharyngeal carriage and, hence, interruption of transmission and establishment of population protection; and are effective in protecting young children (<24 months) who may respond poorly to conventional polysaccharide vaccines [46]. Meningococcal conjugate vaccines are available as monovalent (A or C), quadrivalent (A, C, Y and W), and combined (serogroup C or C and Y, along with *Haemophilus influenzae* b) vaccines [46]. The development of a successful vaccine against serogroup B was challenging due to several factors so, the existent vaccine is based on conserved proteins, using “reverse vaccinology” [46].

According to WHO countries with high (>10 cases/100,000 population/year) or intermediate (2-10 cases/100,000 population/year) endemic rates of invasive meningococcal disease as well as, countries with frequent epidemics should include meningococcal vaccine in their immunisation plan [42]. In the EU/EEA, only 13

countries have the vaccine for meningococcal C in their routine immunisation programmes, and even fewer have meningococcal B vaccine in their routine immunisation plans (Figure 6). WHO recommends that monovalent meningococcal C vaccine is to be administered only once in children aged more than 12 months, and in children between two to 11 months two doses plus a booster should be provided [42]. However, it is not known, for sure, if boosters are needed after primary vaccination at 12 or more months of age [42]. Therefore, Belgium administers it at age 15 months; Cyprus between 12 to 13 months of age; France and Portugal at 12 months of age; Germany between age 12 to 23 months of age; Greece at age two, four, six, and 23 months; Iceland between age six to eight months of age; Ireland at age six and 13 months; Italy between age 13 to 15 months of age; Luxembourg at age 13 months; Netherlands at age 14 months; Spain at age four and 12 months, and the United Kingdom at age three and 13 months of age. Two countries, Ireland and the United Kingdom, have meningococcal B vaccine in their routine immunisation plans, administered at age two, four, and 12 months of age.

Figure 6: Meningococcal C and B primary immunisation schedules across European Union/European Economic Area.



2.11. Measles

Measles is the main cause of vaccine-preventable deaths in infants in the world [47]. Measles is a systemic infection [19], and measles virus is highly infectious. In the pre-vaccination period a high percentage of individuals were infected by age 10 [48]. This disease is transmitted by aerosolised respiratory droplets and by direct contact and it can be prevented with vaccination [48].

Measles virus is an RNA virus composed of eight proteins; the structural proteins are essential in the pathogenesis of measles [2,48]. Thus, lifelong immunity after disease is attributed to antibodies that neutralise one of those proteins [2,48]. The severity of the disease varies considerably depending on several host and environmental factors; for example, age below five years is a risk factor for severe or fatal measles [48]. Infants are usually protected against measles for six to nine months through transplacentally-acquired antibodies [48]. Therefore, vaccination before age six months frequently fails to induce seroconversion due to immune system immaturity and the presence of maternal antibodies [47]. High avidity antibodies are needed to develop protective immunity to measles, and response is usually less avid in children vaccinated at age six months or nine months, compared with the avidity obtained in children vaccinated at age 12 months [48]. WHO recommends that all children should be vaccinated with two doses of measles vaccine [48]. In countries where the risk for measles infection among infants is low, as in EU/EEA countries, the first dose should be given at age 12 months [48]. The second dose should be administered between the age of four to six years [2].

In EU/EEA countries, the age for primary vaccination of measles varies considerably (Figure 7). For example, in Belgium, Croatia, Estonia, Finland, Ireland, Portugal, Romania, and Spain, it is administered at age 12 months. In the Czech Republic, Denmark, Hungary, and Norway, primary vaccination occurs at age 15 months. The primary vaccination is provided at age 18 months in Iceland and Sweden (Figure 7).

Measles vaccine is normally administered in combination with rubella and mumps as a live attenuated combined vaccine, and studies consistently show that the combined vaccines elicit the same high rates of seroconversion observed with each component individually [2]. Measles has been pointed for eradication in EU/EEA [49]. Measles notification was in 2015 below the elimination target (one case per million population) in 14 of the 30 reporting countries [49]. Seven countries reported zero

cases and 16 had the notification rate above the elimination target, with Croatia reporting the highest rate (51.6 cases per million) followed by Austria (35.3 cases per million) and Germany (30.5 cases per million) [49].

