Vaccinology
in Latin America

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Preface

The first edition of this compilation, *Vaccinology in Latin America: A Resource for Immunization Managers*, evolved from Sabin's years of experience of teaching an annual course on the same topics for immunization managers of Latin America. We asked the course presenters to write up their presentations in chapter format to be included in this resource. The topics for the chapters were selected based on the feedback that course participants provided us over the years. In that sense this book is by the participants, for the participants! We have made every attempt to make the book tailor-made for them.

To that end, this book has no chapter on immunology, the basic science of vaccine discovery and development. In the past when the course provided a presentation on immunology, the participants preferred other topics more closely aligned with their day-to-day work. Here-in lies the basic objective of this book, to provide immunization managers of Latin America the basics in vaccinology that will help them better manage their programs with the ultimate aim of reaching every child and every family with available vaccines.

Ultimately, the quality of this book relies heavily on the expertise, experience and skill of every chapter author. We believe we have assembled an extraordinary faculty of course teachers that have captured key points of their lectures in the chapters they have written. Feedback over the years from course participants on their presentations helps confirm this belief. It has been an honor and pleasure to work with them. This book tries to excel in providing immunization managers with tools that will help them ensure the technical and operational success of their programs.

This book is dedicated to our mentor and dear friend, Dr. Ciro de Quadros, who died on May 28, 2014. Ciro had an amazing career in public health and impacted many of the people who were fortunate to work with him. He mobilized resources to launch the annual course in 2011 and to sustain it into the future for immunization managers of Latin America. Ciro envisioned the course to be targeted to the Spanish speaking audience of the Americas. He felt there was a clear gap in support that needed such a targeted approach. He was highly committed to circulating the lessons learned, in particular those involving disease eradication.

Although this book is dedicated to Ciro de Quadros, I want to take this opportunity to extend a heartfelt thanks to all the immunization field workers and staff of developing countries who dedicate their own lives to saving more lives of others more quickly through the provision of vaccines. Often, their work can be dangerous. But their tireless commitment in the most difficult circumstances is an inspiration for all.

Jon Kim Andrus, MD
Editor

Module 1:
Existing Vaccines and Vaccines in Development

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Vaccines to Prevent Cholera

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Introduction

Cholera, the acute diarrheal disease caused by *Vibrio cholerae* serogroups O1 and occasionally O139, is of cardinal public health importance because of the severity of the clinical illness it can cause ("cholera gravis", leading to death if untreated), its explosive epidemic behavior and its propensity to occur in extensive pandemics involving many countries over many years. The oral cholera vaccines that have become available in recent years are employed to dampen the intensity of seasonal disease in endemic areas, protect high risk populations such as refugees interned in camps in cholera-endemic or cholera-proximal areas, and protect travelers from cholera-free countries/regions who must travel to countries/regions where cholera is epidemic or endemic. The remaining potential use of cholera vaccines, arguably the most important, aims to control large explosive epidemics in immunologically-naïve populations (so called "virgin soil" epidemics) such as when cholera returned to South America in 1991 after a century of absence and the 2010 outbreak in Haiti that followed a devastating earthquake.[1,2] Virgin soil epidemics severely strain the resources of national and local public health authorities and disrupt civil society. The control of such epidemics demands a vaccine that can confer a high level of protection upon immunologically-naïve persons within just a few days of administration of a single dose. One of the new vaccines has characteristics (single dose, protection as early as 8–10 days following vaccination) that may allow it to be amenable to the control of virgin soil epidemics,[3] particularly when such epidemics accompany complex emergencies (earthquakes, floods, wars).

Etiologic Agents

Circa 206 O serogroups of *V. cholerae* are recognized but only two, O1 and O139, routinely express cholera enterotoxin and attachment pili and cause epidemic cholera.[4] Within the O1 serogroup there are two main serotypes, Inaba and Ogawa; a third serotype, Hikojima, is rare. Serogroup O1 strains are also classified into two biotypes, classical or El Tor. Almost all cholera disease currently occurring in the world is due to variants of the El Tor biotype. Emerging El Tor variants have been identified that express classical biotype cholera enterotoxin and sometimes classical toxin co-regulated pili (TCP), the organelles by which *V. cholerae* attaches to enterocytes as a key step in the pathogenesis of cholera.[5-8] These "El Tor hybrids" expressing classical enterotoxin may cause more severe clinical disease than bona fide El Tor strains.[9]

Epidemiology

The Ganges River delta in South Asia is the ancestral home of cholera where between pandemics it persists as "Asiatic cholera." The seventh pandemic of cholera, due to *V. cholerae* O1 El Tor, originated in the early 1960s on the island of Sulawesi, Indonesia, and progressively spread in waves over the ensuing six decades to involve at one time or another almost all of the world's developing and transitional countries;[4] in many it has remained endemic in sub-populations and niches.[4] Thus, cholera is now endemic in many countries of South and Southeast Asia, sub-Saharan Africa and a few countries in the Americas. During the early 1990s it was endemic for several years in Peru, Ecuador, and some other Latin American countries.[10,11]

When cholera invades new territory with immunologically-naive populations, the highest incidence of disease is observed in young adult males. If the disease becomes endemic, the incidence increases in women and children and eventually peak incidence is observed in young children. Cholera exhibits a seasonal pattern almost everywhere that it is endemic.[12] When the new season begins, cholera cases emerge simultaneously in multiple geographically separate foci. This pattern has also been observed when cholera invades new territory. In 1991, when cholera re-invaded South America with an explosive and extensive epidemic in Peru, large outbreaks appeared almost simultaneously in three distinct cities spanning a 900-kilometer stretch of the Pacific Coast.[12] The explosive increase of cases observed at the onset of many epidemics may be the consequence of hyperinfective vibrios released into water sources lacking vibriophages. Conversely, curtailment of the epidemic may be the consequence of an increased prevalence of lytic phages in the water.[13,14]

Reservoirs of Infection. Humans are the sole known natural host of *V. cholerae* O1 cholera disease and chronic carriers are rare.[15,16] Thus, it was previously assumed that in endemic areas mild and asymptomatic infections served as the reservoir to maintain the disease until the next cholera season when conditions would once again favor enhanced transmission. However, epidemiologic observations in the 1970s refuted this assumption and ushered in a new understanding of cholera epidemiology that clarified much of the epidemiologic behavior that previously had been puzzling. Confirmation of a single case of cholera in Texas in 1973 in a fisherman caused by an unusual highly hemolytic El Tor Inaba strain,[17] followed 5 years later by an outbreak of approximately two dozen cases of the identical strain in which poorly cooked seafood was incriminated as the vehicle,[18] led to identification of an environmental focus of infection along the Gulf of Mexico coast of the U.S.A.[19] This El Tor Inaba strain was found to constitute autochthonous flora of the brackish waters of Gulf estuaries, where it was associated with crustacea (shrimp, etc.) eaten as local seafood. Identification of a similar environmental focus of free-living enterotoxigenic *V. cholerae* O1 El Tor in Queensland, Australia, supports the hypothesis that brackish water environmental niches can serve as the reservoir of *V. cholerae* O1.[20]

V. cholerae can enter a "viable but nonculturable" state that allows them to survive harsh environmental conditions through a form of bacterial hibernation.[16,21] When the toxigenic *V. cholerae* eventually encounter favorable conditions of temperature, salinity and pH, they can rejuvenate, regaining the potential to actively metabolize and grow.[21] These may also be the conditions under which zooplankton blooms occur.

Modes of Transmission. Our practical knowledge of the vehicles of transmission of cholera stems from case-control investigations that have documented waterborne transmission and an array of food vehicles.[22,23] When El Tor cholera struck the Pacific coast of several Andean countries of South America in 1991, improperly functioning municipal water supplies and sewage systems, contaminated surface waters, and unsafe domestic water storage methods fostered facile waterborne cholera transmission.[1,24] Beverages prepared with contaminated water and sold by street vendors, ice, and even commercial bottled water have been incriminated.[25]

V. cholerae O1 may be associated with seafood vehicles by means of their natural adherence to the chitinous exoskeletons of shrimp, crabs, and oysters in certain estuarine environments,[18,21,26] or food may be secondarily contaminated during preparation or handling.[27] The most commonly implicated food vehicle worldwide has been raw or undercooked seafood, including mussels, shrimps, oysters, clams, cockles, fish, salt fish, and ceviche (uncooked fish or shellfish marinated in lemon or lime juice).

Cooked grains, rice and beans with sauces have also been incriminated in cholera transmission, particularly in Africa. A small inoculum of enterotoxigenic *V. cholerae* O1 introduced by an infected food handler into one of these types of food and stored without refrigeration can increase by several logs within 8 to 12 hours. Cholera has also been transmitted by vegetables and fruit irrigated with raw sewage or "freshened" by dousing with sewage-contaminated water.[28]

During outbreaks or seasonal epidemics, cholera may spread via multiple modes of transmission. Depending on local customs, climate, and other factors, particular modes and vehicles of transmission predominate.[29] Finally, if pathogenic *V. cholerae* O1 and O139 persist in environmental reservoirs, then transmission across long distances can occur via the ballast water of large ships, as they intake ballast water in one port and discharge it prior to entering another port thousands of miles away.[30]

Epidemiologists recognize that person-to-person contact spread of cholera virtually never occurs. Transmission is essentially always via food or water vehicles.

It has been hypothesized that for a few hours after being shed in enormous numbers by cholera patients purging rice water stools, toxigenic *V. cholerae* remain in a hyper-transmissible state.[13,14,31] Thus, if a case of severe cholera occurs in a crowded setting where other susceptible human hosts and facile modes of transmission exist, the infectious dose may be unusually low and spread of disease may be explosive.[13]

Host Risk Factors. Certain host factors markedly increase the risk of developing cholera gravis, including O blood group,[32,33] hypochlorhydria,[34,35] and a lack of background immunity.[36] Persons of blood group O are at increased risk of developing cholera gravis than persons of other blood groups. When cholera invades a new territory with an immunologically naive population, persons with hypochlorhydria from partial gastrectomy, *Helicobacter pylori* chronic gastritis, etc., have frequently been the index case.[37] The highest incidence of cholera in endemic areas is often children 1 to 4 years of age. The age-specific incidence falls thereafter and the prevalence and geometric mean titer of serum vibriocidal antibody rise, as increasing immunity is acquired.[38] One interesting exception to this pattern is women of childbearing age who exhibit an inordinately high incidence.[12]

International Surveillance and Disease Notification. Since cholera was the disease for which modern public health surveillance and reporting was first organized, it bears the code 001 in the international classification of diseases. By international convention, cholera is a notifiable disease along with plague and yellow fever. In 2014, 190,549 cases of cholera were reported to the World Health Organization (WHO) from 42 countries; 55% were from Africa and 15% from the Americas. The true number of cholera cases globally is much higher and the annual burden is estimated to be 1.4–4.0 million cases and 21,000–143,000 deaths. For reasons involving trade, fear of food embargoes and effects on tourism, many countries delay reporting cholera cases to the WHO or do not report at all. For example, international health statistics in the late 1980s and 1990s indicated that Bangladesh had little or no cholera. Yet at the same time large-scale field trials to evaluate cholera vaccines were carried out in which hundreds of confirmed cases were documented.[39]

The Disease

Cholera infection exhibits a spectrum of clinical illness ranging from asymptomatic shedding of vibrios in the stool to life-threatening watery diarrhea accompanied by overt severe dehydration (cholera gravis). Up to three-quarters of cholera infections may be sub-clinical, and among symptomatic patients only a minority may manifest severe purging. The propensity to develop cholera gravis is strongly associated with two host risk factors: blood group O and hypochlorhydria. If the prodigious losses of body water and electrolytes are not promptly replaced in cholera patients who are actively purging "rice water stools" (e.g., at the rate of one liter per hour in an adult), the patient may rapidly dehydrate, suffer renal shutdown, shock and acidosis, and die within hours of the onset of illness. Patients with cholera gravis exhibit the classic signs and symptoms of severe dehydration including weak or absent peripheral pulses, hypotension, sunken eyes, loss of skin turgor, and decreased urine output. Table 1 compares the concentrations of serum electrolytes in normal adult serum and in rice water stools of adults with cholera gravis. The purging of large volumes of rice water stools as evident in cholera gravis is physiologically equivalent to loss of plasma leading to hemoconcentration, hypovolemia, hypotension, decreased renal blood flow, and overt hypovolemic shock.

Table 1. Concentrations of Electrolytes in Normal Adult Sera and in the Rice Water Stools of Adults with Cholera Gravis

	Normal Adult Serum	Rice Water Stools From Cholera Gravis Patients
Na+	135–145 mEq/ml	135 mEq/ml
K+	135–145 mEq/ml	15 mEq/ml
Cl-	95–105 mEq/ml	100 mEq/ml
HCO_3	24–30 mEq/ml	40 mEq/ml

Pathogenesis and Immunity

V. cholerae O1 comprises a sophisticated, multi-step, delivery system for cholera toxin, the virulence attribute responsible for the severe purging of voluminous watery stools characteristic of cholera gravis. In volunteers the ingestion of as little 5 mcg of purified cholera enterotoxin can induce diarrheal illness and 5 mcg has led to a clinical syndrome that closely resembles the severe purging of cholera gravis.[40] Subsequent volunteer studies with *V. cholerae* O1 vaccine strains that harbored deletions in genes encoding the enzymatically active (A) subunit, both A and B (binding) subunits of cholera toxin or the entire cholera toxin virulence cassette (which encodes two other toxins and a minor colonization factor) showed that some strains retained the ability to cause mild diarrhea and other gastrointestinal symptoms,[41] possibly by invoking intestinal inflammation.[42,43] Whereas ingestion of purified cholera toxin alone can induce a syndrome of severe purging, the fully pathogenic vibrios that cause natural cholera encode multiple virulence factors that direct a stepwise progression to severe diarrhea.

