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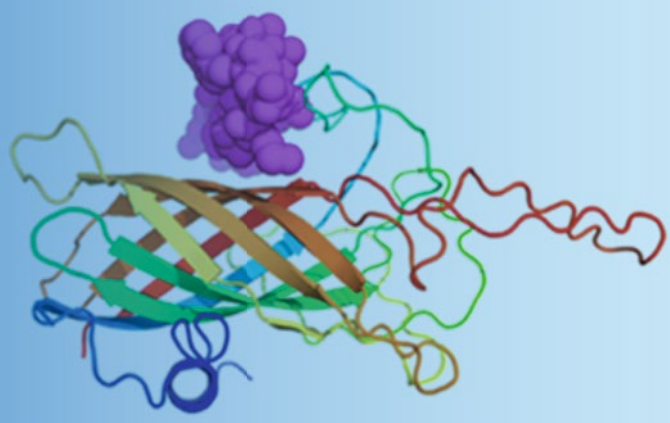

Sunil Thomas *Editor*

Vaccine Design

Methods and Protocols

Volume 1: Vaccines for Human Diseases

Humana Press

METHODS IN MOLECULAR BIOLOGY

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Volume 1: Vaccines for Human Diseases

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Dedication

Dedicated to
Vaccinologists (who work hard developing vaccines)
Healthcare workers in developing countries (who risk their lives vaccinating people)

Preface

"We are protecting children from polio at the cost of our lives."
Sabeeha Begum (a lady healthcare worker providing polio vaccines in Quetta, Pakistan)

Vaccinations have helped in preventing several diseases; however, as yet, there are only two diseases that have been eradicated globally. Mass awareness programs and aggressive vaccination strategies in the twentieth century were able to control smallpox, and the disease was officially declared eradicated in 1980. Rinderpest, a serious disease of cattle, was officially eradicated in 2011, thereby becoming only the second disease to be completely eradicated. Recently, the Americas (North and South America) were declared free of endemic transmission of rubella, a contagious viral disease that can cause multiple birth defects as well as fetal death when contracted by women during pregnancy. The achievement was due to a 15-year effort that involved widespread administration of the vaccine against measles, mumps, and rubella (MMR) throughout the Western Hemisphere.

One of the dreaded diseases—poliomyelitis—is in the last phases of eradication, thanks to the effective vaccines against the disease. The public health effort to eliminate poliomyelitis infection around the world began in 1988, and vaccination strategies have reduced the number of annual diagnosed cases of polio from the hundreds of thousands to couple of hundreds. Nigeria was the last country in Africa to eradicate polio; as of writing this book, no polio is reported in Nigeria since last year. Currently, polio remains endemic in two countries—Afghanistan and Pakistan. Until poliovirus transmission is interrupted in these countries, all countries remain at risk of importation of polio. Illiteracy, ignorance to vaccines, death threats, as well as killing of healthcare workers providing polio vaccines have slowed immunization programs in Pakistan. This toxic scenario coupled with the migration of people has led to the persistence of polio in Pakistan and neighboring Afghanistan. With awareness on the need of vaccination, knowledge on the importance of vaccination, and new rules that may penalize resistance to vaccination, it may be possible to eliminate polio by the end of the decade.

When I was given the opportunity to author this book (*Vaccine Design*: *Methods and Protocols*), I wished to have at least one chapter on vaccine design or vaccine development from every country. Unfortunately, it dawned on me later that not every country invests in science! It was also unfortunate to realize that research and development on vaccines is not a priority even in some developed countries with resources or influence. New sustainable technologies are to be developed to create more jobs and improve the well-being of humans as well as conservation of nature; hence, it is high time countries invest at least 5 % of their GDP for science including vaccine development.

Vaccine Design: *Methods and Protocols* is a practical guide providing step-by-step protocol to design and develop vaccines. The purpose of the book is to help vaccinologists develop novel vaccines based on current strategies employed to develop vaccines against several diseases. The book provides protocols for developing novel vaccines against infectious bacteria, viruses, and parasites for humans and animals as well as vaccines for cancer, allergy, and substance abuse. The book also contains chapters on how antigenic proteins

for vaccines should be selected and designed in silico, vectors for producing recombinant antigenic proteins, and the production of antigenic proteins in plant systems. Most vaccinologists are not aware of the intellectual property (IP) of vaccines, the importance of patents before commercialization, and what components of vaccines could be patented; hence, chapters on these aspects are also included in the book. The book also contains a chapter on the regulatory evaluation and testing requirements for vaccines.

The *Methods in Molecular Biology*™ series *Vaccine Design: Methods and Protocols* contains 103 chapters in two volumes. Volume 1, *Vaccines for Human Diseases*, has an introductory section on how vaccines impact diseases, the immunological mechanism of vaccines, and future challenges for vaccinologists and current trends in vaccinology. The design of human vaccines for viral, bacterial, fungal, parasitic, and prion diseases as well as vaccines for drug abuse, allergy, and tumor are also described in this volume. Volume 2, *Vaccines for Veterinary Diseases*, includes vaccines for farm animals and fishes, vaccine vectors and production, vaccine delivery systems, vaccine bioinformatics, vaccine regulation, and intellectual property.

It has been 220 years since Edward Jenner vaccinated his first patient in 1796. This book is a tribute to the pioneering effort of his work. My sincere thanks to all the authors for contributing to *Vaccine Design*: *Methods and Protocols* Volume 1 (*Vaccines for Human Diseases*) and Volume 2 (*Vaccines for Veterinary Diseases*). The book would not have materialized without the efforts of the authors from all over the world. I would also like to thank the series editor of *Methods in Molecular Biology*™, Prof. John M. Walker, for giving me the opportunity to edit this book. My profound thanks to my wife Jyothi for the encouragement and support, and also to our twins—Teresa and Thomas—for patiently waiting for me while editing the book. Working on the book was not an excuse for missing story time, and I made sure that you were told a couple of stories every day before bedtime.

Philadelphia, PA, USA ***Sunil Thomas***

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Part I

Vaccines: Introduction

Chapter 1

Clinical Impact of Vaccine Development

Puja H. Nambiar, Alejandro Delgado Daza, and Lawrence L. Livornese Jr

Abstract

The discovery and development of immunization has been a singular improvement in the health of mankind. This chapter reviews currently available vaccines, their historical development, and impact on public health. Specific mention is made in regard to the challenges and pursuit of a vaccine for the human immunodeficiency virus as well as the unfounded link between autism and measles vaccination.

Key words Vaccination, Immunization, Vaccine development, Public health, History of medicine

1 Introduction

Vaccination (Latin; *vacca*: cow) and sanitation have saved more lives and improved the public's health than any other medical intervention. Even before the germ theory of disease was established artificial induction was practiced in Asia and Europe [1]. Variolation, the process of obtaining pus from a smallpox vesicle and introducing it into the skin of an uninfected patient, was performed by people in various regions of Asia in the 1500s. This practice was observed by Lady Mary Wortley Montagu in Istanbul and introduced by her to England in 1721. Variolation, while effective, was not reliable and carried the real risk of developing smallpox from the process.

In 1774 an English farmer, Benjamin Jesty, noted that he was immune to smallpox after becoming infected with cowpox; subsequently, he successfully inoculated his wife and children and they were protected from smallpox as well. In 1798, Edward Jenner proved that large-scale inoculation with cowpox was an effective means of combating smallpox. In 1880, Louis Pasteur published work demonstrating that an attenuated form of the bacteria, *Pasteurella multocida*, could be used to produce a protective vaccine in animals. The following year, Pasteur's public demonstration of the effectiveness of an anthrax vaccine in sheep marked the beginning of a new era; it was now possible that vaccines could be reliably produced in a standardized, repeatable fashion.