2.12. Mumps

Mumps is a viral infection that primarily affects salivary glands [2]. Although it is normally a mild childhood disease, with a peak incidence between five to nine years of age, the virus can also affect adults, for whom complications are more frequent and severe than in children [50]. Humans are the only known natural host, and disease transmission is through direct contact or by airborne droplets from respiratory tract [50].

Mumps virus is a negative-strand RNA virus from the Paramyxoviridae family [2]. According to WHO routine mumps vaccination should be implemented in countries with a well-established and effective childhood vaccination schedule, and with the capacity to sustain elevated levels of vaccination coverage [50]. As, insufficient childhood vaccination coverage may lead to an epidemiological shift in the incidence of mumps to older age groups and possibly lead to a more severe disease burden than occurred before immunisation was introduced [50]. WHO considers measles and congenital rubella control a higher priority than mumps control [50]. When the implementation of mumps vaccine is determined, the use of the combined vaccine of mumps-measles-rubella is strongly recommended [50].

Mumps vaccine is normally administered in combination with rubella and mumps in EU/EEA countries. As for measles vaccine, the age for primary vaccination of mumps varies considerably (Figure 7).

2.13. Rubella

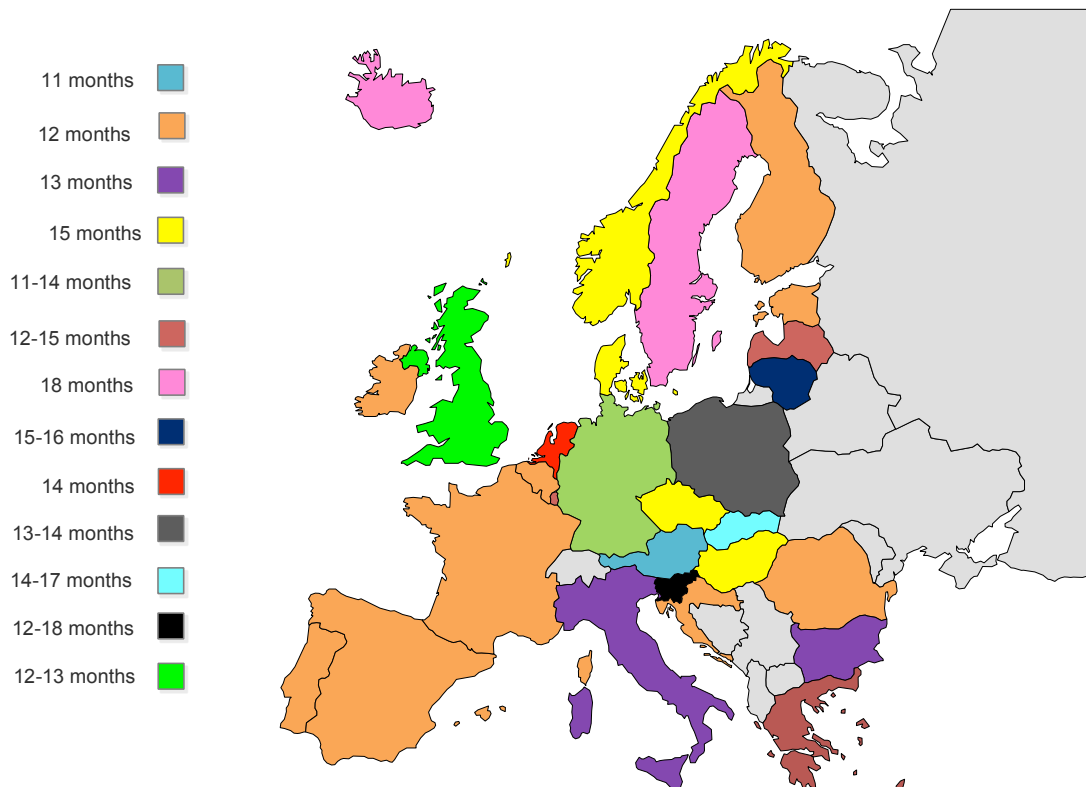
Rubella is an acute; normally mild and self-limited viral disease that usually affects susceptible children and young adults worldwide [51]. However, rubella virus has teratogenicity potential and, thus, is a public health concern [51]. When rubella infection occurs just before conception or during early pregnancy, foetal death or congenital defects, known as congenital rubella syndrome, may happen [51]. Rubella congenital syndrome includes a long list of abnormalities, as all organs of the foetus are affected [2].