Following ingestion, pathogenic *V. cholerae* O1 or O139 must survive the formidable gastric acid barrier and transit the pylorus to reach the proximal small intestine, the critical site of host-parasite interaction. Ingestion without buffer of 10^6 viable pathogenic *V. cholerae* by fasting North American volunteers resulted in neither infection nor diarrhea because the vibrios were destroyed by gastric acid.[44] In contrast, when 10^6 vibrios are administered with sodium bicarbonate buffer or food that protects the vibrios during gastric transit, cholera

develops in approximately 90% of the volunteers.[44] Indeed, when administered with buffer, as few as 10^3 *V. cholerae* O1 El Tor cause diarrhea in ~67% of volunteers,[44] although the stool volume is less than in subjects who ingest higher doses of vibrios.

Once in the small intestine, the vibrios sense their environment by means of ToxR, a protein that is the product of a master regulatory gene, *toxR*.[45] Activation of *toxR* leads to expression of cholera toxin and toxin coregulated pili (TCP), the key intestinal colonization factor,[46,47] and to the indirect activation (via *toxT*) of approximately 17 other genes involved with bacterial adaptation to survival in the human intestine. As neuraminidase and other vibrio enzymes break down the mucus barrier on the surface of the intestine, motility plays a critical role as the unipolar flagellum propels the organisms toward the enterocyte surface, attracted by chemotactins.

TCP constitutes the major intestinal colonization factor for *V. cholerae* O1 and O139.[46,47] TCP of El Tor and O139 are genetically and antigenically identical but differ somewhat from TCP of classical biotype. Genes for TCP biogenesis are found within a 40-Kb *Vibrio* Pathogenicity Island (VPI). A mutant strain of *V. cholerae* O1, unable to express TCP, was unable to colonize the intestine of volunteers or to stimulate good vibriocidal antibody responses.[48]

Experimental challenge studies in volunteers showed that a single episode of clinical cholera due to either serotype (Inaba or Ogawa) within a biotype stimulated 90–100% protection against clinical illness upon subsequent experimental challenge with either the homologous or heterologous serotpye of *V. cholerae* O1 and the protection elicited by classical biotype infection endured for at least three years.[44,49,50] These observations of potent infection-derived immunity were corroborated in the field with natural cholera illness,[12,51] refuting early suggestions that an initial episode of cholera elicited little or only short-lived protection.[52]

Immune Response

Following *V. cholerae* O1 infection, robust serum vibriocidal antibody responses and rises in immunoglobulin G (IgG) cholera antitoxin are observed.[53,54] Approximately 90% of complement-dependent vibriocidal antibodies are directed toward the O antigen with the remaining 10% of antibodies directed against protein antigens. In immunologically primed individuals, strong secretory IgA (SIgA) intestinal antibody responses are recorded following cholera infection. However, significant rises in SIgA anti-LPS and antitoxin are surprisingly sparse in nonprimed individuals. The detection of gut-derived, trafficking IgA antibody secreting cells that make specific antibody to LPS and CT antigens is a good measure of priming of the intestinal immune system.[55]

Whereas infection-derived immunity to cholera is believed to be mediated by intestinal mucosal SIgA antibodies, curiously, serum vibriocidal antibodies are the best correlate of protection.[36,56,57] These serum antibodies may be a proxy for the stimulation of intestinal antibodies. Serum anti-B subunit responses are more prominent in pediatric cholera patients, while serum antibody responses to LPS and TCP are more prominent in adults.[58] Whereas high titers of specific vibriocidal antibodies appear after *V. cholerae* O1 infection, vibriocidal responses following O139 infection are weak and rather nonspecific.[59] A correlate of protection for O139 cholera has not yet been identified.

Diagnosis

The diagnosis of cholera is confirmed by isolating *Vibrio cholerae* from stool cultures on selective media such as thiosulfate-citrate-bilesalt-sucrose (TCBS) both directly and after enrichment in alkaline peptone water;[60] suspicious colonies are agglutinated with typing sera (directly or after sub-culture). Rapid non-culture tests that detect *V. cholerae* O1 and/or O139 lipopolysaccharide antigens are useful in field situations.[61-63]

Treatment

Appropriate antimicrobials are an important adjunct to fluid therapy, as they diminish the volume and duration of purging and rapidly curtail the excretion of vibrios, thereby diminishing the chance of secondary transmission. Patients surviving from hypovolemic shock and severe dehydration manifest certain complications, such as hypoglycemia, that must be recognized and promptly treated. If these fundamental guidelines are followed properly, case fatality, even during explosive epidemics in developing countries, can be kept below 1%.[64,65] Failure to comply with these basic proven clinical rules can result in unacceptably high case fatality.[66,67]

Fluid Therapy. Patients suffering from severe dehydration of cholera with or without overt shock usually lose ~10% of their body weight and must be rapidly rehydrated with intravenous fluids. Fluid therapy is divided into two phases: (1) rehydration phase — the rapid replacement of water and electrolyte deficits, and (2) maintenance phase — the infusion of fluids to replace ongoing losses. Fluid and electrolyte deficits should be replenished as rapidly as possible (within 2–4 hours of initiation). The time recommended for rehydration in adult and pediatric patients is 3 and 6 hours, respectively. In adults, 30% of the total required fluid is administered in the first 30 minutes, while in children this volume is administered over one hour. Patients with cholera gravis generally require multiple liters of intravenous fluids to stabilize them to the point where oral rehydration can begin; at the earliest opportunity, they are carefully weaned from intravenous fluids. Adults with cholera gravis typically require 8–12 liters of intravenous fluids before oral hydration alone can keep up with losses. The most extensively used intravenous rehydration fluid worldwide for treatment of cholera is Ringer's lactate, because it is so widely available. Ringer's lactate contains Na^+ 130 mEq/L, K^+ 4 mEq/L, Ca^{++} 3 mEq/L, Cl^- 111 mEq/L, and lactate (precursor of HCO_3^-) 29 mEq/L. Because the concentration of K^+ in Ringer's lactate is too low, supplemental K^+ must be administered either by adding a sterile KCl (or similar potassium salt) solution to the Ringer's solution to increase the concentration of K^+ to 15–20 mEq/L, or by initiating oral rehydration.

The volume of all diarrheal losses and vomitus must be measured in the patient with cholera. Once the patient has had replacement of his or her deficit and is in the stage of maintenance therapy, fluid management is generally based on 6-hour periods. The total fluid loss during the previous 6-hour period constitutes the volume of fluids that will be administered to the patient during the next 4–6 hours. As diarrheal losses begin to diminish, the 6-hourly replacement requirements decrease accordingly.

Aggressive rehydration therapy with fluid and electrolytes leads to rapid clinical improvement in the patient (e.g., elevation of blood pressure, stronger pulse, improved skin turgor, and enhanced consciousness) reflected in simple laboratory assays (e.g., fall in hematocrit and plasma specific gravity). Once renal perfusion is re-established normal homeostatic mechanisms begin to combat acidosis and regulate serum electrolyte concentrations.

Patients with mild or moderate dehydration and moderate purge rates (< 500 mL per hour) can generally be managed with oral rehydration alone. Oral rehydration therapy is based on the physiological fact that glucose-mediated cotransport of sodium and water across the mucosal surface of the small intestine epithelium remains intact during cholera infection despite the effect of cholera toxin.[68] If the diarrhea is copious, large volumes of oral rehydration fluids must be ingested to keep up with ongoing losses.

The oral rehydration solution (ORS) recommended by WHO for treatment of cholera is composed of Na^+ 90 mEq/L, Cl^- 80 mEq/L, K^+ 20 mEq/L, citrate$^-$ 30 mEq/L, and glucose 111 mmol/L. Packets containing sufficient salts and glucose to prepare 1 liter of rehydration solution are widely available in developing countries. Each packet contains 3.5 g of NaCl, 2.9 g of sodium citrate, 1.5 g of KCl, and 20 g of glucose. In some Asian countries cereal-based oral rehydration solutions that provide multiple actively transported substrates are used to treat cholera;[69] some controlled trials showed no advantage over glucose-based ORS.[70] Reduced osmolarity rehydration solutions (Na^+ 75 mEq/L, Cl^- 65 mEq/L, K^+ 20 mEq/L, citrate$^-$ 30 mEq/L and glucose 75 mmol/L) are controversial for treatment of cholera.[71] Although the rate and volume of purging are reduced versus standard ORS, some patients develop hyponatremia (albeit usually asymptomatic).

The regimen for calculating the amount of oral rehydration solution to be administered to replace ongoing losses differs by age. Since the Na^+ concentration in cholera stools is approximately 135 mEq/L in adults, one-and-a-half volumes of oral rehydration solution containing 90 mEq/L should be given for every volume of watery diarrheal stool passed in order to adequately replace Na^+ losses. In contrast, in young children in whom the Na^+ concentration of cholera stools is only approximately 100 mEq/L, ongoing losses can be replaced on the basis of a 1:1 ratio of oral rehydration solution to volume of diarrheal stool. There is a practical limit to the volume of oral rehydration solution that can be consumed on an hourly basis; in adults and teenagers the upper limit is approximately 750 mL/hour.

Antimicrobial Therapy. Appropriate antibiotics significantly decrease the duration of diarrhea, total diarrheal stool volume, and duration of excretion of *V. cholerae*, and therefore constitute an important adjunct to rehydration therapy. Resistance of *V.cholerae* O1 to commonly used antibiotics is increasing. Tetracycline and its long acting derivative, doxycycline, were used extensively in the past to treat cholera but resistance to these drugs in endemic areas in Asia and Africa has decreased their utility. Nevertheless, they remain useful where monitoring of vibrio strains documents their sensitivity. The regimen for teenagers and adults is 500 mg four times daily for 3 to 5 days and the pediatric dosage for tetracycline is 50 mg/kg/day in four divided doses for 3 to 5 days. Doxycycline requires only once daily administration (300 mg for adults and teenagers and 4 to 6 mg/kg for children, for 3 to 5 days). The very short course of tetracycline therapy used for the treatment of cholera precludes staining of teeth and other adverse reactions otherwise encountered with long courses of this antibiotic.

In areas where tetracycline-resistant *V. cholerae* are prevalent ciprofloxacin 250 mg once daily for 3 days is the preferred regimen;[72] some, but not all, trials with single-dose ciprofloxacin have also given good results.[73-75] Single-dose azithromycin (1 g in adults) has been shown to be effective in treating cholera in both adults and children. In one randomized, controlled clinical trial, a single dose of azithromycin (20 mg/kg, maximum dose 1 g) was as effective as three days of erythromycin therapy (12.5 mg/kg every 6 hours).[76] Trimethoprim–sulfamethoxazole use should be avoided in areas where O139 is known to be prevalent, since *V. cholerae* O139 is typically resistant to this antimicrobial.[77] During epidemics in developing countries, single-day or single-dose antibiotic therapy (such as 1 g of ciprofloxacin or 300 mg of doxycycline for adults or 1 gm of azithromycin) may be necessary in resource constrained settings,[75,78] particularly if antibiotics are in short supply. However, the concern with single-dose therapy is that this may accelerate the emergence of resistance.

Cholera Vaccines

There are currently four licensed cholera vaccines, all administered orally, including:

1. Dukoral® (Crucell) consists of a mix of killed whole cell *V. cholerae* O1 bacteria of both biotypes and serotypes plus 1 mg of cholera toxin B subunit.[79,80]
2. Shanchol™ (Shanta, Hyderabad, India) contains a mix of killed vibrios of both O1 (both biotypes and serotypes) and O139 *V. cholerae*.[81,82]
3. Euvichol® Plus (Eubiologics, Seoul, Korea) contains the identical formulation of vibrios as Shanchol and Euvichol but in a simple, highly practical presentation.[83]
4. Vaxchora® (PaxVax Bermuda, Ltd., Hamilton, Bermuda [part of PaxVax, Redwood City, CA) live single-dose oral cholera vaccine consists of genetically-engineered *V. cholerae* O1 strain CVD 103-HgR.[3,84,85]

A detailed comparison of the salient features of these vaccines is summarized in Table 2.