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The history of vaccination, however, has not been without missteps or controversy. Early vaccines contained cells and bodily fluids and there was the legitimate concern that other infections could be transmitted through vaccination; the use of glycerin reduced this risk. The concept of introducing an infectious agent into healthy persons has been met with resistance from the start. For a time variolation was a felony crime in England. When Pasteur's rabies vaccine saved the life of Joseph Meister there was a public outcry in response to the process of purposefully injecting a lethal pathogen into a human—even one who was suffering from a uniformly fatal disease. The 1955 Cutter Incident, in which recipients of killed polio vaccine developed clinical disease due to the presence of live virus, resulted in 40,000 cases of vaccine-associated abortive polio, 164 cases of paralysis, and 10 deaths [2]. A 1998 Lancet paper by Andrew Wakefield that proposed a link between the measles-mumps-rubella vaccine and autism led to a widespread public loss of confidence in vaccines. The paper was subsequently found to be fraudulent and was withdrawn by the Lancet [3, 4]; however this, combined with the disproven theory that the thimerosal preservative in vaccines caused autism, continues to erode the public's confidence in vaccination [5].

Despite the unquestioned effectiveness and safety of vaccinations there continues to be groups of individuals who eschew vaccination for various scientific and religious beliefs. In the developing world, vaccination rates remain low for many contagious diseases. Until vaccination rates in both of these groups are increased the effectiveness of even the best designed vaccines will be limited and the public will remain at risk. In the following sections we review major vaccine-preventable diseases and the clinical impact that vaccination has had upon them.

2 Adenovirus Vaccines

Human adenoviruses are large, icosahedral, double-stranded DNA viruses belonging to family *Adenoviridae* [6]. They are further classified into seven species (A–G) and 52 serotypes. The history of *Adenoviruses* dates back to the 1950s when they were identified as a common cause for respiratory disease in children and US military trainees [7, 8]. In 1960s, the viruses caused significant morbidity and mortality among US military trainees.

Adenoviruses are spread primarily by respiratory droplets, fecooral route [9], or via direct contact. Close crowding promotes spread of virus. High-risk groups include children in day care centers and military trainees. Clinical syndromes associated with adenovirus infections in humans include respiratory adenovirus in children, acute respiratory disease (ARD) in military recruits, epidemic keratoconjunctivitis, pharyngoconjunctival fever,

hemorrhagic cystitis, infantile gastroenteritis, encephalitis, and opportunistic like infections in immunocompromised humans. Currently, there are no evidence-based guidelines supporting any specific antiviral treatment or prophylaxis for adenoviral illness. The off-label use of ribavirin and cidofovir has produced mixed results in immunocompromised patients with severe life-threatening adenoviral disease.

The first adenovirus vaccine was developed at the Walter Reed Army Institute of Research in 1956. It was an inactivated [10], injectable vaccine that protected against adenovirus infections caused by types 4 and 7. Production of this vaccine was terminated due to manufacturing issues. In 1971, Wyeth Laboratories developed live, oral enteric coated vaccines for adenovirus types 4 and 7. The rates of ARD dramatically reduced in the vaccine era. Unfortunately, the successful immunization program ended in 1999. Increasing mortality from adenovirus ARD in the postvaccination era resulted in the resumption of vaccine production for the military. Ad4 and Ad7 enteric coated live oral vaccines were reintroduced by Teva in 2011.

The CDC recommends two oral tablets to be swallowed [11], one tablet of adenovirus type 4 and one tablet of adenovirus type 7, as part of immunization schedule to military recruits, aged 17–50, who are beginning basic training. The most common side effects include nasal congestion, headache, upper respiratory infections, nausea, vomiting, and diarrhea. This vaccine is contraindicated in pregnancy and in those with anaphylaxis to vaccine components. Adenovirus vaccine in addition to secondary preventive measures including frequent hand washing, reducing crowded conditions, and cohorting has shown considerable reduction in ARD rates. A cost–benefit analysis estimated prevention of 4555 cases and $2.6 million savings with year-round vaccination [12]. Clinical trials have shown 94.5 % seroconversion, 99.3 % efficacy with Ad4 vaccine, and 93.8 % with Ad7 vaccine [13].

3 Anthrax Vaccines

Anthrax is a zoonotic disease caused by a spore-forming gram-positive bacilli *Bacillus anthracis* found in the soil. Human disease presents in three distinct clinical forms: cutaneous, inhalational, and gastrointestinal anthrax [14]. Injectional anthrax has also been described in intravenous heroin users [15]. Additionally, *Bacillus anthracis* is a Category A agent of bioterrorism.

Historically, researchers believe that anthrax originated in Egypt in 1250 BC and was responsible for the fifth and sixth biblical plagues. Clinically the disease was first described in the 1700s. In 1877, the German microbiologist, Robert Koch, studied *Bacillus anthracis* and described the causal relationship between

this specific bacterium and anthrax. In 1881, Louis Pasteur created the first vaccine using an attenuated strain of anthrax bacteria. Human anthrax was reported worldwide in the 1900s with industrial cases arising in developed countries and agricultural cases in developing Asian and African countries. With the advent of the first animal vaccine by Max Sterne in 1937 the number of human cases of anthrax dwindled. The first human vaccine against anthrax was created in the 1950s. Even though the incidence of human disease remains low, the use of *Bacillus anthracis* as a biologic weapon created the driving force for an improved human vaccine.

Anthrax is rare in the USA owing to vaccinations of livestock but remains common in developing countries that lack animal vaccination programs [16]. The bacterium produces highly resistant spores that can survive extreme environmental conditions for prolonged periods of time. The pathogenesis of disease in humans is attributed to the virulence factor of the capsule and production of two exotoxins. The anthrax toxin has three components—protective antigen (PA), lethal factor (LF), and edema factor (EF). The protective antigen with the edema factor forms the anthrax edema toxin responsible for cyclic AMP-mediated tissue swelling either in skin or mediastinum. The protective antigen with the lethal factor forms the anthrax lethal toxin responsible for cell death.

All three clinical presentations of anthrax have an incubation period of approximately 2–5 days. The cutaneous form of anthrax initially presents as a small, painless, pruritic papule that subsequently develops into a 1–2 cm large fluid-filled vesicle associated with surrounding edema, erythema, regional lymphadenopathy, and mild systemic symptoms. The vesicle ruptures in 5–7 days leaving behind an ulcer with black eschar which eventually falls off without a scar in 2–3 weeks. Antibiotics do not alter the development of cutaneous lesion. Inhalational anthrax manifests with nonspecific symptoms of myalgias, fever, and upper respiratory infection within 1–5 days of inhalation of infectious doses of *B. anthracis.* Patients then acutely develop respiratory distress syndrome from pulmonary hemorrhage and edema, and may die within 24 h. Widening of the mediastinum is a classic radiographic finding that develops secondary to lymphatic and vascular obstruction. If left untreated inhalational anthrax is 100 % fatal. Gastrointestinal anthrax develops after ingestion of anthrax-infected meat. Symptoms include abdominal pain, nausea, vomiting, diarrhea, and hematemesis with progression to septic shock and death. All three primary forms of anthrax can also manifest with bacteremia and secondary meningitis. Anthrax does not spread from person to person. Treatment involves decontamination and use of antibiotics such as ciprofloxacin, doxycycline, and penicillin. Passive immunization with human monoclonal anti-PA antibody, raxibacumab, has been approved for use in inhalational anthrax.