Rubella virus is a togavirus [2]. Humans are the only known host, and it is transmitted by respiratory route [2]. Rubella vaccine is normally administered with mumps and measles in a combined live attenuated vaccine, and single or combined vaccines are highly efficacious [51]. Although a high response to a single dose of rubella vaccine and long-term persistence of protection do not support the need of a second dose, this second dose is, generally, administered based on the indications for a second dose of measles and mumps vaccines [51]. The main aim of rubella vaccination is the prevention of congenital rubella infection [51].

Rubella vaccine is normally administered in combination with measles and mumps in EU/EEA countries. As for measles and mumps vaccine, the age for primary vaccination of rubella varies considerably (Figure 7).

Rubella has been pointed for eradication in the EU/EEA [49]. In 2015, rubella notification rate was lower than the elimination target (one case per million population) in 25 of the 28 reporting countries in 2015 [49]. The higher rates were observed in Poland (53.4 cases per million), followed by Germany and Ireland (1.1 and 1.3 cases per million, respectively) [49].

Figure 7: Measles, mumps, and rubella primary immunisation schedules across European Union/European Economic Area.



3. Conclusion

The EU/EEA is composed of a diverse group of countries and a large variation of early childhood immunization schedules and included vaccines were observed across countries in this review. The considerable variation observed in the early childhood immunization series among different EU/EEA countries, concerning both vaccines included and immunisation schedules, have raised some questions and concerns. One such concern is the mobility across the EU/EEA of young children travelling or moving, since they can easily miss a dose or booster. An ideal solution would be an uniform EU/EEA immunisation schedule (Table 2).

WHO recommends that rotavirus vaccine should be included in all national immunisation programmes, and considers it a priority. Therefore, we would also suggest considering the inclusion of this vaccine in the EU/EEA immunisation schedule. The vaccine would be given at age two and four months to coincide with DTPa schedule and to reduce the number of visits to medical facilities.

Concerning DTPa vaccine, some interference is likely between maternal antibodies and diphtheria vaccine [22]. Thus, it would be better to only administer diphtheria vaccine at age two months or after. However, since it is a combined vaccine, pertussis vaccine should be given as early as possible, at the age of six weeks according to WHO, since pertussis is of particularly risk for young infants. For tetanus vaccine, age at first dose does not seem critical, which is owed to its great immunogenicity and because maternal antibodies presumably do not interfere with neonatal responses [22]. We suggest that the primary schedule for DTPa should be provided at two, three, and four months, as protection for pertussis is reached earlier [22]. An alternative is maternal immunisation between 28 and 38 weeks' gestation, which is already being executed in countries such as the United Kingdom and Portugal.

Since the WHO region was officially declared polio-free, no special schedule is needed for polio vaccine. Therefore, and due to the inherent convenience of reducing the number of visits to medical facilities, polio vaccine would have the same schedule of DTPa vaccine: two, three, and four months. Hib vaccine schedule should also be the same.

WHO recommends the administration of hepatitis B vaccine as soon as possible after birth, preferable in the first 24 hours, even in low-endemicity countries [36]; this

dose should be followed by two or three doses with a minimum interval of four weeks to complete the primary series. Based on this hepatitis B vaccine, the primary schedule in the EU/EEA would be: birth, two, and four months.

The first dose of pneumococcal vaccine should be, according to WHO, administered as early as six weeks of age, with the second dose at an interval of eight weeks [37]. Based on this, the primary series of this vaccine in the EU/EEA immunisation schedule would be at two and four months of age.