Table 2. Salient Characteristics of Four Licensed Oral Cholera Vaccines

Parameter of Comparison	Dukoral	Shanchol	Euvichol Plus	Vaxchora (CVD 103-HgR)	PxVx0200 (CVD 103-HgR) high dose (~ 10^9 cfu)
Components	Heat inactivated *V. cholerae* O1 classical Inaba ($2.5x10^{10}$), classical Ogawa ($2.5x10^{10}$), formalin-inactivated classical Ogawa ($2.5x10^{10}$), formalin-inactivated El Tor Inaba ($2.5x10^{10}$) and 1 mg of recombinant cholera toxin B subunit suspended in 3 ml of buffer	Heat inactivated *V. cholerae* O1 classical Inaba ($2.5x10^{10}$), classical Ogawa ($2.5x10^{10}$), formalin-inactivated classical Ogawa ($2.5x10^{10}$), formalin-inactivated El Tor Inaba ($2.5x10^{10}$) and 1 mg of recombinant cholera toxin B subunit suspended in 1.5 ml of buffer	Heat inactivated *V. cholerae* O1 classical Inaba (300 Elisa units [EU]), classical Ogawa (300 EU), formalin-inactivated classical Ogawa (300 EU), formalin-inactivated El Tor Inaba (300 EU) and formalin-inactivated O139 (300 EU) suspended in 1.5 ml of buffer	Recombinant *V. cholerae* O1 classical Inaba strain CVD 103-HgR with deletion of *ctxA* and insertion of a Hg++ resistance marker in *hlyA* (inactivating Hemolysin A) (~10^8 colony forming units [cfu])	Recombinant *V. cholerae* O1 classical Inaba strain CVD 103-HgR with deletion of *ctxA* and insertion of a Hg++ resistance marker in *hlyA* (inactivating Hemolysin A) (~10^9 colony forming units [cfu])
No. of doses	2	2	2	1	1
Interval between doses	2 weeks	2 weeks	2 weeks	—	–
Well tolerated	Yes	Yes	Yes	Yes	Yes
Efficacy or effectiveness in endemic populations	~ 50%	~ 65%	~ 65% (by extrapolation from Shanchol)	The high-dose (10^9 cfu) formulation will be used in endemic populations	Yes. (79% efficacy by extrapolation from Orochol E)[97]
Efficacy in industrialized country adults	Yes	No	No	Yes[3,85]	Yes (by extrapolation from Vaxchora[3,85])

Parameter of Comparison	Dukoral	Shanchol	Euvichol Plus	Vaxchora (CVD 103-HgR)	PxVx0200 (CVD 103-HgR) high dose (~ 10^9 cfu)
Duration of efficacy	3–4 yrs[106]	5 years[92]	Extrapolation from Shanchol data	At least 6 months (by extrapolation from Mutacol)[96]	At least 6 months (by extrapolation from Mutacol)
Onset of efficacy following first dose	Not known. Likely ≥ 21 days	Not known. Likely ≥ 21 days	Not known. Likely ≥ 21 days	8–10 days[3,96]	8–10 days[3,96]
Herd immunity	Yes	Yes	Likely	Likely	Likely
Boostable immune responses	Yes	Yes	Extrapolation from Shanchol data	Yes, but only after at least 4 months following primary immunization	Yes, but only after at least 4 months following primary immunization
Immunogenicity in toddlers and pre-school children	Yes	Yes	Extrapolation from Shanchol data	Yes (extrapolation from Orochol E data)[107-109]	Yes (extrapolation from Orochol E data[107-109])
Efficacy in toddlers and pre-school children	Yes	Yes (lower than in older children and adults)	Extrapolation from Shanchol	?	Age ≥2 years (extrapolation from Orochol E data[97])
Safety & immunogenicity in pregnant women	Yes[110]	Yes[111]	Extrapolation from Shanchol	?	?
Safety & immunogenicity in HIV-positive persons	Yes	Yes[112]	Extrapolation from Shanchol	Yes (extrapolation from Orochol E data)	Yes
Presentation	Liquid suspension of vaccine in a glass vial containing a single dose and accompanied by an aluminum foil sachet with buffer. The buffer sachet is emptied into a cup with 150 of cool water, stirred and the 3 ml of vaccine suspension is added and further mixed. For children (age 2 years and above, one-half of the 150 ml buffer solution should be discarded (leaving 75 ml) before adding the 3 ml of vaccine.	Liquid suspension of vaccine in glass vials containing a single dose. The cap of the vial is removed by hand or with a forceps and the 1.5 ml contents of the vial are transferred to the mouth of the vaccinee.	Liquid suspension of vaccine in plastic tubes with easily removal tips for direct transfer of the 1.5 ml of liquid vaccine directly into to the mouth of the vaccinee.	Double sachets, one sachet containing lyophilized vaccine and the other sachet containing buffer powder. The contents of the buffer sachet is put into a cup and 100 ml of water is added and the suspension stirred. The contents of the vaccine sachet are then added to reconstitute the lyophilized vaccine. The resultant 100 ml vaccine cocktail is then ingested.	Double sachets, one sachet containing lyophilized vaccine and the other sachet containing buffer powder. The contents of the buffer sachet is put into a cup and 100 ml of water is added and the suspension stirred. The contents of the vaccine sachet are then added to reconstitute the lyophilized vaccine. The resultant 100 ml vaccine cocktail is then ingested.
Strategy for delivering the vaccine	Mostly via campaigns	Mostly via campaigns	Mostly via campaigns	Travel clinics	Mostly via campaigns

Non-Living Oral Vaccines. Dukoral is the commercial product of a non-living oral cholera vaccine prototype that was tested in U.S. volunteers and then in a randomized controlled field trial in Bangladesh in the 1980s.[39,86] The prototype vaccine contained purified B subunit prepared from holotoxin by biochemical separation of the B subunit from the toxic A subunit. The current commercial formulation, Dukoral, contains recombinant B subunit.[87] Dukoral has been shown to be well tolerated and protective against cholera in post-licensure evaluations.[88,89] The B subunit enhances Dukoral's anti-bacterial immunity by adding antitoxic immunity that is also effective against enterotoxigenic *Escherichia coli* producing heat-labile enterotoxin; however, the additive protection of antitoxic immunity is short-lived, lasting only 4–6 months.[90,91] Dukoral, administered as two doses 2 weeks apart, is used by European and Canadian travelers for protection against travelers' diarrhea caused by LT-producing *E.coli*. Although Dukoral has been pre-qualified by the World Health Organization for procurement by U.N. agencies, heretofore it has been little used for control of endemic or epidemic cholera other than in demonstration projects.

Shanchol demonstrated its ability to diminish the incidence of cholera in highly endemic neighborhoods of Kolkata, India.[92] Two doses of Shanchol administered two weeks apart conferred 65% efficacy (95% CI, 52–74%) against cholera overall (all ages combined).[92] However, there was a clear hierarchy of protection with young children 1–4 years of age (who suffer the highest incidence of cholera) having the lowest level of efficacy. Over the 5 years of surveillance, the efficacy was 75% in persons ≥ 15 years of age, 68% in children age 5–14 years, and 42% in children 1–4 years of age at the time of enrollment and vaccination.[92] The impact of prior immunologic priming was evident during the first year of follow-up when the point estimate of efficacy was only 17% in children age 1–4 years but was 81% in older children age 5–14 years and 66% in individuals age 15 years and above.[82] Shanchol was also efficacious in a nested case/control study following a mass vaccination to control seasonal cholera in Guinea;[93] this trial also highlighted the complexities of organizing reactive immunization campaigns and the desirability of a single-dose regimen.[94] A single-dose of Shanchol was systematically evaluated in a massive randomized placebo-controlled field trial in urban slums in Dhaka, Bangladesh. A single dose gave 63% (95% CI, -39–90%) protection among children 5–14 years of age, 56% (16–77) protection among persons ≥ age 15 years but only 16% (-49–53%) efficacy among children <5 years of age.[95] The incidence of cholera in children <5 years ($1.75/10^5$ person days) was 8.3-fold higher than among children 5–14 years ($0.21/10^5$ person days) and 5.8-fold higher than among persons age ≥ 15 years, presumably indicating the ability of single-dose Shanchol to work well in persons with considerable prior background immunity to cholera but not performing well in immunologically less-primed hosts.[95] Heretofore, Shanchol has been the oral cholera vaccine most extensively utilized from the WHO cholera vaccine stockpile.

There are no pre-licensure efficacy or post-licensure effectiveness data yet on Euvichol or Euvichol Plus. They were licensed based on their identity of formulation to Shanchol and clear demonstration of non-inferiority in eliciting seroconversion of serum vibriocidal antibody titers.[83] Euvichol and Euvichol Plus received rapid WHO prequalification and will now be able to expand the supply of oral cholera vaccine in the WHO stockpile. In Table 2 it is assumed that Euvichol Plus will provide similar effectiveness as Shanchol.

Vaxchora™ (strain CVD 103-HgR) was licensed by the U.S. FDA in June 2016 and in the U.S.A. and other industrialized country markets will provide a single-dose, rapidly acting (strong protection evident in 8–10 days) oral cholera vaccine for immunologically-naïve persons who must travel on short notice to places of high risk.[85] CVD 103-HgR has a deletion of the gene encoding the enzymatically-active A subunit of cholera toxin, while leaving intact the immunogenic B subunit. It also has a Hg^{++} resistance marker inserted into *hlyA*, thereby inactivating Hemolysin A expression. In persons from industrialized countries, a single oral dose containing ~10^8 colony forming units (cfu) of CVD 103-HgR is well tolerated, elicits serum vibriocidal antibody seroconversion in >90% of vaccinees, has only modest excretion (18–25% have positive coprocultures from day 1–4 post-vaccination) and confers 90% efficacy

against challenge with wild type *V. cholerae* O1 10 days after vaccination. Upon challenge at 3 months following ingestion of a single dose, 80% vaccine efficacy was recorded.[3] The volunteer challenge studies with Vaxchora identified serconversion of vibriocidal antibody as a strong correlate of protection.[3]

CVD 103-HgR was originally manufactured by the now defunct Swiss Serum and Vaccine Institute and commercialized under the trade name Orochol® in many countries and as Mutacol® in Canada. This earlier formulation protected volunteers against challenge with *V. cholerae* O1 of either El Tor or classical biotype and either Inaba or Ogawa serotype and conferred protection against challenge as soon as 8 days and as long as 6 months after vaccination.[96] A formulation containing one-log higher vaccine organisms, Orochol E (~10^9 cfu), was commercialized for use in developing countries.[97] The reason for the one-log higher dosage for developing country populations is that environmental enteropathy, which is highly prevalent in the low socioeconomic levels of the population at highest risk of cholera and other enteric infections, dampens the immune response to the live oral vaccine. The higher number of cfu per dose overcomes this intestinal barrier.[98-100] The biology of the need for the higher dose in impoverished developing country populations has been reviewed.[101]

Orochol E was evaluated in a large-scale, randomized, placebo-controlled, double-blinded field trial in North Jakarta neighborhoods where cholera was hyperendemic.[102] Randomization was at the level of the individual in the Jakarta trial when the critical role of indirect protection was not yet appreciated.[103,104] In this venue the vaccine did not show evidence of significant protection but shortly after the enrollment and vaccination, cholera incidence dropped by >80% in what was previously a hyperendemic ecology. One interpretation is that the live oral vaccine via indirect protection lowered the overall incidence in the community to a point where efficacy could not be demonstrated but the cholera burden was greatly diminished for four years.[102] Orochol E's ability to protect populations in developing countries was later shown in a post-licensure reactive vaccination undertaken by the WHO during a cholera epidemic in Micronesia where 79% vaccine efficacy was calculated.[97] Clinical trials have begun with a high-dose formulation of CVD 103-HgR (PXVX0200) prepared by the manufacturer of Vaxchora to explore its utility for reactive vaccination.[85,105] In one preliminary study in Mali, West Africa, a single dose of the high-dose formulation was significantly more immunogenic in stimulating serum vibriocidal antibodies than one or two doses of Shanchol used as the immunologic comparator.[105]

Prevention and Control

Safe Water and Food. Since enteric fever pathogens are typically acquired via the ingestion of contaminated water or food, enteric precautions should be taken when living or traveling in endemic areas. Only treated (boiled or chemically treated) water should be consumed. Foods that may be fecally contaminated (e.g., uncooked salad vegetables) should be avoided. Travelers to cholera-endemic areas should be particularly careful of eating seafood dishes unless they are cooked to a high temperature.

Conclusion

Both for the prevention of disease in populations in cholera-endemic countries and for travelers from industrialized countries to cholera-endemic and epidemic regions of the world, several new and improved oral vaccine options now exist to prevent cholera disease. The global supply of cholera vaccines in also increasing. Judicious use of these vaccines can diminish the risk of cholera worldwide.

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Vaccines as a Control Strategy Against Viral Hepatitis Infections

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Introduction

Hepatitis is an inflammatory process of the liver, whose etiology may be infectious and also related to toxins such as alcohol, drugs, or autoimmune reactions. Within the sources of infection, viruses are the main etiology.

Currently, viral hepatitis infections are a global health problem despite progress made in the areas of diagnosis, prevention, and treatment. Based on 2015 estimates by the World Health Organization (WHO), this situation translates into 325 million people with chronic hepatitis infections worldwide, 1.34 million deaths a year — similar to the number of deaths by the human immunodeficiency virus[1] (HIV) — and has high morbidity amongst patients and high costs for public health systems, in addition to long-term complications. In 2013, hepatitis viruses were the seventh cause of mortality in the world. For this reason, the WHO has emphasized the importance of generating a comprehensive approach in the fight against these diseases, and helping countries strengthen their strategies against viral hepatitis infections.[2]

The cluster of "viral hepatitis" comprises various hepatotropic viruses, whose transmission route, evolution, treatment, and eventual complications differ based on the types of viruses. These specific characteristics translate into a lack of uniformity in prevalence globally. Despite the fact that several viruses can impair liver function temporarily, currently there are at least five known viruses that primarily infect the liver, with hepatitis as their main clinical manifestation. They comprise hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D or delta (HDV), and hepatitis E (HEV) viruses.[3]

The transmission route for hepatitis A and E is mainly the fecal-oral route through contaminated water and food, hence prevalence increases in places with poor sanitation. The main transmission route for hepatitis B, C, and D is sexual, vertical (mother to child) or through blood and blood products. Hepatitis B, C, and D distribution is heterogeneous based on practices that favor transmission, such as unsafe sexual intercourse, sharing of needles amongst intravenous drug users or unmonitored blood transfusions.

Currently, vaccines are one of the prevention tools used to control these diseases. Monovalent and combined vaccines are available against the hepatitis A and B viruses, while a vaccine against hepatitis E is under development. Further information on hepatitis A, B, and E is presented in this chapter. To date, there are no vaccines against the hepatitis C and D viruses.