The human anthrax vaccine, anthrax vaccine adsorbed (AVA), is produced from a cell-free culture filtrate of attenuated,

non-encapsulated strain V770-NPI-R of *B. anthracis*. It predominantly contains the protective antigen adsorbed to aluminum hydroxide. This vaccine is mainly recommended for certain members of the US military, laboratory workers who work with anthrax [17], and individuals who work with animal and animal products. The vaccine is an intramuscular injection given as five shots at 0 and 4 weeks and 6, 12, and 18 months with annual booster [17]. For postexposure prophylaxis [17], three injections of AVA at 0, 2, and 4 weeks plus 60 days of antibiotics have been recommended. Side effects of the vaccine include mild local reaction and nonspecific systemic symptoms such as low-grade fever, headache, and myalgia. The only contraindication is hypersensitivity to the vaccine. There have been no controlled clinical trials in humans to determine either the efficacy of AVA or its use along with antibiotics for postexposure prophylaxis. However the use of AVA has reduced the incidence of anthrax among industrial and agricultural workers.

4 Cholera Vaccines

Cholera is an acute diarrheal illness caused by the bacterium *Vibrio cholerae*. It is one of the oldest infectious diseases known to mankind. In the eighteenth century the disease spread from its original reservoir, the Ganges Delta in India, causing epidemics and pandemics resulting in the death of massive numbers of people across the globe.

Cholera is an intestinal infection with toxigenic strains of *V. cholerae* serogroups O1 and O139. *V. cholerae* O1 serogroup is further classified into two serotypes—Ogawa and Inaba—and two biotypes—classical and El Tor. The mode of transmission is through ingestion of contaminated food and water [18]. The disease occurs in children and adults especially in the lower socioeconomic groups. The short incubation period of 2 h to 5 days is responsible for exponential wave of this disease. Following consumption of infected food, the bacterium uses its virulence factors—toxin-coregulated pilus (TCP) [19], hemagglutinin [20], and single flagellum to colonize the small intestine and secretes cholera enterotoxin (CT). The "-B-" subunit of CT binds to the GM1 ganglioside receptor, facilitating entry into the intestinal mucosal cells and "-A-" subunit activates adenyl cyclase leading to excess fluid and salt secretion. Clinical symptoms include acute diarrhea and vomiting rapidly leading to electrolyte imbalances, hypovolemic shock, multiorgan failure, and death. Cholera can be fatal if there is a delay in replacement of fluid and electrolytes. Diagnosis is made clinically and by identifying the bacterium in stool cultures. Serologic tests are available but are nonspecific.

The global annual incidence of cholera is uncertain but the approximate cases may be 3–5 million causing 100,000–120,000

deaths. More than half the cases occur in Africa and remainder in Asia. There have been sporadic cases along the US Gulf Coast associated with undercooked or contaminated seafood. The majority of other cases in the developed countries are secondary to travel to endemic areas.

The preparation of the earliest vaccine against cholera began in the late eighteenth century. Initial studies were made with a parenteral killed whole-cell cholera vaccine in the 1880s which had limited use owing to short-term efficacy. The currently licensed cholera vaccines contain either genetically attenuated strains, killed organisms, or antigens. Three oral vaccines—two killed and one live—have been developed and licensed in several countries. The whole-cell killed vaccine plus CTB (WC-rCTB/Dukoral) contains killed strains of *V. cholerae* O1 (classical, El Tor, Ogawa, and Inaba) with B subunit of cholera toxin. The vaccine is given as two oral doses combined with a liquid oral buffer, 7–14 days apart in adults and in three doses in children 2–6 years of age with need for further booster doses. The vaccine is WHO prequalified but remains experimental in the USA. The reformulated bivalent killed whole-cell-only vaccine (WC-only/Shancol in India/mORCVAX in Vietnam) contains killed whole cells of *V. cholerae* O1 and O139. It is given as two doses 2 weeks apart with further boosters at 3-year intervals. Since the vaccine does not contain the gastric acid-labile cholera toxin subunit, it does not have to be coadministered with a buffer. The only live oral cholera vaccine is CVD103-HgR (Orochol or Mutachol). The vaccine is a live attenuated Inaba strain, which is genetically engineered to express CTB subunit and not the active CTA subunit. The vaccine is administered as a single oral dose with a buffer and does not require booster doses. The live vaccine has not been prequalified by WHO.

The WHO recommends the use of the two killed oral vaccines in cholera endemic areas and areas at risk for outbreaks [21]. The cholera vaccine is unavailable in the USA and CDC does not recommend cholera vaccines to most travelers owing to short-term and incomplete protection. These vaccines however cannot replace the pivotal role played by hygiene and proper sanitation in the control of cholera outbreaks.

5 Diphtheria Toxoid

Diphtheria is an acute toxin-mediated disease caused by *Corynebacterium diphtheriae*, a gram-positive bacillus that is acquired via the respiratory tract. The disease has been well described throughout history with Hippocrates famously writing about it in the fifthcentury BC. Outbreaks throughout Europe occurred as early as the fifteenth century. Spain experienced a major epidemic, in 1613, known as "El Año de los garrotillos," the year of strangulation [22].

Corynebacterium diphtheriae is a toxin-producing gram-positive bacillus. It has three biotypes: gravis, intermedius, and mitis, with the most severe forms of disease being produced by the gravis serotype. Susceptible persons may acquire toxigenic bacillus in the nasopharynx. The organism produces a toxin that inhibits protein synthesis and is responsible for local tissue disease and membrane formation. The locally produced toxin is absorbed into the bloodstream and transferred to other tissues.

The clinical presentation of diphtheria can be insidious with an incubation period of 1–5 days. Usually the symptoms are nonspecific and mild in the initial stages with fever and mild pharyngeal erythema being common. Within 3–4 days patches of exudate appear that coalesce to form membranes covering the entire pharynx [22]. As the disease progresses, large adenopathies become evident and patients begin to appear toxic. Attempts to remove the membranes often result in bleeding. Patients may recover following this stage. If enough toxin has been produced, patients may develop acute disease with prostration, coma, and high fevers. Marked edema and adenopathy may result in the classic "bullneck" appearance. Although pharyngeal diphtheria is the most common form of the disease in unimmunized populations, other skin or mucosal sites may be involved. This includes the nasopharynx, cutaneous, vaginal, and conjunctival forms. Invasive disease is very rare and is due to nontoxigenic strains of *C. diphtheriae*. Most complications of diphtheria, including death, are attributable to the toxin. Myocarditis can occur early in the disease process or appear weeks later. When it does occur, it is often fatal. Neuritis often affects motor nerves and can cause pharyngeal paralysis. The overall mortality of diphtheria is 5–10 % with rates as high as 20 % in those younger than 5 years or older than 40 [22].

Diphtheria antitoxin produced from horses was first used in the USA in 1891 and it was commercially produced in Germany in 1892. Equine diphtheria antitoxin is produced by hyperimmunizing horses with diphtheria toxoid and toxin. To prevent reactivity from horse serum, current preparations are semi-purified by techniques that concentrate IgG and remove as much extraneous proteins as possible. Diphtheria antitoxin is used for the treatment of infected patients and, in the past, for persons with high-level exposures.

The development of an effective toxoid, a combination of toxin-antitoxin, was achieved in the 1920s. Beginning in the 1940s, this was combined with the pertussis vaccine and became widely used. Diphtheria toxoid is produced by growing toxigenic *C. diphtheriae* in liquid medium. The filtrate is incubated with formaldehyde to convert toxin to toxoid and is then adsorbed onto an aluminum salt. Diphtheria toxoid is available combined with tetanus toxoid as pediatric diphtheria-tetanus toxoid (DT) or adult tetanus-diphtheria (Td), and with both tetanus toxoid and acellular pertussis vaccine as DTaP and Tdap. Diphtheria toxoid is also

available as combined DTaP-HepB-IPV (Pediarix) and DTaP-IPV/Hib (Pentacel) [23].