Meningococcal C vaccine would be included in the immunisation plan and administered at age 12 months, as recommended by WHO [41]. Since the majority of cases are now caused by serogroup B in the EU/EEA, particularly in countries that have introduced serogroup C meningococcal conjugate vaccine [41], the inclusion of meningococcal B vaccine should be considered as part of the EU/EEA immunisation plan with the primary schedule of two, four, and six months. Measles, mumps, and rubella vaccine primary immunisations would be administered at age 12 months, as high avidity antibodies for measles are achieved at age 12 months [47].

Given the growing number of people moving around the EU/EEA, including young couples with young children, a common schedule might have a positive impact on vaccinations [21]. Therefore, even though early childhood immunization schedules in the EU/EEA are safe and effective, the significant difference observed between countries might cause constraints in vaccination. Therefore, we present a recommendation, based on the available knowledge, for the early childhood immunization schedule the EU/EEA. To the best of our knowledge, this is the first review presenting a suggestion for the early childhood immunization schedule in the EU/EEA.

Table 2: Primary immunization schedule proposal for countries that for European Union/European Economic Area.

Tuberculosis	Rotavirus	Diphtheria - Tetanus - Pertussis	Poliomyelitis	Hib	Hepatitis B	Pneumococcal disease	Meningococcal disease C	Meningococcal disease B	Measles - Mumps - Rubella
0*	2, 4 months	2,3 and 4 months + 18 months	2,3 and 4 months + 18 months	2,3 and 4 months + 18 months	0, 2 and 4 months	2 and 4 months + 12 months	12 months	2, 4 months + 18 months	12 months

* as soon as possible after birth in countries with high incidence. Hib= Haemophilus influenzae type B. Hexavalent vaccine (combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis, Hib and hepatitis B) can be used to reduce the number of injections at 2 and 4 months and heptavalent (combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis and Hib) at 3 months.

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Supplementary Material

Vaccination effectors

Introduction

Active immunity refers to the process of exposing the body to an antigen to generate an adaptive immune response that is long-lasting [1]. Active immunity can be acquired by vaccination or through exposure to the disease [1]. Vaccines interact with the immune system and usually create a response similar to those produced by the infection without subjecting an individual to the disease and its complications [2]. Antigens, which are present in vaccines, can be live (as viruses or bacteria), or inactivated or genetically-engineered vaccines. The immune system will develop a specific response to the antigens, and it normally involves the production of antibodies (immunoglobulins; humoral immunity) and of specific cells, including T and B cells (cell-mediated immunity), whose aim is to facilitate the elimination of a pathogen [2].

Vaccine types

Vaccines can be categorised into wide groups: live attenuated vaccines, and inactivated (subunit, toxoid carbohydrate, and conjugate) vaccines [3]. Subunit vaccines can be subdivided into those for which the antigen is produced using recombinant DNA technology, and those based on normal bacteriological growth processes [1].

The first group of vaccines contains attenuated forms of the pathogen that mimic the immune response elicited by the pathogen itself [3]. Live attenuated vaccines (viruses or bacteria) cause a strong cellular and antibody response; their immunity lasts for several decades, even with only a single dose (except for those administered orally) [3]. Nevertheless, a percentage of receivers do not respond to the first dose of an injected live vaccine, thus, a second dose is recommended to provide a high level of population immunity [2]. The development of active immunity to a certain live vaccine can be affected by circulating antibodies to the vaccine virus, as the ones transferred through placenta [2]. This may lead to a poor or no response to the vaccine [2]. This group of vaccines can provide a lifelong memory [3].

The vaccines of the second group can be composed of either whole viruses or bacteria or fractions of them [2]. Fractional vaccines are protein-based or polysaccharide (PS)-based [2]. Protein-based vaccines include toxoids (inactivated