Hepatitis A

The hepatitis A virus is a hepatovirus of the picornaviridae family, of small molecular size, with single-stranded RNA, and is non-enveloped which allows it to survive in low-pH media as well as in mild temperatures for extended periods of time.[4] There are seven genotypes with only one serotype; only four out of the seven genotypes affect humans (genotypes I and III are the most common).[5]

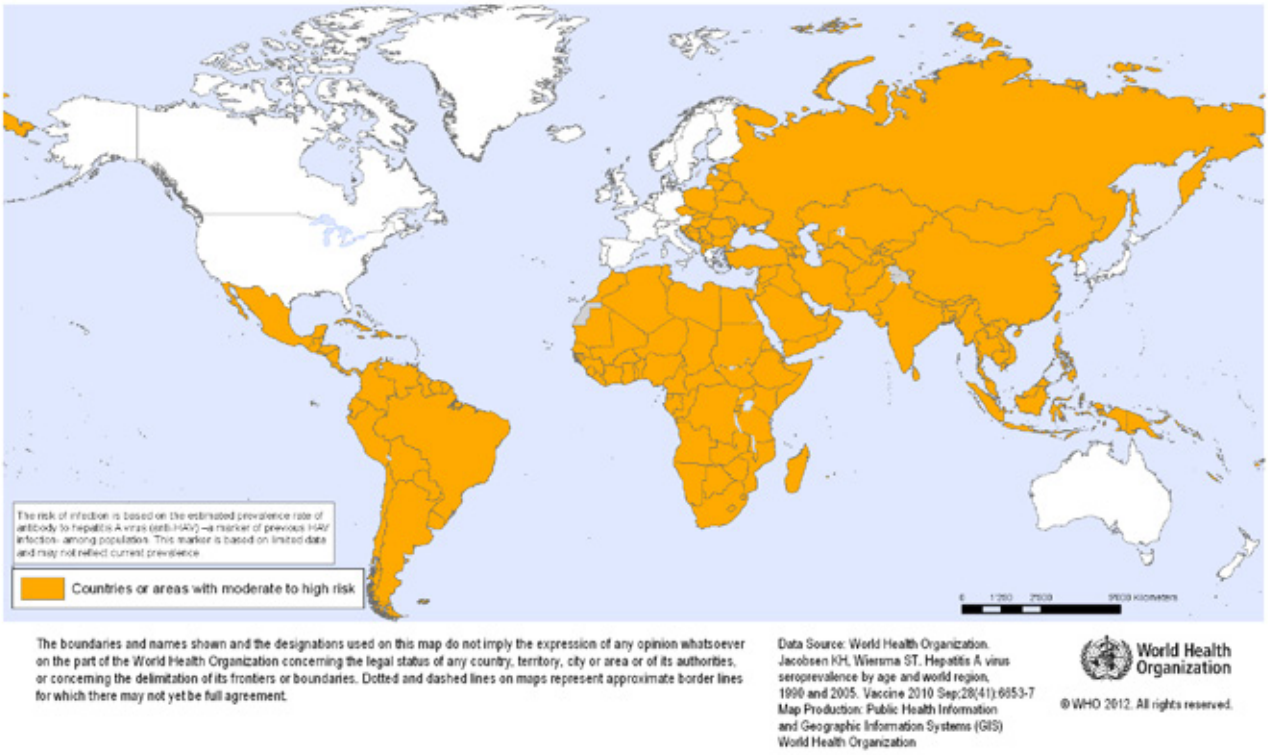
HAV is transmitted primarily via the fecal-oral route along with contaminated food or water. It spreads fast since its viral excretion takes place 10–15 days before the onset of symptoms and up to 7–10 days after the onset of jaundice. Viral excretion in feces prevails in the prodrome of the disease, since the viral load is lower during the symptomatic phase and undetectable for the resolution of symptoms.[4]

Infections due to the hepatitis A virus affect 1.5 million people a year. Estimates indicate that about 70% of the children infected before three years of age suffer from asymptomatic, but productive, infections with the potential to generate outbreaks involving large several cases. These asymptomatic cases in highly-endemic areas result in the underreporting of cases.[6] Fortunately, 99% of the patients spontaneously overcome the condition within 2 to 4 weeks and maintain lifelong immunity against all genotypes. Despite most of the cases often being asymptomatic or having mild gastrointestinal symptoms with or without jaundice, there are acute fulminant manifestations that may require urgent liver transplantation as the sole viable treatment. One percent of cases are estimated to be fulminant hepatitis due to HAV with an incidence rate of 1–3 individuals every 1,000 population, with an 80% mortality rate.[7] Older age is the main risk factor associated with the severity of the infection.[8]

Infection due to HAV has global distribution but its prevalence differs significantly based on the environmental and socioeconomic conditions in every region (Figure 1). Upon introduction of mass vaccination against the HAV infection, the incidence of the infection was significantly reduced all over the world.[9] Therefore, it is extremely important to continuously update information on the estimated risk of disease, as well as national prevention and control strategies such as vaccination coverage, since such interactions translate into permanent shifts in the risk situation.[10]

In most of the countries of the Americas Region and the Caribbean, more than 50% of the population has acquired natural immunity against the hepatitis A virus by 15 years of age. Hepatitis A endemicity is moderate to high and varies between regions, for example with anti-HAV seroprevalence in countries of the Caribbean and the Andean sub-region (Peru, Ecuador, and Bolivia) of 57% and 96% respectively, for individuals aged 15–19.[11] However, endemicity in the Americas Region as well as exposure to the virus are decreasing, thus increasing the risk of outbreaks in older age groups.

Figure 1. Global Risk-Level of Hepatitis A



Source: World Health Organization, 2012.

Diagnosis is based on the detection of specific serum antibodies (anti-HAV IgM) two weeks before the onset of symptoms. In cases in which the onset of symptoms has been within the first 5 to 7 days, viral detection and genotyping can be performed using a fecal sample.

Treatment of hepatitis A is supportive and symptomatic. In cases of fulminant liver failure, access to high-complexity centers for liver transplantation will determine the prognosis of the patient.

Prevention and control measures include sanitary and food-safety measures (hand washing, caution around water and food-handling, and hygiene measures) and prevention based on vaccines and immunoglobulin. Gammaglobulin (igG) is indicated as a post-exposure measure for pregnant women and children up to 1 year of age without previous protection.

Starting in the 1990s, various vaccine formulations have been marketed (attenuated live and inactivated) including hepatitis A vaccine formulations and schedules, as mentioned in Table 1 below. The combined formulations for hepatitis A and hepatitis B, as well as hepatitis A and typhoid fever are sometimes used in travelers.[6] Despite the two dose recommendations by manufacturers, in 2012, WHO endorsed the single-dose immunization strategy as of one year of age.

Table 1. Availability of Vaccines to Prevent Hepatitis A

Vaccine	Trade Name (Manufacturer)	Age (Y)	Dose	Route	Schedule	Booster
Hepatitis A vaccine, inactivated	Havrix® (GlaxoSmithKline)	1–18	0.5 mL (720 ELU)	IM	0, 6–12 mo	None
		≥19	1.0 mL (1,440 ELU)	IM	0, 6–12 mo	None
Hepatitis A vaccine, inactivated	Vaqta® (Merck & Co., Inc.)	1–18	0.5 mL (25 U)	IM	0, 6–18 mo	None
		≥19	1.0 mL (50 U)	IM	0, 6–18 mo	None
Combined hepatitis A and B vaccine	Twinrix® (GlaxoSmithKline)	≥18 (primary)	1.0 mL (720 ELU HAV + 20 µg HBsAg)	IM	0, 1, 6 mo	None
		≥18 (accelerated)	same as above	IM	0, 7, 21–30 d	12 mo

Source: Noele P. Nelson, Trudy V. Murphy. "Table 3-02. Vaccines to prevent hepatitis A." Hepatitis A. Chapter 3. Yellow book.

In certain instances, hepatitis A vaccination is recommended for adults, including:[6,12]

- Travelers to sites of intermediate or high endemicity
- Chronic liver disease
- Individuals with clotting disorders
- Men who have sex with men
- Laboratory personnel exposed to the hepatitis A virus
- Food industry personnel
- Childcare personnel in charge of children < 1 year

Morbidity has fallen globally since the licensure of hepatitis A immunization for infants as of 12 months of age. In 2004, the U.S. had an overall rate of 1.9/100,000 population, the lowest rate ever recorded and 79% lower than any previously recorded rate.[13] Similar examples have been observed in countries from various regions such as Argentina, Australia, Israel, Italy and Spain.[14,15] The experience in Argentina is highlighted below, since their vaccination schedule comprises a single dose at 12 months of age.

Similarly, this vaccination strategy has modified the age at which onset of the disease occurs, with an observed increase of incidence amongst adults, as well as higher morbidity. Scientific evidence indicates that these immunization programs may result in a significant reduction of hepatitis A incidence due to acquired immunity. Follow-up of the vaccinated population to assess seroprotection in the long term is key to be able to avoid infection at an older age. National health policies need to include hepatitis A immunization within the framework of public health policies.[16]

Country Spotlight: Single-Dose Hepatitis A Immunization at One Year of Age in Argentina

As of 2005, the epidemiology of the hepatitis A virus (HAV) in Argentina has shifted due to the introduction of single-dose HAV immunization at 12 months. Local evidence showed a dramatic decline in the number of hepatitis A cases after vaccine introduction and up to the present, as well as a reduction in the number of hospital admissions due to this pathology.[17] Similarly, there has been an impact on cost reduction in the public health sector as determined by medical and social savings resulting from this strategy.[18]

Based on the evidence from Argentina, the WHO recommended the single-dose strategy be implemented by other countries as part of their national immunization schedules in a June 2012 vaccine position paper.[19] Therefore, several countries including Brazil, Colombia, Mexico and Paraguay have implemented this strategy to control the disease. Within this context, Argentina has committed to strengthening surveillance of this pathology as part of the follow-up and assessment of their single-dose strategy.

In 2011, two multicenter studies were performed in Argentina to assess the strategy of a single-dose hepatitis A immunization (HA) at one year of age which was implemented in 2005, in coordination with the National Program for the Control of Vaccine-Preventable Diseases (ProNaCEI), within the National Ministry of Health.[20] In 2011, a seroprevalence study was performed in the short-to-intermediate term to measure anti-HAV antibodies in children four years after immunization with one dose of the hepatitis A vaccine. In the study, 93% (95% CI: 91.7–94.6) of the children maintained protective antibody titers (anti-HAV IgG>10 mUI/ml), indicating that a single-dose HAV vaccine in our environment is highly immunogenic in the intermediate term. In 2013, these studies were repeated and showed 97% seroprevalence of protective anti-HAV antibodies in children vaccinated with a single dose more than seven years prior. In 2016, a new seroprevalence study showed 87% of the children still present protective antibody level, supporting the local strategy. Currently, Argentina is running a study on the "humoral and cellular immune memory response 10 years following single dose vaccination against hepatitis A in Argentinian children," regarding the effective protection of the vaccine in the population.

To date, HAV vaccination coverages at the country level have been satisfactory since the introduction of the vaccine into the National Immunization Schedule. Despite this progress, isolated cases continue to be reported in children under nine years of age without HAV vaccination history, specifically in departments with low coverages. Steadily declining rates have been observed in all age groups and in all regions of the country. A slight increase has been observed in case reporting amongst adults, but no vaccinated children have presented liver failure or the need for transplantation.

Hepatitis B

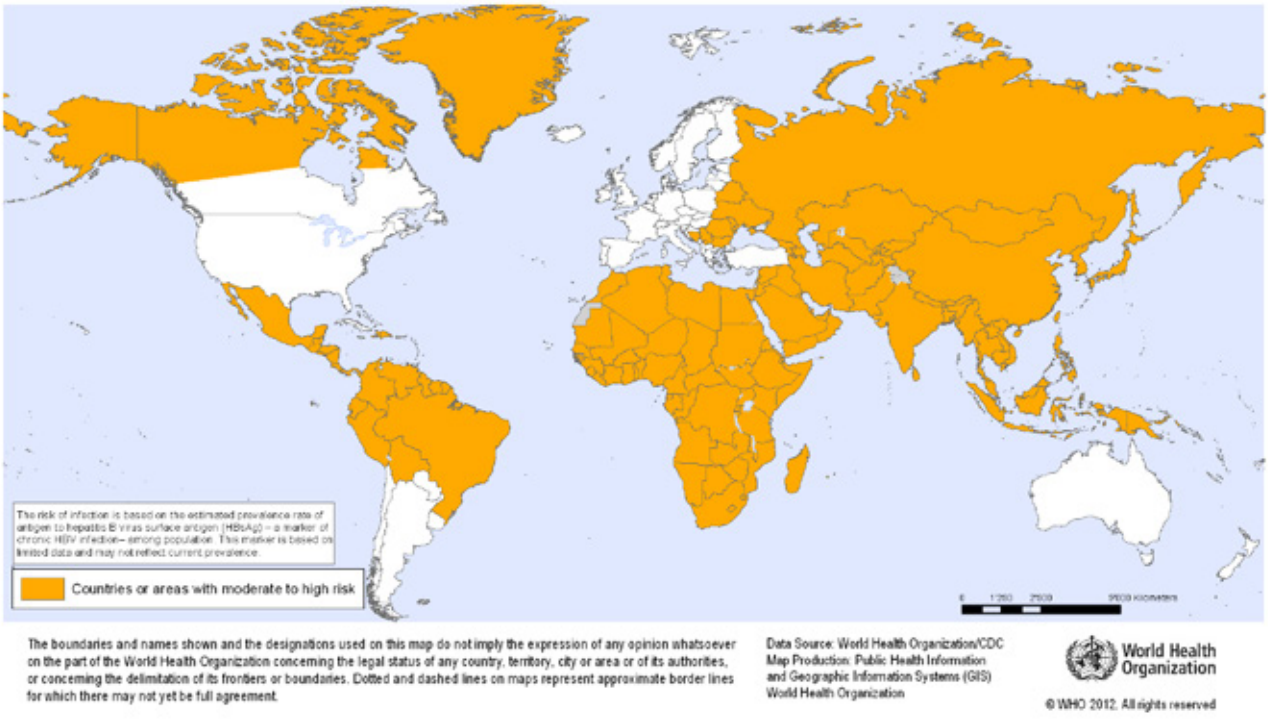
The hepatitis B virus (HBV) infects more than 500 million persons globally. It is the main cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. About 2 billion individuals are estimated to have previous or current HBV infection worldwide, an estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive) and more than 240 million are chronic HBV carriers.[21] In 2015, hepatitis B resulted in 887,000 deaths, mostly from complications (including cirrhosis and hepatocellular carcinoma).[22] Acute hepatitis B resulting in fulminant liver failure produces 130,000 deaths a year globally.[23] The high economic cost of this virus is expressed in the years of life lost due to liver pathology accounting for 5% to 10% of liver transplantations.[24,25]

HBV transmission, through the sexual, vertical, and parenteral routes, is very efficacious (10% to 30% if the source is HBsAg positive and 30% to 60% if the source is HBeAg positive). The incubation period is long, between 1 and 4 months. The most common clinical manifestation is acute hepatitis, which is spontaneously resolved within 1 to 3 months. Additionally, there are asymptomatic manifestations that can be observed in up to 60% of cases. Between 6% and 10% of infected individuals will evolve to chronicity. Age is the determining factor for chronicity, and it is common in newborns after an acute infection (90%) and in children < 5 years of age (20%–60%), but it is unusual when the infection is acquired in adulthood (<5%).[26,27]

Due to the virus' human reservoir, it is possible to control, eliminate, and eradicate HBV. Based on studies performed in the United States in 2007, the risk factors to acquire the virus include the use of intravenous drugs (15%), sexual intercourse with persons infected with HBV (6.2%), men who have sex with men, hemodialysis, multiple sexual partners and injuries with sharp elements.