After a primary series of three properly spaced diphtheria toxoid doses in adults or four doses in infants, a protective level of antitoxin (defined as greater than 0.1 IU of antitoxin/mL) is reached in more than 95 %. Diphtheria toxoid has been estimated to have a clinical efficacy of 97 % [2]. Revaccination is recommended every 10 years.

6 Haemophilus Influenza Vaccines

Haemophilus influenzae is an important cause of severe bacterial infections in children younger than 5 years. It was first identified by Koch in 1883 but it was not until the influenza pandemic in 1918 that *H. influenzae* was recognized as a cause for secondary infection and not the primary cause of influenza [24]. In 1931, Pittman [25] demonstrated two categories of *H. influenzae*-encapsulated and nonencapsulated forms and further designated six serotypes (a–f) [25] on the basis of capsular properties. *H. influenzae* type b (Hib) was responsible for 95 % [26] serious invasive bacterial infections in the prevaccine era.

Haemophilus influenzae is an aerobic, non-spore-forming gram-negative coccobacillus. It requires two factors "X" (hemin) and "V" (nicotinamide adenine dinucleotide) [27] for in vitro growth, a property that distinguishes it from other *Haemophilus* species. The polyribosyl-ribitol-phosphate polysaccharide capsule is responsible for virulence and immunity. Hib colonizes nasopharynx (asymptomatic carriers) and is spread through respiratory droplets. Antecedent viral infections may play a role in invasive disease. Common invasive presentations include meningitis, pneumonia, otitis media, epiglottitis, septicemia, cellulitis, and osteoarticular infections. Non-type-b-encapsulated *H. influenzae* rarely causes invasive disease. A positive culture of *H. influenzae* from infected sterile body fluid or detection of Hib polysaccharide antigen in CSF is diagnostic. Serotyping is extremely important as type b isolated in children younger than 15 years is a potentially vaccine-preventable disease.

The first-generation pure polysaccharide vaccine (HbPV) was introduced in the early 1980s in the USA but was not immunogenic in children younger than 18 months and had variable efficiency in older children (age-dependent vaccine response). It was used until 1988 and is no longer available in the USA. The conjugation of the polysaccharide to the "carrier" protein results in a T-dependent antigen and increases immunogenicity and boosts response. The annual incidence of invasive Hib disease before the use of conjugate vaccine was 20–88 cases per 100,000 cases in the USA and has dramatically reduced ever since its introduction. Four conjugate Hib vaccines have

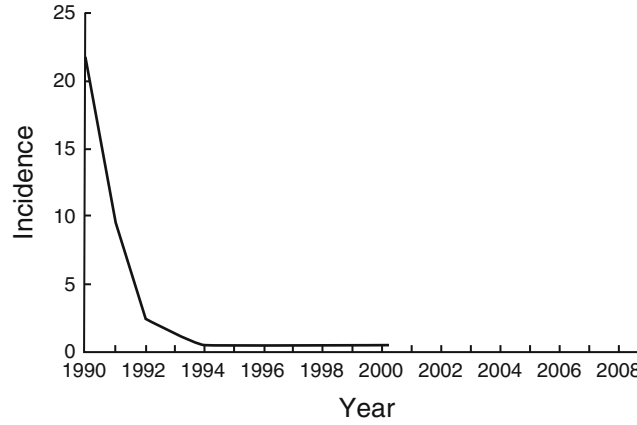

Fig. 1 Incidence of invasive Hib disease 1990–2009 (rate per 100,000 children less than 5 years of age). Graph from CDC/vaccines/pinkbook/hib

been developed [28]. The first *H. influenzae* type b polysaccharide-protein conjugate vaccine (PRP-diphtheria toxoid conjugate) was licensed in 1987 and is no longer available. The Haemophilus b oligosaccharide conjugate (HbOC) licensed in 1990 contains oligosaccharides from purified PRP from Hib Eagan strain coupled with nontoxic variant of diphtheria toxin isolated by *Corynebacterium diphtheriae*. The PRP-OMP vaccine was also licensed in 1990 and is a purified PRP from Hib Ross strain covalently bonded to an outer membrane protein complex of *Neisseria meningitides* strain B11. PRP-T is covalently bound PRP to tetanus toxoid and was licensed in 1993. The three HiB conjugate vaccines licensed for use are interchangeable. The Advisory Committee on Immunization Practices (ACIP) recommends start of immunization as early as 6 weeks of age with total of three doses of any combination HiB vaccines before the first birthday and a booster dose at 12–15 months of age. The only contraindication is hypersensitivity to vaccine components. HiB is not routinely recommended for persons 5 years and older; it can be considered in special situations such as asplenia, sickle cell anemia, or HIV infection (Fig. 1).

The routine use of HiB conjugate vaccine has dramatically decreased disease in developed countries and shown to be highly effective in reducing the incidence of disease in developing countries. Efforts are under way by the WHO to increase awareness and global availability of this effective vaccine especially in resource-limited countries.

7 Hepatitis A Vaccines

The first description of hepatitis or "episodic jaundice" dates back to the time of Hippocrates, and the earliest outbreaks were reported in Europe in the seventeenth and eighteenth centuries [29].

During World War II the scientific details regarding this disease were obtained. Hepatitis A was epidemiologically differentiated from hepatitis B in 1940s but it was only in 1970s that serological tests were developed to definitively diagnose this disease.

Hepatitis A occurs worldwide but is endemic in Central, South America, Asia, the Middle East, and Africa. It is caused by *hepatitis A virus (HAV)*, a non-enveloped RNA virus belonging to the family of *Picronaviridae*. Humans are the only natural host. HAV is resistant to most organic solvents and detergents and can survive at a pH as low as 3 but can be inactivated by high temperature (>85 °C), chlorine, and formalin [30]. *HAV* infection is acquired through fecal-oral route either by person-person contact or through ingestion of contaminated food or water. The incubation period is approximately 28 days [31]. *HAV* replicates in the liver; infected persons shed the virus for 1–3 weeks and have a very high risk of transmission 1–2 weeks prior to the onset of symptoms. Risk factors for *HAV* infection include international traveling, men who have sex with men, intravenous drug users, and persons with chronic liver disease or with clotting disorders. The clinical features are similar to other types of acute viral hepatitis. HAV infection presents as an acute febrile illness with nausea, abdominal discomfort, and jaundice. Other atypical manifestations include vasculitis, cryoglobulinemia, and neurologic, renal, and immunologic reactions. HAV is a self-limited disease that does not produce chronic infection or chronic liver disease. Fatality from acute liver failure occurs in 0.5 % of those infected. Diagnosis is made on clinical, epidemiologic, and serologic basis. The antibody test for total anti-HAV measures both IgM-HAV and IgG-HAV. IgM becomes positive in acute HAV infection within 5–10 days before the onset of symptoms and can persist up to 5–6 months. IgG appears in the convalescent phase of the disease and confers lifelong protection.