bacterial toxin) and subunit or subviral products [2]. PS-based vaccines are composed of pure cell wall PS from bacteria [2]. Conjugate polysaccharide vaccines contain PS that are chemically linked to a protein, which causes the PS to be more potent [2]. Normally, these vaccines contain substances named adjuvants that increase the magnitude and quality of the immune response [3]. The circulating antibodies do not influence the response to an inactivated vaccine and, therefore, they can be administered when the antibody is present in the blood [2]. This group of vaccines do not provide a lifelong memory [3]. Thus, several boosters are needed to maintain protective immunity [3]. A protective immune response is not developed in the first dose, and usually only occurs after the second or third dose [2]. Furthermore, the immune response to these groups of vaccines is mostly humoral, and little or no cellular immunity is observed [2]. The response to a pure PS vaccine is classically a T-independent response [2]. Therefore, these vaccines can stimulate B-cells without the assistance of T-helper (Th) cells [2]. These types of vaccines and all T-cell-independent antigens are not consistently immunogenic in children below two years of age, as the immune system is still immature [2]. In addition, PS vaccines predominantly produce IgM and little IgG antibodies, resulting in less effective vaccines [2]. To solve this problem, PS began to bind to protein in a process called conjugation. Through this process, the immune response changes from T-independent to T-dependent [2].

Vaccines mechanisms

A prerequisite of a vaccine is to elicit adequate alert signals through antigens or adjuvants that trigger inflammatory response mediated by cells of the innate immune system [4]. Various cells, such as dendritic cells (DCs), monocytes, and neutrophils, express pattern recognition receptors (PRRs) as toll-like receptors (TLRs) that are capable of reacting to a signal [3,4]. Through these receptors, after encountering the pathogen, the cells can become activated and produce pro-inflammatory cytokines and chemokines that will attract monocytes, granulocytes, and natural killer cells [4]. These organisms generate an essential inflammatory microenvironment in which monocytes differentiate into macrophages and immature DCs become activated [4]. After activation, DCs will migrate to lymph nodes and present the antigen to B- and T- cells that are activated [4]. Live viral vaccines trigger the innate immune system

more efficiently through pathogen-associated signals that are recognised by PRRs [4]. After the administration of a live vaccine, the pathogen rapidly disseminates and DCs are activated at multiple sites [4]. Therefore, the early diffusion of live vaccines causes the site and route of administration to be less important than for non-live vaccines [4]. Non-live vaccines mainly activate innate immune responses at their site of injection [4]. Thus, their site and route of administration are very important. For example, the high number of DCs in dermis permits a reduction of the antigen concentration in intradermal vaccines [4].

B-cells are mainly activated in the lymph nodes [4]. Then, B-cells engage with T-cells and initiate their proliferation [4]. Consequently, antigen-specific B-cells interact with activated DCs and T-cells [4]. T-cells contribute to B-cell differentiation and production of immunoglobulin-secreting plasma cells that produce antibodies [4]. During B-cell differentiation, immunoglobulin IgM undergoes recombination and switches to IgG, IgA, or IgE. This allows the production of highly specific antibodies [4]. T-cells CD4+, Th1, and Th2 have an essential role as helpers, as their CD40L molecules engage with CD40 of B-cells, allowing the class switch of immunoglobulins [4]. Antigen-specific B-cells, which receive help from antigen-specific activated T-cells, endure differentiation into plasma cells or memory B-cells [4].

The conjugation of a pathogen PS with a protein carrier elicits antigen-specific CD4+ Th cells, a T-dependent antibody response [4]. This response produces highly-differentiated B-cell differentiation that allows the production of antibodies, as previously mentioned [4]. The T-dependent response can be provoked by toxoid, protein, inactivated, or live attenuated viral vaccines, and is capable of producing higher affinity antibodies and immune memory [4]. In addition, live attenuated vaccines normally generate CD8+ cytotoxic T-cells [4]. Currently, the majority of the existent vaccines mediate their protective efficacies through the induction of highly specific IgG antibodies [4]. Antigen-specific antibodies have been confirmed to confer vaccine-induced protection against many diseases [4]. The removal of a pathogen from mucosal surface implies the presence of vaccine-induced IgG antibodies, and its concentration needs to be in great abundance and possess much affinity to facilitate the antibody titre in saliva and mucosal secretions [4]. Usually, this response is elicited by a glycoconjugate vaccine. Normally, vaccination does not prevent local infection of the mucosa if it is only after the infection that vaccine-

induced IgG serum antibodies neutralise the pathogen and limit its proliferation and spread [4].

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