Worldwide prevalence varies by region and within the regions as observed in Figure 2. However, out of the total global population, about half of the population is located in highly-endemic areas.[8]

Figure 2. Global Risk-Level of Hepatitis B



Source: World Health Organization, 2012.

Information obtained between 1990 and 2005 shows a prevalence of 2% in the central and tropical regions of Latin America, and ranging between 2% and 4% for the Caribbean and the Andean sub-regions.[28] There is higher prevalence of co-infection with the hepatitis D and B viruses, as observed in the Amazon sub-region.[29,30]

Globally, nine HBV genotypes (A-I) have been identified, with at least an 8% difference in their genomic sequence.[31,32] Higher hepatocarcinoma rates have been observed in patients infected with the C and F

genotypes, and certain A subgenotypes found in Southern Africa. Virus genotyping is extremely important to determine its regional characteristics. In the Americas Region several genotypes co-exist, out of which genotype F is the main one.[33] Antivirals as well as the protection conferred by the licensed vaccines at present have proven to be effective against all genotypes.[34]

There are several supplementary ways to control hepatitis B as detailed by WHO in a 2009 vaccine position paper, including: vaccination of newborns, completion of a 3–4 dose schedule, catch-up vaccination in cohorts of children with low coverage, vaccination of adolescents and adults included in the high-risk groups in countries of low or moderate endemicity, and improvement of coverage in children from highly-endemic countries.[35] The vaccination schedule should have three doses and, for infants, the recommendation is to administer the first dose as soon as possible, preferably within twelve hours after birth.[36]

There are various hepatitis B vaccines, including monovalent or combined with hepatitis A. Vaccines use the recombinant hepatitis B surface antigen (recombinant DNA vaccines), achieving immunogenicity above 90% that decreases in adults older than 40 years, immunosuppressed hosts and tobacco users. Efficacy ranges between 80% and 100%, and its correlate of protection is Anti-HBs >10 UI/L with a recommendation for routine testing solely in special hosts. Several studies have analyzed the vaccine safety profile.

By 2008, 177 of the 193 WHO member states (92%) had incorporated hepatitis B immunization schedules into their national childhood immunization schedules.[37] All of the countries of the Americas have officially introduced the hepatitis B vaccine into their childhood immunization programs.

There are specific indications for the hepatitis B vaccine in adults as shown below:[36]

- Individuals at risk due to sexual exposure: HBsAg-positive sexual partner, individuals with more than one sexual partner over the last 6 months, sexual contact with individuals under follow-up due to sexually-transmitted infections, men who have sex with men.
- Individuals at risk of infection via percutaneous route or mucous exposure to contaminated blood: frequent or recent use of intravenous drugs, close contacts with HBsAg-positive individuals, residents and staff at care centers, health providers, individuals with diabetes mellitus aged 19 to 59.
- Others: travelers to highly-endemic sites for hepatitis B, persons with chronic liver disease, persons living with HIV.

In spite of the known risk factors, epidemiological surveillance in the United States during 2007 has not shown a high percentage of patients with any of the risk factors known for the infection.[38] Overall, 58% of the population did not have a known predisposing factor in this surveillance report.

A multicenter study performed in Argentina has shown that HBV is currently the most frequent cause of fulminant hepatic failure.[39] In this low-endemicity country for HBV, vaccination was implemented as a public health strategy for health personnel in 1992, and a three-dose schedule was implemented for all live births in 2000. Satisfactory and constant coverages were maintained with a steady decline in HBV cases amongst the younger age groups, coinciding with the protection conferred by the vaccine. An increase of reported cases amongst young adults was observed. In 2012, scientific evidence and disease surveillance led to the recommendation of universal hepatitis B immunization for the entire population that had not previously received a complete schedule based on the epidemiological evidence of local and international data. This recommendation was added to the National Immunization Schedule in 2014. Immunization was deemed mandatory and provided free of charge for the whole population, making Argentina the first country to introduce this strategy for the control and elimination of hepatitis B.[40,41]

Hepatitis E

Every year there are 20 million infections due to the hepatitis E virus, leading to an estimated 3.3 million symptomatic cases.[42,43] WHO estimates that hepatitis E caused approximately 44,000 deaths in 2015 (accounting for 3.3% of the mortality due to viral hepatitis). Three thousand newborns are infected yearly.[44] The route of transmission is fecal-oral with outbreaks involving many cases. Currently, there are four known genotypes, of which 1 and 2 affect mainly humans.[45,46] There is evidence that immuno-suppressed individuals, for example solid-organ transplant recipients, would be more vulnerable to developing a chronic and lethal liver disease due to any of the four genotypes.[47]

Mortality ranges between 0.1% and 4% but the main risk factor for complications is the third trimester of pregnancy when the mortality rate reaches 10% to 50% among pregnant women. Distribution is global, and there are differences by region as observed in Figure 3 below.[48]

Source: Centers for Disease Control and Prevention, 2018.

Figure 3. Global Risk-Level of Hepatitis E

The Region of the Americas has low prevalence of the virus but cases and outbreaks have been reported in some countries. Studies conducted in Brazil show prevalence close to 3% in adults, and 1.7% to 16.2% in Bolivia.

To date, only one vaccine against hepatitis E (Hecolin) has been licensed based on the ORF2 239 protein. ORF2 codes for the viral capsid protein, and thus, the neutralizing antibodies. It is derived from a genotype 1 Chinese strain, and it contains aluminum and thimerosal as adjuvants. It is supplied in a pre-filled syringe for a three-dose schedule (0, 1, 6 months) in individuals aged 16 to 65. The vaccine is stable between 2 and 8°C, out of direct sunlight. It has demonstrated 98% (0–6m) immunogenicity versus 100% (0m, 1m, 6m) in a Phase IIa study, and presents a 98.7% seroconversion rate with three doses in Phase III studies (N= 113,000 participants). Its efficacy in Phase II and III studies has shown protection against G4, but evidence is scarce in relation to G1. There is no information regarding G2 and G3. Cross protection has been demonstrated against G4 but there is no evidence in connection with genotypes G1, 2, and 3. Currently, the duration of antibodies is up to 4.5 years. To date, no safety data have been published.[49]

Based on the abovementioned, WHO established in the hepatitis B vaccine position paper that despite HEV being a public health problem, in particular for some countries, there is limited information on global incidence as well as morbidity and mortality. In spite of having a promising vaccine with a good proven response in individuals aged 16 to 65, given the insufficient nature of the data (in particular in individuals <16 years or in connection with cross reaction with G1-2-3), WHO does not recommend routine use in national immunization programmes. However, a country may adopt the most convenient strategy given the local epidemiological situation. Routine use is not recommended in the following populations given insufficient evidence on immunogenicity, effectiveness, and safety profile: pregnant women, individuals <16 years, chronic liver disease patients, patients on organ transplantation lists and travelers. The administration of this vaccine may be considered in outbreak situations, mainly in high risk groups. Studies on immunogenicity, efficacy and safety profile should be performed in groups with limited data.[18]

Conclusion

Immunization continues to be the most important and cost-effective preventive intervention to reduce morbidity and mortality amongst children. In an era of new vaccines, countries across all regions, including the Americas Region, need to make great efforts to document the epidemiology of these diseases before and after vaccine introduction. Their experiences with the challenge of introducing new vaccines into their national immunization schedules should also be documented. The experience of each country becomes essential and extremely important to spread knowledge locally, regionally, and globally for other countries and regions to benefit from the documented lessons learned and to make evidence-based decisions. Countries also need to decide on the inclusion or exclusion of a specific vaccine based on epidemiological data on disease burden, other available interventions, and the economic cost of the strategy.

Viral hepatitis cases continue to be a great challenge for public health. They demand a joining of public health forces to fight against these viruses with the purpose of improving the quality of life of the population, along with the understanding that vaccines are tools for social equity and equality.

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HIV, Tuberculosis, and Malaria

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Introduction

The acquired immunodeficiency syndrome (AIDS), tuberculosis (TB), and malaria, collectively, account for over 5 million deaths a year. However, because of their genetic instability, great variability or ability to hide within host cells, they have been able to avoid the conventional development of effective vaccines. Globally they represent one of the greatest challenges for public health in the second decade of the XXI century. Recent trials have evidenced the feasibility to develop vaccines that can prevent the infection caused by the human immunodeficiency virus (HIV) and malaria.

Furthermore, advances in vaccine development, including new adjuvants, new administration schedules, and strategies for the presentation of antigens at the intracellular level have led to progress in the development of a better tuberculosis vaccine. New tools, such as the so-called systems biology and vaccine design based on the structure of antigens, will hopefully deepen the understanding of the protection mechanisms which, in turn, will result in the development of vaccines against these pathologies.

Overview of HIV, TB, and Malaria

What do these three microorganisms have in common? They all pose a challenge for humanity. To date, HIV/AIDS has caused over 25 million deaths; 33 million people are currently living with HIV, and 2.6 million new cases emerge every year, resulting in 1.8 million deaths annually.[1,2] In the case of malaria, 225 million new cases and one million deaths occur every year.[3] Finally, tuberculosis impacts a third of the world population, results in 9.6 million new cases and 1.7 million deaths a year; treatment has been challenged by the emergence of multidrug-resistant tuberculosis.[4] Broad genetic heterogeneity and ability to hide at intracellular level are common among the three pathogens.

Structural Vaccinology and Systems Biology

However, new technological advances provide hope. New immunization schedules, new adjuvants, and new methods for antigen presentation are being tested. Moreover, innovative ways to treat these illnesses have resulted in the identification of adequate protection markers and clinical and regulatory innovations. Structural Vaccinology comprises the design of new antigens based on the already-known structure of surface proteins while exposing preserved epitopes or creating molecules with multiple immuno-dominant epitopes to induce better protective immune responses.[5] Systems Biology is a strategy to address biological problems by collecting and integrating data at various levels, thus revealing properties that cannot be demonstrated or predicted otherwise, such as for example the response to a new vaccine dependent on genetic, molecular, and environmental factors and their interaction.[6] By means of a computational analysis, models can be developed to forecast whether a vaccine will produce an adequate protective response or not.[7,8]

Current Status of HIV, TB, and Malaria Vaccines

HIV

Why don't we already have a vaccine against HIV? The absence of a vaccine has not been because of a lack of effort, rather the capacity HIV has to escape: immediate and final integration with the genome in the host cells, variability of the epitopes to which the antibodies and T cells bind, and weak neutralizing antibodies, as illustrated by the absence of a spontaneous cure or recovery from the HIV infection.

The genome sequence of the virus is highly variable. The world population of HIV viruses is divided into four main groups (A, B, C, and E) mainly present in Africa, North America and Europe, Asia, and Africa, respectively. Within each group, the sequence varies tremendously and the virus continues to evolve and mutate in every patient infected with the virus. Neutralizing antibodies against the virus and the T-cells induced by the natural infection or conventional vaccination generate a narrow immune response, which is inadequate to confer protection against all of the virus variants.

The HIV protective immunity lacks proper markers. Regarding the antibodies, no significant relationship exists between the neutralizing antibodies and the viral control; however, some response has been observed following the passive transfer of anti-HIV and anti-simian immunodeficiency virus (SIV) neutralizing monoclonal antibodies. As to specific anti-HIV CD8 T-cells, depletion of CD8 cells in rhesus monkeys results in the immune system losing control over SIV, the magnitude of the response being inversely proportional to the viral load (VL) in acute and chronic patients and in elite controllers. The quality of the lymphocytic response, as expressed in its multi-functionality, differentiation and avidity, is also important. HIV-specific CD4 T-helper-1 cells exhibit the inverse relationship to the viral load, both in the acute infection phase and in the dormant infection phase.