In the prevaccine era, the only methods for prevention of hepatitis A were hygienic measures and use of protective immunoglobulins. Two inactivated whole-virus hepatitis A vaccines, VAQTA and HAVRIX [32, 33], were licensed in 1995 in the USA and approved for use. The other vaccines used worldwide are AVAXIM, EPAXAL, and Heavile. All these vaccines are made from different strains of the HAV; VAQTA is based on strain CR326F, and HAVRIX is based on strain HM175 and contains a preservative unlike VAQTA. Both vaccines are highly immunogenic. ACIP recommends vaccination for all children at 12–23 months of age. Adults who are at increased risk of infection or complication from HAV infection should be routinely vaccinated. HAVRIX is administered intramuscularly as a single primary dose in children 1–18 years (0.5 ml) and adults above 19 years (1 ml) followed by a booster at 6–12 months. VAQTA is administered similarly to HAVRIX; however the booster is administered 6–18 months after primary dose. In 2001, Twinrix—a combination vaccine with adult dose of hepatitis B vaccine (Engerix-B)

and pediatric dose of HAVRIX—was approved for adults greater than 18 years of age; it is given intramuscularly at 0, 1, and 6 months. Contraindications to the vaccine include allergic reactions or moderate-to-severe illness. Adverse reactions include pain at injection site but systemic side effects are rare. The wide use of vaccines has resulted in a sustained reduction of disease in most of the developed world; however hepatitis A infection remains an ongoing issue in the developing world.

8 Hepatitis B Vaccines

Hepatitis has been recognized as a clinical entity since the times of Hippocrates when he dubbed it epidemic jaundice. However, the wide diversity of viruses that can be responsible for this entity has only recently begun to be recognized. The first case of "serum hepatitis" or what was believed to be hepatitis B was first described during an epidemic which resulted from vaccination against smallpox in shipyard workers in late nineteenth-century Germany. Jaundice developed in 15 % of the inoculated workers. The role of blood as a vehicle became clearer in 1943 when Beeson described the transmission of hepatitis to recipients of blood transfusions [34].

Hepatitis B virus is a small double-shelled DNA virus of the family *Hepadnaviridae* [34]. It has a small circular DNA genome. It contains several antigens including the hepatitis B surface antigen, hepatitis B core antigen, and the hepatitis B E antigen. Humans are the only known host to the virus.

Hepatitis B virus is primarily hepatotropic; although hepatitis B surface antigen (HbSAg) has been recovered from other organs, there is little evidence that it replicates outside of the liver. Most experimental data supports the notion that the virus is not directly cytopathic but rather the damage to tissue is mediated by an immune response to the virally infected hepatic cells. Infection can range from being asymptomatic to causing a fulminant hepatitis. Persons infected with hepatitis B can also progress to a chronic infection resulting in cirrhosis and hepatocellular carcinoma [34].

The clinical course of hepatitis B is indistinguishable from other causes of viral hepatitis. The incubation period ranges from 40 to 160 days. Definitive diagnosis requires serological assays to distinguish it from other causes of hepatitis. The preicteric phase which occurs a week before the onset of jaundice is characterized by malaise, fatigue, nausea, vomiting, and right upper quadrant pain. The icteric phase lasts from 1 to 3 weeks and is characterized by jaundice, hepatomegaly, and acholia. Approximately 40 % of people in the USA that develop acute hepatitis B are hospitalized. Fulminant hepatitis occurs in 0.5–1 % of cases and is more common in adults than children. During the convalescent phase, all symptoms resolve but fatigue may linger for weeks.

Approximately 5 % of cases will progress to chronic infection with the risk of chronic infection decreasing with age. As many as 90 % of infants who acquire the virus from their mothers progress on to chronic infection. Persistent infection is defined as having a positive HBSAg for more than 6 months. Viral replication persists throughout the course of chronic hepatitis B infection and disease progression depends on interactions between the virus and host immunity. It is a dynamic process that may span over the course of decades. Most patients can be asymptomatic but continue to spread infection. This carries a 25 % risk of developing cirrhosis and dying of liver cancer.

The incidence of hepatitis B peaked in the 1980s. Approximately 10,000 or less cases are now reported annually in the USA. Before routine childhood immunizations, most infections occurred in adults. The highest risk groups are those between 20 and 45, those who engage in high-risk sexual practices, and those who use injection drugs. Up to 16 % of patients who acquire the disease deny any risk factors [35].

Hepatitis B vaccine has been available in the USA since 1981. It consists of a 226-amino acid S gene product. This gene is injected via plasmids into *Saccharomyces cerevisiae* which produce a recombinant HbSAg protein. The final product contains 95 % purified protein surface antigen but no yeast DNA. Thus, infection cannot result from hepatitis B vaccination. The vaccine has a proven efficacy of around 90–95 % [35, 36] in normal populations with lower rates of immunogenicity in subsets of patients. A particularly challenging group has been patients with *HIV* in whom vaccine efficacy can be as low as 30 %. This is worrisome as patients with HIV are to be considered high risk for acquiring the infection [37].

9 Human Papillomavirus Vaccines

Human papillomavirus (HPV) is a DNA virus that causes epithelial lesions of mucous membranes ranging from benign papillomas to carcinoma [38–40]. The association of HPV and cancer was first described by Orth [41] in 1970s. In the 1980s, zur Hausen [42] identified HPV 16 and HPV 18 in cervical cancer cells. The introduction of screening and use of HPV vaccines have decreased the incidence of HPV-associated cervical cancers in the developed world [43]. However, the incidence of HPV-associated anal and oropharyngeal cancer is on the rise.

HPV is the most common sexually transmitted disease in the USA. It is estimated the prevalence varies from 14 to more than 90 %, the highest rate occurring in the age group 20–24. HPV is transmitted through vaginal sex, anal sex, genital-genital contact, and oral sex and rarely from pregnant women with genital HPV to their babies causing recurrent respiratory papillomatosis (RRP) in

the child. HPV replicates in the nuclei of stratified squamous epithelial cells. In majority of individuals HPV is spontaneously cleared but in small number of cases HPV persists with risk of progression to high-grade dysplasia or invasive carcinoma of the cervix, vulva, vagina, penis, anus, and oropharynx [44]. In the USA, there are approximately 17,000 women and 9000 men affected with HPV-related cancer yearly. The Pap smear used as a screening tool helps prevent HPV-associated cervical cancer in women but unfortunately the lack of screening for other HPV-related cancers results in increased morbidity and mortality.

There are two available HPV vaccines—Gardasil and Cervarix. Gardasil is a recombinant human papillomavirus quadrivalent vaccine produced in the yeast *Saccharomyces cerevisiae* [45]. It contains viruslike particles of types 6, 11, 16, and 18 which together cause around 90 % of genital warts. Cervix is a recombinant bivalent vaccine composed of viruslike particles of types 16 and 18, which causes approximately 70 % of cervical cancers worldwide. In young females, either of the vaccines may be used. The target age group is 9 through 26 years of age to prevent HPV-related genital warts, cervical intraepithelial neoplasia, and cancers. In young males, Gardasil is the only approved vaccine, with target age group being 11–12 years. HPV vaccination is also recommended for older teens who are not vaccinated when younger. Both HPV vaccines are administered intramuscularly as a three-dose schedule—with the second dose being given 1 or 2 months after the initial dose and third dose 6 months after the first dose. The vaccine series does not have to be restarted if interrupted and can be interchanged with either HPV vaccine product to complete series. The most commonly reported side effects are nausea, headache, dizziness, and local reactions at injection site. The vaccine is contraindicated in persons with history of hypersensitivity to vaccine components and in pregnancy owing to limited efficacy data. Its use is safe in immunocompromised hosts as both Gardasil and Cervarix are noninfectious vaccines.

Despite the safety and efficacy, HPV vaccines remain underutilized. It is estimated that only 57 % of adolescent girls and 35 % of adolescent boys received one or more doses of HPV vaccine. CDC data and statistics [46] illustrate that if clinicians give a stronger recommendation for adolescent HPV vaccinations before the age of 13, 91 % of adolescent girls would be protected from HPV-related cancers.