Scientists from all over the world have been attempting to develop an effective HIV vaccine for over two decades. In the nineties, Phase I and Phase II clinical studies were conducted with subunit vaccines from the HIV envelope. However, the neutralizing antibodies (Nabs) *in vitro* only neutralized the vaccine strains, which is unacceptable for HIV given the high-frequency at which the virus mutates surface antigens.[9, 10] Later on, recombinant subunit vaccines were tested but the results were negative due to the antigen diversity and the Nabs inability to neutralize wild strains.[11, 12] Studies geared to activate T-cell immunity against HIV, such as STEP or the RV144 study, attained 30% protection or lower.[13, 14]

Nowadays it is clear that neither the humoral protection nor the cellular protection by themselves will be sufficient to develop a protective vaccine. Unfortunately, combined strategies attained only marginal protection. Through systems biology, immunogenicity markers have been studied (CD4-specific, CD8-specific, viral load) to generate more stable gp120 and gp41 molecules, with preserved epitopes, which in turn has translated into broad-spectrum Nabs as the only strategy proven to prevent the HIV infection.[15]

The identification of immuno-dominant neutralizing epitopes from HIV variants will likely be the basis for the development of new membrane proteins for broader protection. Other supplementary strategies include non-neutralizing antibodies against preserved antigens to broaden immunity (due to mosaic antigens or preserved chimeric antigens), T-cell-based vaccines to control viruses that mutate due to the selective pressure of the neutralizing antibodies and new vectors.

Despite the significance and hope behind the progress made in recent years, all of the forecasts for the availability of an effective HIV vaccine have failed, which makes it impossible to speculate how far we are from the attainment of the goal.

Tuberculosis

TB is caused by the bacteria *Mycobacterium tuberculosis* that infects the lungs by penetrating them and growing inside the macrophages. The immune cells surround the infected macrophages and form granulomas where the bacteria may remain dormant for a while. The weakening of the immune system with the HIV infection paves the way for the reactivation and ensuing disease.

On an annual basis 9.6 million individuals will develop TB and 1.5 million will die. However, TB incidence has been diminishing by 1.5% every year since 2000 and mortality has diminished by 47% since 1990. The greatest problem is the emergence of multidrug resistant TB (MDRTB). Annually 3.3% new cases emerge, 20% relapse and almost 10% of MDRTB is extremely resistant or utterly impossible to treat. MDRTB poses a challenge for the development of new TB vaccines.

M. tuberculosis poses multiple difficulties for the development of an ideal vaccine. At the outset, the antigens are complex: different proteins on the cellular wall and inside, some secreted at various stages of the infection; glycoproteins; sugars; microlipids, and lipids (lipids do not present themselves as protein antigens traditionally do and there are no lipid vaccines [Koch removed the lipids from purified protein derivative (PPD)]). Furthermore, *M. tuberculosis* presents a complex vital cycle: log growth, multiple immuno-dominant antigens secreted at each stage, ability to stay in immuno-salient latency (latency genes, latency antigens) and subsequently reactivate as an active disease.

Do we need four different TB vaccines? The question is valid since non-immune patients present the primary disease; immune and sensitized patients experience the post-primary disease, the dormant disease, and the need to optimize treatment for the disease. Therefore, the immunization strategies proposed to control the disease are infection prevention (*prime*), disease prevention (*booster*) and prevention of relapses (therapeutic). The tuberculosis vaccine based on an attenuated *Mycobacterium bovis* strain, or bacille Calmette–Guerin (BCG), has been used for close to 100 years but its efficacy is controversial.[16, 17]

The BCG vaccine may prevent the spread of the disease and deaths in children but not chronic infection or pulmonary tuberculosis in adults. However, depending on the studies, efficacy ranges between 0% protection against any disease (study conducted in Madras/Chennai, India)[18] and up to 80% against miliary TB and meningeal TB in children (study conducted in the United Kingdom)[19] through 50% protection against pulmonary TB (study conducted in the United States).[20-23] Furthermore, currently there are several BCG strains but the BCG manufacturing techniques are not part of the existing production practices and we are unaware if the BCG vaccine generates a proper primary immune response against *M. tuberculosis*.[24]

At this time, 16 new vaccines are undergoing clinical trials (proof-of-concept or Phase IIb studies): with recombinant antigens, DNA or viral vectors and subunit vaccines as BCG booster (to prevent chronic infection or avoid reactivation).[25-27] However, the most advanced vaccine is the one that entails the reengineering of the BCG vaccine itself.[28]

Challenges for future studies of TB vaccines include geographical diversity in terms of risk of infection and TB disease, definition of clinical target (infection, disease, latency or cure, duration, level of acceptable efficacy, integration or replacement of BCG vaccine, prioritization of potential vaccines and impact of HIV epidemiology).

Malaria

Malaria is caused by the *Plasmodium* parasite, which infects humans through a mosquito bite. The mosquito injects the parasite in the form of a sporozoite that quickly migrates to the liver. Following 6 to 7 days it is released in a different form, as an amerozoite, infecting and multiplying inside red blood cells. Finally, a new form of the parasite (gametocyte) is generated in the human host and acquired once again through mosquito bites. *Plasmodium falciparum* and *Plasmodium vivax* are the main human pathogens. The various stages of the parasite have different antigen compositions; antigen variability within each stage has been one of the main obstacles to the development of a vaccine.

Natural immunity against malaria is specific to each stage of the disease, but naturally-acquired immunity develops slowly, and incompletely for a limited amount of time. In spite of recent advances in the reduction of malaria mortality due to other interventions (48% reduction since 2000), every minute a child in Africa dies due to malaria. Moreover, success is undermined by the financial instability of the affected countries and resistance to artemisinin and insecticide. Therefore, vaccines are urgently needed to reduce the incidence and deaths caused by the disease as well as to block transmission of the parasite through herd immunity and allow for the elimination and eradication of the disease.

The most advanced vaccine in clinical trials (RTS, S) completed a Phase III evaluation in African children from 13 centers, in eight countries. Over 12 months of follow-up, RTS, S demonstrated approximately 50% protection against the clinical disease caused by *Plasmodium falciparum* in children aged 5 to 17 months and about 30%

protection in children aged 6 and 12 weeks, when administered together with vaccines from the Immunization Program.[29] In spite of waning immunity [In participants aged 5–17 months, the half-life of the short-lived component of the antibody response was 45 days (95% credible interval 42–48) and that of the long-lived component was 591 days (557–632)], there is a clear benefit to the vaccine.

An average of 1,363 cases of clinical malaria are estimated to have been prevented over 4 years of follow-up per 1,000 vaccinated children and 1,774 cases are estimated to have been prevented amongst those who received the booster.

The World Health Organization (WHO) monitored a process to enable understanding of the differences in the epidemiological models developed by four different groups (Imperial College, Swiss TPH, Intellectual Ventures, GSK) intended to reach consensus on impact and cost-effectiveness. All the models forecast a 10% to 28% reduction in malaria-related mortality in children < 5 years who received the full schedule. In areas with moderate-to-high transmission, this translates into the prevention of 116,500 cases of clinical malaria and 484 deaths every 100,000 children vaccinated.

At a hypothetical price of US$ 5/dose, the average incremental cost-effectiveness rate of the vaccine is US$ 87 ($48–$244) per DALY prevented and US$ 25 ($16–$222) per clinical case prevented, which is favorable when compared to the global cost-effectiveness estimated for other vaccines. Based on a comparative cost-effectiveness study conducted by the Imperial College of London, long-lasting insecticide nets (LLINs) are the most cost-effective initial intervention in all of the scenarios, followed by seasonal malaria chemoprophylaxis where recommended and, lastly, RTS, S in places with parasite prevalence > 10%.

WHO recommended conducting pilot studies with RTS, S/AS01 in 3 to 5 sites with a high burden of disease in Africa. The studies should assess the operational feasibility of providing the vaccine to the target population under the four-dose schedule recommended within the context of the local health services, the impact of the vaccine on all-cause child mortality when implemented concomitantly with other interventions recommended against malaria and surveillance of adverse events following vaccination, in particular meningitis and cerebral malaria, before considering coverage escalation.

A drop in the disease caused by *P. falciparum* will prioritize the development of a vaccine against *P. vivax*. However, work is being conducted to improve human immunization models (issues with relapses and lack of *P. vivax* cultures). The first *P. vivax* trial used a recombinant *P. vivax* CS protein in AS01, but the clinical evaluation may be difficult given the potential interactions with *P. falciparum* and the differentiation of new infections from hypnozoite reactivation.

The development of more efficient vaccines to prevent the clinical disease caused both by *P. falciparum* and *P. vivax*, as well as vaccines to help eliminate the parasite by blocking its transmission, is a priority. The barriers to the development of these vaccines have been the shortage of clearly-identified immunogenic antigens for all the stages of the parasite life cycle, the absence of clearly-defined protection markers, a limited number of safe and effective delivery systems (adjuvants inducing a potent and lasting humoral or cellular immune response) and, for vaccines designed to attain herd protection targeting the developmental stages of the parasite or mosquito antigens, the absence of a pre-established clinical and regulatory roadmap to pave the way for vaccine licensure by the regulatory authorities.

Conclusions

Historically, successful vaccines have been effective against pathogens treatable with antibodies and with a stable antigen repertoire. HIV, malaria, and tuberculosis have broad antigen variability and require T-cell immunity to obtain protection against these diseases. The development of vaccines against these pathogens requires new approaches such as structural vaccinology (a branch of structural biology that is emerging as a promising platform for the identification of effective protective antigens) and systems biology (computational and mathematical modeling of complex biological systems).

Moreover, we are entering an era where the extended use of a vaccine requires more than only safety and efficacy data. Recommendations for the use of new vaccines will be considered in terms of implementation studies that determine the most effective forms of widespread use. Otherwise, the vaccines most likely to fail are the ones developed mainly for the poorest peoples of the world.

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Current Situation of Human Papillomavirus Vaccines

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Introduction

One of the most outstanding scientific discoveries for vaccine-preventable diseases has been the identification of the causal relationship between the human papillomavirus (HPV) and cervical cancer. This discovery was made in 1977 by Harold Zur Hausen, who was awarded the Nobel Prize in Physiology and Medicine in 2008.[1,2]

Infectious Agent Profile

The human papillomavirus (HPV) is a member of the *Papillomaviridae* family. Its genome consists of double-stranded deoxyribonucleic acid (DNA), containing approximately 8,000 base pairs covered by the major and minor structural proteins, L1 and L2, respectively. The capsid proteins L1 and L2 develop structures that interact with the cellular surface molecules and, therefore, facilitate cell penetration by the virus DNA; moreover, their respective late genes (L) encode the proteins. Early genes (E) control virus replication during the virus cycle. The study of L1 genome sequencing[3,4] has led to the identification of more than 190 virus types, which have high affinity to specific tissue and infect the cutaneous and mucosal epithelium without invading connective tissue or spreading regionally or systemically. The transmission path is mainly sexual, and hard to prevent. The virus incubation period is estimated to be three weeks to eight months; condyloma acuminata may occur at two or three months after infection.[5]

Viruses are classified as low-risk HPV or high-risk HPV, depending on their potential to induce cancer. Currently, the International Agency for Research on Cancer (IARC) defines 12 high-risk virus genotypes associated with cancer in human beings: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Additionally, there is some evidence about the oncogenic potential of two genotypes: 68 and 73.[6] Most of the infections are temporary in nature, and about 70% to 90% of them clear within 1 to 2 years.[7,8] Histopathologically, the lesions of the cervix, referred to as cervical intraepithelial neoplasia (CIN), fall into one of three categories: cervical intraepithelial lesion 1 or CIN1, involving mild dysplasia; CIN2 or moderate to severe dysplasia, and CIN3 or severe dysplasia.[9]

Progression of the lesions has been described as a potentially reversible phenomenon up to CIN3, the stage at which neoplastic growth penetrates the basement membrane invading the stroma. Persistent infection and integration of genetic material within the cells are the main factors contributing to oncogenesis.[9-14] Progression from CIN1 to CIN3 may take about 10 years and progression from CIN3 to cervical cancer may take about two years.[10] The etiological role of HPV in cervical cancer has been demonstrated biologically and epidemiologically.[10,13-14]

Epidemiology

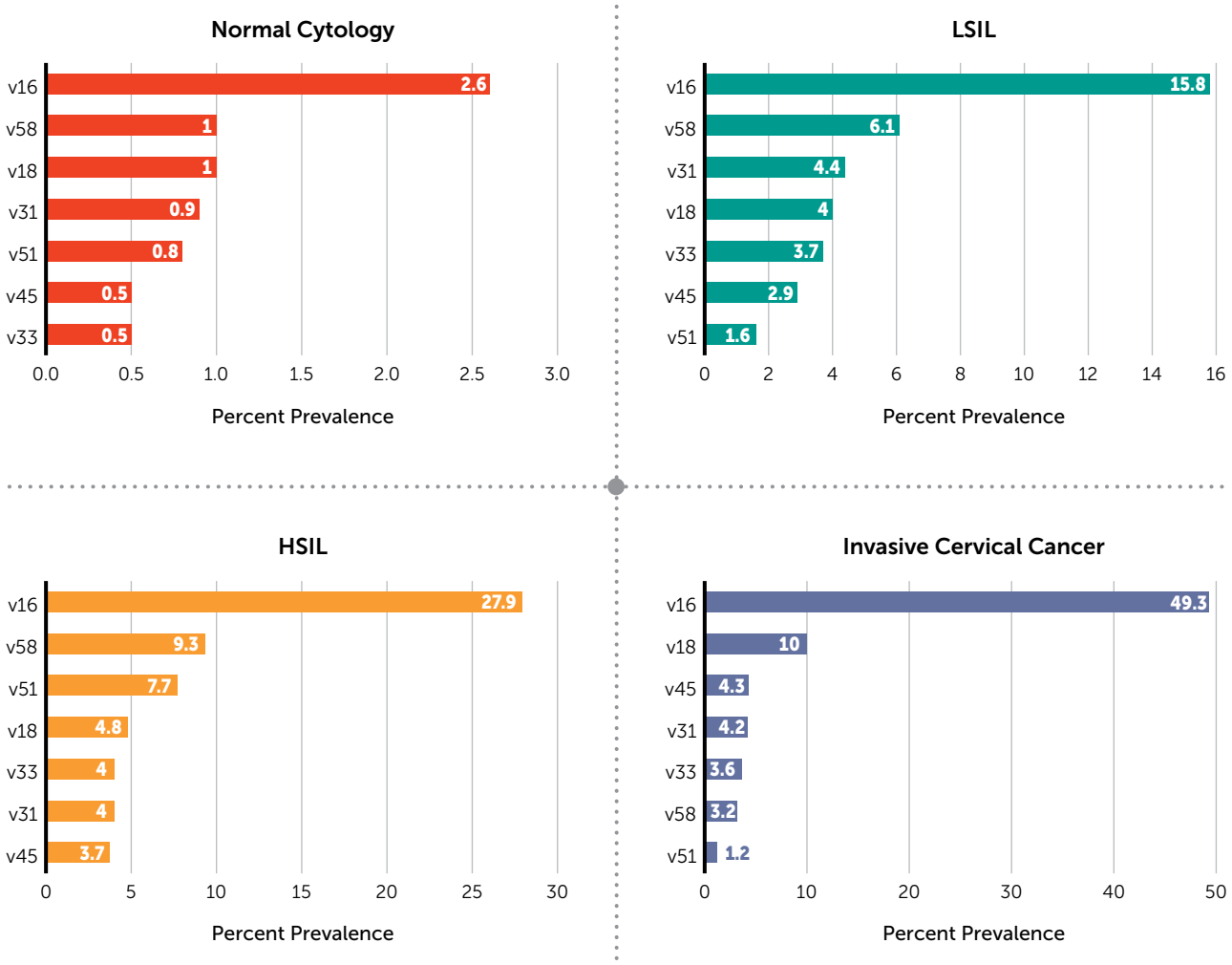
Based on data provided by IARC, over 100,000 cases of HPV-related cancer are diagnosed yearly in Latin America: cervical cancer (80%), oropharynx cancer (6.5%), as well as the remaining HPV-related cancers of the anus, penis, vulva, and vagina.[14]