10 Influenza Vaccines

Influenza is a highly contagious viral disease caused by the single-stranded RNA *influenza virus*. Descriptions of pandemic influenza can be found in many places throughout history and its name is

derived from an epidemic in fifteenth-century Italy which was thought to have occurred under the influence of the stars [47]. At least four pandemics occurred in the nineteenth century and three occurred in the twentieth century. The infamous Spanish influenza which occurred in the early twentieth century was responsible for at least 18–19 million deaths which dwarfed World War I which was occurring at the time and may be partially responsible for ending that conflict. The virus itself was first isolated in the 1930s for the first time by Smith, Andrews, and Laidlaw. The first inactivated vaccine was first created in the 1950s and that was followed by a live attenuated vaccine in 2003.

Influenza virus is a single-stranded RNA virus of the family *orthomyxovirus* [47]. Basic antigen types A, B, and C are determined by nuclear material. Influenza A can be further characterized by two components, hemagglutinin (H1, H2, and H3) and neuraminidase (N1, N2) which play roles in viral cell penetration. Influenza A naturally infects humans, swine, and poultry among other birds and the virus can freely exchange genetic material in these hosts. The H and N antigens vary and are part of the reason why the virus is so successful at evading immunity and the reason vaccines need to be reformulated annually. The virus undergoes antigenic drift which is a minor variation of its surface antigens caused by point mutations in a gene segment. These can result in epidemics since the protection that has been conferred by prior years of infection is incomplete. Antigenic shift on the other hand is a major change in one or both H and N antigens that are likely the result of a recombinant virus exchange between those who affect birds and humans. These major changes occur at varying intervals and are responsible for worldwide pandemics.

Following respiratory transmission, the virus proceeds to invade respiratory epithelial cells in the trachea and the bronchi. This replication itself results in cellular death. Of those infected, 30–50 % will not experience symptoms and those who go on to develop them can have a wide spectrum of manifestations ranging from mild respiratory complaints to a rapidly evolving febrile illness complicated by secondary bacterial infections [48]. Primary influenza is characterized by the abrupt onset of fever, chills, myalgias, headache, sore throat, and extreme fatigue. The presence of fever and respiratory symptoms are the most sensitive indicators of illness. Fever may range from 38 to 40 °C but may vary. Symptoms usually improve within 1 week but cough and fatigue can persist for 2 weeks or more. Gastrointestinal symptoms, croup, and otitis media can occur and are more common in children. Complications from influenza tend to occur in the extremes of age and those with comorbidities. The most common complications are exacerbations of underlying conditions such as COPD, congestive heart failure, and coronary artery disease. This is coupled with the development

of bacterial pneumonia caused by usual community pathogens as well as an increased incidence of *Staphylococcus aureus* pneumonia. In the USA influenza is responsible for over 200,000 hospitalizations and 30–5000 deaths annually with the largest impact on the elderly and the very young. A greater number of hospitalizations occur in years when influenza H2N3 is the predominant strain. An increase in mortality typically accompanies influenza epidemics and a large number of these deaths are not directly related to the infection but rather to its complications such as cardiac events and exacerbation of other chronic medical conditions.

Two types of influenza vaccines are currently available: an inactivated trivalent or quadrivalent vaccine containing influenza A H3N2 and H1N1 plus influenza B-inactivated viruses, and an attenuated live virus vaccine that has the equivalent components of the inactivated trivalent vaccine. The inactivated vaccine is injected intramuscularly or intradermally. Hemagglutinin is the main component and immunogen in these vaccines. In 2003 the FDA approved a live attenuated vaccine. It contains the same components of the trivalent inactivated vaccine; they are cold adapted and reproduce effectively in the nasopharynx of the recipient. It is administered as a single dose of a spray through each nostril.

The immunity conferred by the inactivated virus vaccine is deemed to last for less than a year. On years when there is a good match between the circulating strain and the vaccine, protection can be as high as 90 % among those younger than 65 and around 40 % in older patients [49, 50]. This usually yields a vaccine efficacy close to 50–60 %. Vaccination has also been shown to be effective at preventing complications of influenza. Inactivated vaccine should be administered on a yearly basis to eligible patients which now includes all patients older than 6 months.

The live attenuated virus vaccine is 87 % effective in decreasing disease and close to 30 % effective in decreasing otitis media. The live attenuated vaccine should be administered to patients older than 2 years up to age 49 [51].

Recommendations for the antigenic composition of the vaccines are made annually to ensure that the vaccines are effective against recently circulating strains of the virus. This is subject to antigenic drift and shift which explains why certain influenza seasons feature strains not anticipated by vaccine makers. The timeline for production of the vaccine is similar each year and hinges on the activity of the WHO influenza surveillance network. Because production of the vaccine requires several months, data collection must be balanced with manufacturing times. If recommendations are made too early, then antigens could change rendering the vaccine ineffective. If recommendations are made too late, timely vaccine manufacture may be impossible.

11 Japanese Encephalitis Vaccines

Japanese encephalitis (JE) is a vaccine-preventable mosquito-borne viral infection that occurs in the developing countries of Asia. Outbreaks consistent with JE were reported as far back in 1871 in Japan and the virus was first isolated from *Culex tritaeniorhynchus* in 1938.

JE virus (JEV) is a single-stranded RNA *flavivirus* closely related to West Nile and Saint Louis encephalitis virus. JEV is transmitted through the bite of infected Culex species of mosquitoes. The natural cycle of the JEV is enzootic consisting of bird-mosquito-bird or pig-mosquito-pig circulation of the virus. Humans are incidental or dead-end hosts. Human-to-human transmission is rare but cases from vertical transmission and through organ transplant have been reported. Transmission usually occurs in rural agricultural areas, mainly associated with irrigated rice fields in the tropical and temperate regions of eastern and southern Asia. Epidemic activity is highest in summer and early fall while endemic activity is sporadic and not associated with any seasonal pattern. JE is primarily a disease of children as adults acquire immunity through natural infection. As per the CDC, the incidence of JE among travelers to Asia from non-endemic areas is less than one case per million travelers [52].

The majority of human infections with JEV are asymptomatic with less than 1 % of developing clinical symptoms. The incubation period is 5–15 days. The most common presentation is that of acute encephalitis with sudden onset of fever, headache, vomiting, and mental status changes. Other manifestations include seizures, a parkinsonian syndrome, and acute flaccid paralysis [53] resembling poliomyelitis. IgM antibody of CSF and serum samples is currently the standard test for diagnosis. Viral isolation and nucleic acid amplification tests are insensitive tools for diagnosis. There is no specific treatment and therapy consists of supportive care and managing complications.

The incidence of JE has drastically decreased over the last few decades owing to vector control programs and vaccinations. The three most important types of vaccines currently used are purified, mouse brain-derived, inactivated Nakayama or Beijing strains of JEV; cell culture-derived inactivated JE vaccine based on Beijing P3 strain; and cell culture-derived live attenuated JE vaccine from SA 14-14-2 strain. The only licensed vaccine in the USA is the inactivated vero cell culture-derived vaccine branded as Ixiaro, approved in 2009. The ACIP recommends vaccination [54] for travelers spending more than 1 month in endemic areas during the JEV transmission season or short-term travelers with high risk or uncertain activities or traveling to a region with a JE outbreak. The primary immunization schedule includes two intramuscular

injections given on days 0 and 28 to be completed at least 1 week prior to travel date. There is limited data on efficacy and use in pregnancy. The common adverse reactions include local reaction and flu-like illness.

12 Meningococcal Vaccines

Meningococcal disease is an acute, potentially life-threatening disease caused by the gram-negative, endotoxin-producing bacteria *Neisseria meningitidis* or the meningococcus. It causes meningitis, sepsis, and focal infections. Epidemics of meningococcal meningitis were first described in the early eighteenth century. Prior to the advent of antibiotics, the case fatality rate was as high as 70–85 % (Fig. 2).

Meningococcus is an aerobic, gram-negative diplococcus and is a normal commensal of the human nasopharynx. The organism has an inner cytoplasmic membrane and outer membrane separated by a cell wall. The outer membrane proteins and polysaccharide capsule serve as antigens and are responsible for the pathogenicity of the organism. Meningococci are classified on the basis of characteristics of the polysaccharide capsules—at least 13 serogroups have been described and most invasive disease is caused by serogroups A, B, C, Y, and W-135.

The meningococcus colonizes only the nasopharynx and carriage rates are highest among adolescents and young adults [55–57]. It is transmitted through aerosol droplets or direct contact with respiratory secretions. Risk factors for infection include complement deficiency [58, 59], asplenia [60], HIV, recent viral illness, and tobacco smoking. In less than 1 % of colonized humans the organism invades to cause bacteremia and around 50 % of the bacteremic patients develop meningeal involvement. The incubation period is around 2–10 days. Clinical presentations include meningitis and bloodstream infections, called meningococcemia, characterized by fever, hypotension, petechial rash, and multiorgan failure. Less common manifestations include otitis media,

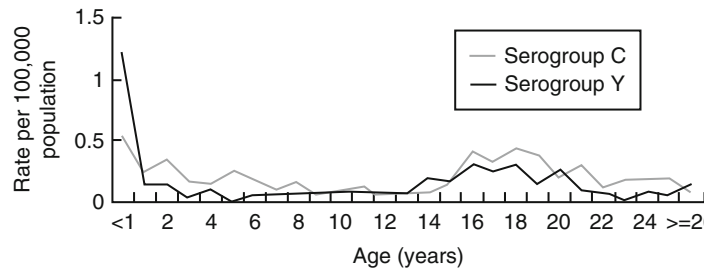

Fig. 2 Rates of meningococcal disease by age, USA, 1999–2008. Source: CDC/vaccines/meningococcal; CDC Active Bacterial Core Surveillance

pneumonia, and arthritis. Diagnosis is made by a positive gram stain and bacterial culture from a normally sterile site. Detection of polysaccharide antigen in CSF and serology may also be used in evaluation. Intravenous aqueous penicillin is considered therapy of choice.

Vaccination is the most effective method to prevent meningococcal disease. The first meningococcal polysaccharide vaccine (Menomune; MPSV4) was licensed in the USA in 1978 and is a quadrivalent A, C, Y, W-135 polysaccharide vaccine administered subcutaneously. Three meningococcal conjugate vaccines (MCV4-Menactra, Menveo, MenHibrix) are available in the USA. Menactra was licensed in 2005 [61, 62] and Menveo in 2010 [63]. Both vaccines are quadrivalent A, C, Y, W-135 conjugated to diphtheria toxoid, approved for persons 2–55 years of age. MenHibrix is a meningococcal serogroup C, Y, Haemophilus B tetanus toxoid conjugate vaccine. It is indicated to prevent meningococcal and Haemophilus disease in children 6 weeks through 18 months of age. The first meningococcal serogroup B vaccine available in the USA called Trumenba was licensed in late 2014 for individuals 10–25 years in a three-dose series at 0, 2, and 6 months. The vaccine is indicated [64] in persons aged 11–18 years, first dose at age 11–12 years and a booster at age 16 or first dose if given at 13–15 years then booster at 16–18 years. No booster is indicated if primary dose was given on or after age 16 years. Other indications [64] include persons aged 2–55 years or 9 months–2 years with functional or anatomical asplenia or complement deficiency, with increased risk for exposure or travel to hyperendemic areas. Bexsero, a second meningococcal serogroup B vaccine, was approved by the FDA in January 2015. It is administered in two doses 1 month apart. At the time of this writing, the CDC has not yet published recommendations on the use of the serogroup B vaccines; these recommendations are expected to be released in June 2015. Adverse reactions include local reactions, fever, and mild systemic symptoms. Contraindication [64] to the vaccine is moderate-severe illness or allergy to vaccine component. In most areas, invasive meningococcal disease is a reportable condition. Antimicrobial chemoprophylaxis (ciprofloxacin, rifampin, ceftriaxone) is recommended for close contacts with exposure to index patient given the high rate of secondary disease.

12.1 Measles, Mumps, and Rubella Vaccine

Measles is a ubiquitous, highly contagious disease caused by the measles virus. It was recognized as early as seventh century. Measles was described as severe disease, “more to be dreaded than smallpox”—for the first time by Persian physician Rhazes in the tenth century [65, 66]. In the pre-vaccine era, school-aged children had the highest risk of infection and more than 95 % of cases occurred by 15 years of age [67, 68] (Figs. 3, 4, and 5).

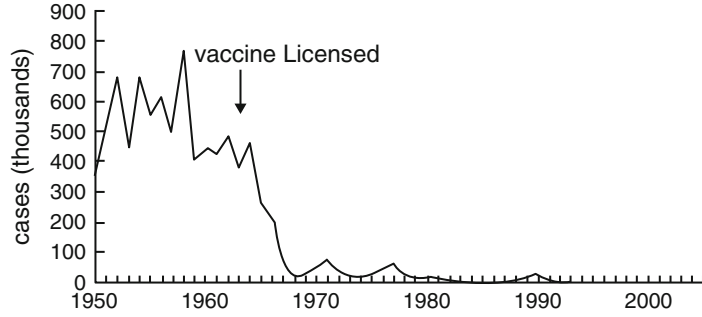

Fig. 3 Measles—USA, 1950–2009. Graph from CDC/vaccines/pinkbook/measles

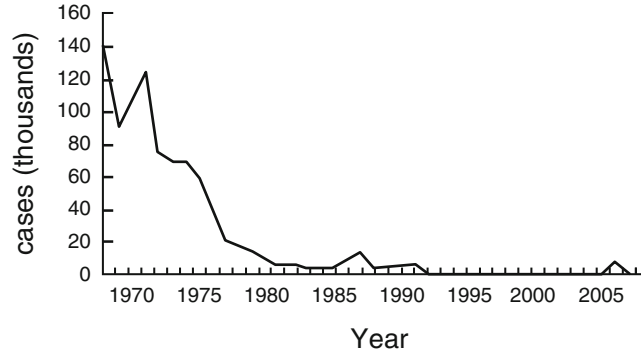

Fig. 4 Mumps—USA, 1968–2009. Graph from CDC/vaccines/pinkbook/mumps

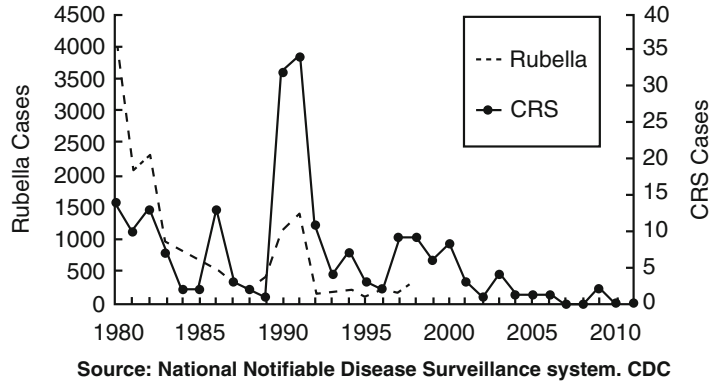

Fig. 5 Rubella cases in the USA, 1966–2009. Graph from CDC/vaccines/rubella

Measles virus is a single-stranded RNA virus member of the genus Morbilliform in the family *Paramyxoviridae*. Two membrane envelope proteins—fusion protein (F) and hemagglutinin (H)—are responsible for pathogenesis. There is only one antigenic type of measles virus. Measles is an airborne disease and is spread via respiratory transmission. The primary site of invasion and

replication is the respiratory epithelium. The incubation period is 10–12 days followed by a prodrome consisting of fever, cough, coryza, conjunctivitis, and Koplik spots—punctate bluish-white spots on red background on buccal mucosa which are pathognomonic of measles. The rash of measles is a maculopapular rash that develops 2–4 days after prodrome or 14 days after exposure and spreads from the head over the trunk to the extremities during a 3–4-day period. The rash fades over next 3–4 days in the order of its appearance. The complications of measles include diarrhea, otitis media, pneumonia, encephalitis, seizures, and rarely death. Diagnosis is clinical and is confirmed by serological testing—most commonly by ELISA.