Mortality caused by cervical cancer varies in the different regions of the world, presumably due to differences in health care systems, screening and access to health care. The highest mortality rate is observed in Africa, at 27.6 per 100,000 women, and the lowest rates occur in East Asia, Europe, Australia, and New Zealand, at 2 per 100,000 women.[14]

Most sexually-active individuals will have an infection at some point in their lives due to at least one HPV genotype. A meta-analysis published in 2007, which included 157,879 women from 36 countries, estimated a 10% global prevalence of HPV infection in women with normal cytology,[15] with marked geographical differences: higher frequency in Africa (22.9%) and Latin America (18.6%), and less frequency in Southeast Asia (8.3%) and Europe (6.6%). In 2007, HPV-16 was considered the most prevalent genotype in every region (3–4% in North America; 2% in Europe) followed by genotype 18. Similar results were derived from other studies,[16] and from surveillance conducted by IARC in 2005 in women aged 15–74 years from 11 countries.[17] In every region, a peak in the infection rate was observed at age 25, followed by a decrease and a subsequent increase at age 45.[16,17]

The distribution of HPV genotypes is variable amongst the populations even within the same region.[18] A meta-analysis of HPV-infection and HPV-associated cervical cancer surveillance, including reports between 1990 and 2007 in Latin American and Caribbean women, also showed that a comparison of genotype prevalence in women with normal cytology and prevalence in women with a lesion or cervical cancer, yields significant differences in the HPV types detected. In all cases, type 16 was the most frequently identified and accounted for 2.6% in women with normal cytology, 15.8% in low-grade intraepithelial lesions, 27.9% in high-degree CIN, and 49.3% in invasive cancers.[19] Figure 1 illustrates HPV-genotype distribution based on cytology status as established in the meta-analysis. The full report is available online at: www.sabin.org.

Figure 1. Distribution of Prevalence of Specific HPV Genotypes by Type of Lesion or Cytology Status Among Women in Latin America and the Caribbean[19]



Source: Valenzuela MT et al., 2009.[19]

Notes: LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion.

A similar study published in 2011 regarding HPV prevalence in Canadian women concluded that type 16 was the most common; however, 36 HPV types were isolated in 873 women with CIN and 252 women with cervical cancer. The HPV types identified, and their frequencies, differed based on the extent of the lesion. The most frequent genotypes in order of decreasing frequency were HPV-16, 51, 52, 31, 39, 18, and 56 in women with

CIN1; HPV-16, 52, 31, 18, 51, 39, and 33 in women with CIN2; HPV-16, 31, 18, 52, 39, 33, and 58 in women with CIN3; and HPV-16, 18, 45, 33, 31, 39, and 53 in women with invasive cervical cancer.[20]

In a study regarding the prevalence and genotype distribution of HPV infection in Chinese women who were asymptomatic, HPV was found in 10.3% women (9.5% low-risk types and 1.1% high-risk types). HPV genotypes 16, 52, and 58 were found most frequently in 26.2%, 19.45%, and 13.8% in the study population, respectively.[21]

Prevalence data in men are sparse and difficult to assess. It is estimated that the frequency of infection in men is typically 50%, with a higher rate of low-risk HPV infection when compared to women. However, genotype distribution may change based on the sample collected and the technique used for analysis.[16,22]

Available Vaccines

HPV vaccines are synthesized from the L1 protein. Five proteins are assembled in highly immunogenic, non-infecting virus-like particles (VLPs).[23] In 1993, researchers in the United States from the National Cancer Institute (NCI) discovered a way to synthesize VLPs with the same structure as HPV-16, and it was later used by Merck to manufacture the first quadrivalent vaccine.

Currently, three vaccines have been registered (Table 1), two of them manufactured by Merck/Co., Inc. (quadrivalent vaccine and nonavalent vaccine) and the other one manufactured by GlaxoSmithKline (bivalent vaccine).

As of May 2017, the WHO supports the recommendation for a 2-dose schedule with adequate spacing between the first and second dose (with a 6-month interval) in those aged 9–14 years.[24]

Table 1. Vaccination Characteristics and Schedules for the VLP HPV-16/18, VLP HPV-6/11/16/18, and VLP HPV-6/11/16/18/31/33/45/52/58 Vaccines

VACCINE (MANUFACTURER)	Cervarix® HPV-16/18 (GSK)	Gardasil® HPV-6/11/16/18 (Merck)	Gardasil 9® HPV-6/11/16/18/31/33/45/52/58 (Merck)
Vaccination schedule recommended by manufacturers	9–14 years: 2 doses (0.5 mL at 0 and 5–13 months) ≥15 years: 3 doses (0.5 mL at 0, 1, 6 months)	9–13 years: 2 doses (0.5 mL at 0 and 6 months or 0 and 12 months) Alternative 3-dose schedule: (0.5 mL at 0, 2, 6 months)	9–14 years: 2 doses(0.5 mL at 0 and 5–13 months) Alternative 3-dose schedule: (0.5 mL at 0, 2, 6 months) ≥15 years: 3 doses (0.5 mL at 0, 2, 6 months)
WHO recommendation (Global)	Enroll the high priority population: girls 9–14 years of age, before extending coverage to other groups or males. For individuals receiving the first dose before 15 years: 2-dose schedule with a 6-month interval between doses. If the interval between doses is shorter than 5 months, a third dose should be given at least 6 months after the first dose. There is no maximum interval (no more than 12–15 months is suggested). For individuals receiving the first dose ≥15 years: 3-dose schedule (0, 1–2, 6 months). The 3-dose schedule should be used for those younger than 15 years known to be immunocompromised and/or HIV-infected.		
PAHO/WHO TAG recommendation (Americas)	TAG reiterates the importance of prioritizing high coverage in girl cohorts aged 9–14 years to ensure full protection against HPV among girls and induce herd immunity among boy populations. Following the WHO recommendation, countries and territories should implement and monitor the two-dose strategy (with HPV2 or HPV4) with a six-month interval between doses for individuals receiving the first dose before age 15 years. Intervals no greater than 12–15 months are suggested. Three-dose schedules are only recommended for individuals that initiate vaccination at >age 15 years, or those of any age who are immunocompromised and/or HIV-positive.		
Adjuvant	500 µg aluminum hydroxide & 50 µg of 3-O-desacyl-4-monophosphoryl lipid A (AS04)	225 µg amorphous aluminum hydroxyphosphate sulfate (AAHS)	500 µg amorphous aluminum hydroxyphosphate sulfate (AAHS)
Substrate system with recombinant technology	Baculovirus expression system (*Trichoplusia ni cells*)	Yeast substrate (*Saccharomyces cerevisiae*)	Yeast substrate (*Saccharomyces cerevisiae*)
Intramuscular	X	X	X

Source: World Health Organization, 2017.

Bivalent HPV Vaccine

The bivalent vaccine, Cervarix®, includes two antigens: genotypes 16 and 18. The L1 purified proteins in both genotypes are absorbed onto aluminum hydroxide, with the addition of the AS04 adjuvant.[25,26] A special characteristic of this vaccine is the AS04 adjuvant, comprised of deacylated monophosphoryl lipid A (MPL), a non-toxic derivative from *Salmonella Minnesota* R595 lypopolysaccharide, which activates the humoral immune and cell-mediated response and induces the activation of antigen-presenting cells (APC).[27]

The Phase I study of this vaccine was conducted in 49 North American women aged 18–30 years. The results were favorable in terms of immunogenicity and safety.[28]

Phase II studies were conducted in a method similar to studies for the quadrivalent vaccine. The first study was a randomized, double-blind study in 61 women aged 18–30 years.[28] The experimental group received the bivalent vaccine and the control group only received aluminum hydroxide. The second study was also randomized, double-blind in 60 women aged 18–30 years to compare the safety and immunogenicity of the bivalent vaccine with two different adjuvants.[28] One group received the vaccine with AS04 while another group received the aluminum hydroxide vaccine and the third had no addition of adjuvant. In a third study, 209 women aged 18–30 years were randomized to study the effect of dosing.[28] The fourth randomized, double-blind, placebo-controlled study included women aged 15–25 years (560 participants received the vaccine and 553 participants received the placebo).[28,29]

Phase III studies demonstrated an efficacy of 98.1% (95% CI: 88.4–100) against CIN3 caused by HPV-16/18 based on a causality algorithm. In 2010, the bivalent vaccine was registered and recommended by ACIP.[30]

The vaccine is marketed in vials of one or two doses or in pre-filled syringes. It is administered intramuscularly. Each 0.5 mL dose has 20 µg HPV-16 L1 protein and 20 µg HPV-18 L1 protein absorbed onto 500 µg aluminum hydroxide, and 50 µg monophosphoryl lipid A (MPL). The vaccine is indicated for girls starting at 9 years of age for the prevention of premalignant cervical, vulvar, and vaginal genital lesions and type-specific cervical cancer, in a two-dose schedule at 0 and 5–13 months.[24,30] The immune response to the bivalent vaccine is measured through a type-specific enzyme-linked immunosorbent assay (ELISA) using a technology adapted by GSK.[31]

Vaccine efficacy was assessed through the PApilloma TRIal against Cancer In young Adults (PATRICIA) study in three cohorts of women aged 15–25 years. This randomized, double-blind, controlled trial intended to assess vaccine efficacy for type-specific CIN2+ against HPV-16 and 18 (Table 2). Mean follow-up for these cohorts was 34.9 months (SD: 6.4) after the third dose.[32]

Table 2. Results of the PATRICIA Study in Women aged 15–25 Years

Cohorts	ATP*	TVC**	TVC-Naive***
Vaccinated (n)	8,093	9,319	5,822
Controls (n)	8,069	9,325	5,819
Vaccine efficacy (%) **96.1% CI**	92.9 79.9–98.3	30.4 16.4–42.1	70.2 54.7–80.9

Source: Paavonen et al., 2009.[32]

Notes: *According-to-protocol analysis (primary analysis). **Total vaccinated cohort (TVC): included all women receiving at least one vaccine dose, regardless of their baseline HPV status; represents the general population, including those who are sexually active; therefore, it is representative of the general population. ***Total vaccinated cohort: no evidence of oncogenic HPV infection at baseline; represents women before sexual debut.

Additionally, cross-protection against CIN2+ associated with HPV-31, 33, and 45 was seen.

It is possible to extrapolate the efficacy results for both vaccines from studies performed in women over 15 years of age to girls 9 to 15 years of age through immunogenicity bridge studies, since performing efficacy studies in underage girls is unethical. Immunogenicity studies in girls have demonstrated a response in antibody titers at least two folds the levels seen in women over 15 years.

Quadrivalent HPV Vaccine

The quadrivalent vaccine has four genotypes: 16, 18, 6, and 11 — the first two being the main high-risk oncogenic viruses and the last two being the low-risk viruses. These VLPs are absorbed onto aluminum hydroxyphosphate.[33-36]

Phase I studies conducted in approximately 290 individuals established that 20 µg, 40 µg, and 50 µg doses generated a significant immune response as compared to 10 µg.[37] Phase II studies for vaccine administration in approximately 6,000 individuals across Europe, Australia, North America, and Latin America, established that the vaccine is safe, and immunogenic as compared to the placebo.[38,39] Subsequently, Phase III studies were conducted in 17,500 individuals in North America, Latin America, Asia, and Australia and established the efficacy and safety of the vaccines.

In 2006, the FDA authorized the first prophylactic HPV vaccine, Gardasil®, which contains the two major oncogenic genotypes, 16 and 18, accounting for about 60% of cervical intraepithelial lesions at risk of progressing to cancer and the two low-risk genotypes, 6 and 11, accounting for approximately 90% of genital warts (i.e., condyloma accuminata) as well as other pathologies such as recurrent respiratory papillomatosis.