Prior to 1963, approximately 500,000 cases and 500 deaths were reported annually in the USA [69], with epidemic cycles every 2–3 years. Following vaccine licensure in 1963, the incidence of measles decreased by more than 98 %. Between 1989 and 1991, there was a resurgence of measles with 55,622 cases in children less than 5 years of age with 123 reported deaths. Measles incidence then declined rapidly post-resurgence period owing to increased vaccination programs of preschool children, adolescents, and young adults. The Centers for Disease Control reported a total of 911 cases of measles from 2001 to 2011; however owing to vaccination delay and misguided ideas about vaccination, 159 cases have been reported in the USA in 2015, the greatest number of cases reported since measles elimination was documented since 2001.

In 1963, both a killed and a live attenuated Edmonston B strain of measles virus were licensed in the USA. The killed vaccine was withdrawn in 1967 owing to development of atypical measles. The Edmonston B strain was withdrawn in 1975 due to a high incidence of post-vaccination fever and rash. A live, further attenuated Schwarz strain was licensed in 1965, but is no longer used in the USA. The only available measles vaccine is a live, further attenuated Edmonston-Enders strain (Moraten). The vaccine is available combined with MMR, or combined with measles, mumps, rubella, and varicella as MMRV (ProQuad).

Mumps was first described by Hippocrates in the fifth century BC and scientifically detailed by Robert Hamilton, a British physician in 1790 [70]. In 1935, Johnson and Goodpasture [71] proved viral cause for this disease. Although mumps is a benign disease of childhood, it was a major cause of morbidity among soldiers during American Civil War and World Wars I and II [72–74].

Mumps virus, a *Paramyxovirus* with a single-stranded RNA genome, causes a communicable acute viral illness via airborne transmission or by direct contact with infected saliva. After acquisition, the virus replicates in the nasopharynx and regional lymph nodes. Viremia develops 12–25 days after exposure affecting the meninges and various glandular organs such as the salivary glands,

pancreas, testes, and ovaries. Prodromal symptoms include low-grade fever, myalgia, anorexia, and headache. Parotitis is the most common clinical finding occurring in 30–40 % of infected persons, although up to 20 % mumps infections are asymptomatic. Complications of mumps include aseptic meningitis, orchitis, oophoritis, pancreatitis, and rarely deafness and death. Laboratory diagnosis is made by using serology or PCR detection of the mumps virus. An estimated 212,000 cases of mumps occurred in the USA in 1964. After the licensure of the Jeryl Lynn Strain of attenuated mumps virus vaccine in 1967, the number of reported mumps cases has steadily declined except for sporadic resurgences.

The first mumps vaccine was developed in 1948 but it was withdrawn in mid-1950s owing to limited temporal immunity. All mumps vaccines currently in use contain live viruses. The various mumps vaccine strains available are Jeryl Lynn, Urabe AM9, Leningrad-Zagreb, and Leningrad-3. The currently used mumps vaccine in the USA is the Jeryl Lynn strain, a live attenuated mumps vaccine. It is available combined with measles and rubella as MMR, or combined with measles, rubella, and varicella vaccine as MMRV (ProQuad).

Rubella was initially described in the late eighteenth century and was differentiated from other exanthems by German physicians, hence the name German measles [75]. The term rubella, meaning "little red" [76], was coined by a British physician in 1841 during an outbreak in India. However it was only in 1941 that Norman McAlister Gregg [77], an Australian ophthalmologist, recognized congenital rubella syndrome (CRS). The rubella virus was first isolated by Parkman and Weller in 1962. The pandemic in Europe during 1962–1963 and in the USA in 1964–1965 spurred work on the rubella vaccine. The highest incidence of rubella in the USA was in 1969. The licensure of the vaccine that year led to a marked decrease in the incidence of rubella and CRS. A record low of seven cases was reported in 2003 and in the year 2004 rubella was no longer considered to be endemic in the USA, but it remains an ongoing problem in many parts of the developing world. On April 29, 2015, the World Health Organization declared the elimination of rubella in the Americas.

Rubella virus is an RNA virus belonging to *Togaviridae* family and genus *Rubivirus*. Rubella spreads by respiratory aerosols and primary replication occurs in the nasopharynx and regional lymph nodes. The incubation period is 14–21 days. The first week after exposure is usually symptom free followed by second week of viremia, low-grade fever, malaise, and lymphadenopathy. The characteristic maculopapular erythematous rash develops 14–17 days after exposure; it begins on the face and spreads downward. Other symptoms include arthralgia, arthritis, conjunctivitis, testalgia, or orchitis. The complications of rubella are chronic arthritis, thrombocytopenia purpura, encephalitis, orchitis, neuritis, and a rare late

syndrome of progressive panencephalitis. Congenital rubella syndrome affects 85 % of infants infected during first trimester but congenital defects are rare if infection occurs after the 20th week of gestation. The virus may affect all organs and cause congenital defects, the most common of which is deafness. Other prominent clinical findings include cataracts, glaucoma, retinopathy, patent ductus arteriosus, ventricular septal defect, pulmonic stenosis, coarctation of aorta, and neurologic and boney abnormalities. The laboratory diagnosis of rubella is made by isolation of the virus from clinical specimens or by serology using enzyme immunoassay.

In 1969, three rubella vaccines were licensed: HPV-77:DE5 (Meruva), HPV-77:DK-12 (Rubelogen), and GMK-3:RK53 (Cendevax). RA 27/3, a human diploid fibroblast strain (Meruvax-II, Merck), was licensed in 1979 and all other strains were discontinued. RA 27/3 rubella vaccine was first isolated from a rubella-infected aborted fetus in 1965. The virus was attenuated using human diploid fibroblasts. The vaccine is available combined with measles and mumps vaccines as MMR, or combined with measles, mumps, rubella, and varicella as MMRV (ProQuad).

MMR or MMRV vaccine is routinely recommended for all children 12 months of age or older [78]. The first dose of MMR should be given on or after first birthday and the second dose is given between ages 4 and 6. High schools and colleges in the USA and other countries frequently require students to have received two doses of vaccine at some point in their lives prior to matriculation. The adverse reactions include fever, rash, thrombocytopenia, arthritis/arthropathy, encephalopathy and rarely parotitis, or deafness. MMR vaccine is contraindicated in pregnancy, immunocompromised patients, during acute illness, or those with severe allergic reaction to vaccine components which include neomycin.

13 Pertussis Vaccines

Pertussis or whooping cough is an infectious disease caused by *Bordetella pertussis*, a gram-negative bacillus.

Before the introduction of the whole-cell vaccine, there were over 250,000 cases of whooping cough per year and 10,000 deaths worldwide. The incidence of pertussis declined significantly with the implementation of universal vaccination. Pertussis incidence has been gradually increasing since the early 1980s. A total of 28,000 cases were reported in 2014, the largest number since 1959. The reasons for the increase are not clear. A total of 27,550 pertussis cases and 27 pertussis-related deaths were reported in 2010. The increase in disease incidence in the USA has mostly been seen in older children and adults, likely reflecting waning