The vaccine is marketed in single-dose vials or pre-filled syringes. It is administered intramuscularly and each dose contains 0.5 mL of 20 µg HPV-6 L1 protein, 40 µg of HPV-11 L1 protein, 40 µg of HPV-16 L1 protein, and 20 µg of HPV-18 L1 protein absorbed onto 225 µg of adjuvant. The vaccine is indicated for women and men as of 9 years of age for the prevention of premalignant genital lesions (cervical, vulvar, and vaginal), premalignant anal lesions, cervical cancer, anal cancer causally related to oncogenic HPV-16 and 18, and the prevention of condyloma accuminata.[40] The vaccine was registered with a three-dose administration schedule, but is currently being recommended for use with a two-dose schedule with a 6-month interval between doses.[24]

A specific type immunoassay (Luminex) was conducted to assess vaccine immunogenicity.[41] Two Phase III studies, referred to as Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) I and II, were conducted to assess efficacy with a mean follow-up of 42 months. The studies demonstrated high efficacy (Table 3): 100% (95% CI: 92.9–100.0) against cervical intraepithelial lesions type 2/3 or CIN2/3 caused by genotypes 16 and 18, in receptors uninfected by HPV. Clinical efficacy against vaginal and vulvar cervical infections and HPV-16 and 18 associated lesions was also demonstrated.[42,43] Intention-to-treat analysis (ITT) demonstrated efficacy significantly lower than 45.1% (95% CI: 29.8–57.3), which could be explained by the inclusion of HPV-infected women.[42]

Table 3. Results of the FUTURE I and II Studies in Women Aged 16–26 Years

Women Aged 16–26 Years	Follow-Up of 42 Months
Impact on Lesions	**Efficacy % (95% CI)**
Cervical intraepithelial lesion 2/3 caused by HPV-16/18	100.0 (93–100)
Vulvar or vaginal intraepithelial lesions 2/3 caused by HPV-16/18	100.0 (82.6–100)
Cervical intraepithelial lesions 1 caused by HPV- 6/11/16 or 18	96.0 (91–98.4)
Vulvar lesions I caused by HPV- 6/11/16 or 18	100.0 (74–100)
Vaginal lesions I caused by HPV-6/11/16 or 18	100.0 (64–100)
Vaginal warts caused by HPV-6 or 11	99.0 (96–100)

Source: Schiller et al., 2012.[43]

In 2007, the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommended the vaccine for women of 9 to 26 years of age[44], and the American Cancer Society recommended routine vaccination for women ranging between 9 and 18 years of age.[45]

Nonavalent HPV Vaccine

The nonavalent (9-valent) vaccine, Gardasil 9®, is now available and it adds five new HPV virus genotypes to the four already included in the quadrivalent vaccine. These genotypes are: 31, 33, 45, 52, and 58. An efficacy and immunogenicity study was conducted in women aged 16–26 years, by applying a series of three intramuscular injections on day 1, month 2 and month 6. In connection with antibody response, the demonstrated outcome is non-inferior to the one generated by the quadrivalent vaccine. Regarding efficacy, in an per-protocol analysis, the rate of high-grade cervical, vulvar, or vaginal disease associated with HPV- 31, 33, 45, 52, and 58 was 0.1 per 1,000 person/year in the 9-valent group and 1.6 per 1,000 person/year in the quadrivalent vaccine group, thus demonstrating a 96.7% efficacy (95% CI: 80.9% – 99.8%).[46]

Follow-Up of the Vaccinated Cohorts

The bivalent and quadrivalent vaccines were initially registered with a three-dose schedule and, later on, studies were conducted to assess the presence of neutralizing antibodies. For the bivalent vaccine, 100% of women remained seropositive at 8.4 years of follow-up. For the quadrivalent vaccine, seropositivity measured as IgG class antibodies was 94.3%, 89.4%, 99.5%, and 88.8% for HPV-6,11,16, and 18, respectively at 8 years of follow-up.[47] To date, 9.4–years of follow-up data of the bivalent vaccine have been reported.[48]

Two-Dose Versus Three-Dose Schedule

At the global level, there is interest in simplifying vaccination schedules to increase compliance and the advantages of vaccine adherence, including reduced logistical challenges of vaccinating in schools and lowering related costs and resources.

Studies conducted to demonstrate the non-inferiority of the immune response with a two-dose schedule as compared to a three-dose schedule are valid provided they are conducted concurrently, using the same protocol, in girls and women of the same age, enrolled and randomized to one of the two vaccination schedules.

Non-inferiority of a treatment group is understood as the lower bounds of the multiplicity adjusted 95% Confidence Intervals (CI) for the Geometric Mean Titers (GMT) ratio resulting (girls or women) greater than 0.5. The ratio is estimated for each alternative schedule and every specific genotype.

A study conducted in Vietnam[49] was intended to assess the non-inferiority of alternative vaccination schedules by comparison with the standard three-dose schedule using the quadrivalent vaccine in girls aged 11–13 years. The alternative schedules with the quadrivalent vaccine were administered at 0, 3, 9 month intervals; 0, 6, 12 month intervals; and 0, 12, 24 month intervals. Non-inferiority criteria were met with the first two schedules for the four vaccine genotypes; however, this criterion was not met for genotypes HPV-16 and HPV-6 a month upon conclusion of the schedule at 0, 12, and 24 month intervals. The cohort of girls was followed for 36 months to establish the duration of the antibodies based on these three different schedules. Results demonstrated that there was no inferiority in the response to the alternative schedules as compared to the standard schedule.[50]

In another study, a two-dose versus a three-dose schedule in girls aged 9 and 13 years was compared as well as the response to the two-dose schedule in girls and the three-dose schedule in women aged 16–26 years. The GMT were measured at 7, 18, 24, and 36 months after the last vaccine dose. The results established that the only differences observed in terms of inferiority were in girls that received the two-dose schedule versus the girls that received the three-dose schedule against genotype 18 as of the 18 month and against genotype 6 as of the 36 month. The antibodies response expressed as GMT was non-inferior in a two-dose schedule in girls as compared to a three-dose schedule in women.[51]

In May 2017, the WHO stated the current evidence supports the recommendation for a 2-dose schedule with adequate spacing between the first and second dose in those aged 9–14 years.[24]

Vaccine Impact

Vaccination impact data is derived from information on HPV epidemiology before and after vaccination and vaccine coverage (even with one or two vaccine doses higher than 50%). A recently published meta-analysis reports the following data: A) in girls aged 13–19 years, infections caused by HPV-16/18 have decreased by 64 % ($p = 0.01$); infections caused by HPV-31/33/45 have decreased by 28% ($p= 0.44$); infections caused by HPV-31/33/45/52/58 show basically no decrease ($p = 0.32$); B) in women aged 20–24 years, infections caused by HPV-16/18 have decreased by 31% ($p=0.00001$). [52]

Australia is the country with the most extensive HPV vaccination experience, since their National Immunization Program started administering the quadrivalent vaccine in girls and boys in 2007. Five years after vaccination, the condyloma acuminata in women under 21 years of age decreased from 11.7% in 2007 to 0.85% in 2011.[53] Another researcher in Australia measured HPV genoprevalence amongst women aged 18–24 years who attended family-planning centers. The 2005–2007 pre-introduction data was compared to the 2010–2011 post-introduction data. The number of infections caused by HPV-16/18/6/11 decreased from 28.7% to 6.7%, p<.001; infections caused by high-risk genotypes were reduced from 47.0% to 34.2%, p<.05.[54]

In the United States, prevalence of HPV-16 and 18 in CIN2/3 and adenocarcinoma in situ (CIN2+) in women has been compared via the epidemiological surveillance system through population-based sentinel centers from 2008 to 2012. The prevalence of CIN2+ lesions caused by HPV-16/18 decreased from 53.6% to 28.4% amongst women who had received at least one vaccine dose. This decrease, however, was observed in unvaccinated women (57.1% vs 52.5%). Estimation of vaccine efficacy in the prevention of CIN2+ was 21% (95% CI: 1–37); 49% (95% CI: 28–64) and 72% (95% CI: 45–86) in women who had initiated the schedule 25–36 months, 37–48 months, and more than 48 months before screening, respectively. [55]

These findings confirm the following:

1. An extended vaccination schedule administered at 0, 1 and 12 month intervals or at 0, 2 and 12 month intervals does not yield lower immunogenicity than a traditional schedule administering the last dose at 6 months. Moreover, higher GMT levels may be obtained with an extended schedule.
2. A two-dose schedule administered at 0, 2 months vs. 0, 6 months shows that the latter with a 6-month interval had higher Geometric Mean Concentrations (GMC) in girls aged 9–14 years.

Immune Response

The HPV infection caused by any genotype is quite common. Between 50% and 80% of women are expected to be infected at some point in their lives.[56] After infection, the first barrier the virus encounters is innate immunity — phagocytes, soluble proteins (such as cytokines and the epithelial barrier) — which clears the virus in almost 90% of infections. However, innate immunity does not demonstrate specific memory. The other defense mechanism, adaptive immunity, is activated by natural immunity, which is characterized by high-specificity and immune memory. Antibody response to L1 after vaccination affords protection against HPV infection via adaptive immunity. The antibody-mediated humoral immunity can prevent viral reinfections, while cell-mediated immune responses are key to clearing temporary infections. CD4(+) T-lymphocytes play a central role in humoral immunity and cell-mediated immunity. Seroconversion and generation of antibodies against the major virus proteins or the L1 protein occur simultaneously upon activation of cell-mediated immunity or shortly thereafter.[5]

The generation of the secondary antibody response to exposure as well as the preservation of antibody levels at all times are the main roles of memory B cells. High levels of memory B cells, for example, may represent a biomarker indicative of high levels of long-lasting serum antibodies.

Natural immune responses to HPV infection are weak due to HPV evasion mechanisms. The natural infection does not cause viremia or the elimination of cells thus resulting in a minimally inflammatory process.[57]

To date, no protection marker or antibody concentration indicative of protection has been established. [57]

In connection with the HPV-vaccine-induced immune adaptive response, the following has been described[58]:

1. The VLPs, with no viral genome, activate CD4(+) helper lymphocytes which go into a proliferation and differentiation state and interact with B cells. The activated CD4(+) lymphocyte cytokines contribute to maturation of B cells, which generate specific antibodies against the virus VLPs.
2. Virus-specific T-lymphocytes and memory B cells are generated for the VLPs.
3. On the next contact with the virus VLP or HPV, a T-cell dependent immune response is generated in a short period ranging between 24 and 48 hours.
4. The VLPs in HPV vaccines generate a significant immune response, with antibody titers 10 to 100 folds higher than the response induced by natural infection.[59]
5. Immune response in girls aged 9–14 years is higher than in women over 15 years. A significant difference has been shown to exist between receptors in girls and receptors in adult women, with a higher number of memory B cells in the former group, suggesting that at least for the purpose of inducing memory B cell creation, immunization of girls aged 9–13 years could be advantageous to maximize the response to HPV vaccines and to obtain higher efficacy.[59-61]
6. The bivalent vaccine which has an aluminum hydroxide-adjuvant with the addition of AS04 generates a higher antibody response than the quadrivalent vaccine.[62–65]
7. A "head-to-head" study comparing the immune response generated by the bivalent versus the quadrivalent vaccine against HPV-16 and 18 demonstrated that the bivalent vaccine generated 3.7 and 7.3 folds more neutralizing antibodies respectively in women aged 18– 26 years at 7 months after the introduction of the three-dose schedule. After 48 months of follow-up, the GMT remained 2.0 and 5.2 folds higher against HPV-16 and HPV-18, respectively. However, to date there is no clarity as to the clinical impact these differences may have, i.e., how it translates clinically into protection against infection.[63,66]

Adverse Events

The World Health Organization (WHO), through its Global Advisory Committee on Vaccine Safety, concluded in March 2014 that available HPV vaccines have an excellent safety profile.[67] The vaccine efficacy studies have included an assessment of potential short-term (assessments at 7 and 30 days after vaccination) and long-term (follow-up of 39 months) adverse events.[48,68] Local events at the HPV injection site, including pain and edema, occur more frequently and some systemic events, such as fatigue and headaches, are less frequent when compared to the control group.[69] However, no statistically significant differences have been shown in the occurrence of other adverse events as a result of HPV vaccination as compared to the control group.[68] Some reports have related the onset of some autoimmune diseases to vaccination; however, properly conducted population-based studies have ruled out such associations. In a study published in the British Medical Journal in 2013, no difference was observed in the number of autoimmune diseases, neurological changes or thromboembolic vein disease in 300,000 girls who received the HPV quadrivalent vaccine when compared to the control group.[70]

Vaccine safety and efficacy in individuals less than 9 years has not been established. As a precautionary measure, the vaccine is not recommended for administration in pregnant women.

Vaccine Coverage in Latin America

In July 2017, the Technical Advisory Group (TAG) on Vaccine-preventable Diseases of the Pan American Health Organization (PAHO) provided an update on the use of HPV vaccines in the Region of the Americas. As of June 2017, 29 countries and territories in the Americas have introduced the vaccine into their national immunization programs. Through routine immunization, an estimated 80% of the adolescent female cohort has access to the HPV vaccine. The worldwide administration of approximately 1.7 million HPV doses has been reported, yet there is a paucity of country-level vaccination coverage data, including in the Region.[71]

Per the 2017 TAG meeting report, "In 2016, only 14 of 29 countries and territories reported HPV vaccination coverage for the full recommended series in their national schedules, either two or three doses. Among these countries, the highest full-series coverage reported was 86% and the lowest 6%, with a median range of 47–55%. There is confusion regarding the selection of denominator populations for each dose in the series as well as additional challenges in making inter- or intra-country comparisons because of differing target populations."[71]

Conclusion

The role of HPV as a cause of cervical cancer and the risk attributable to this virus on other types of cancers such as oropharynx, penis, anal, vulvar, and vaginal cancers are undisputable. Cervical cancer is one of the main causes of death amongst women in every region of the world, with the highest impact in Africa. This complex virus has more than 190 genotypes, out of which 12 are high-risk based on their oncogenic potential. The epidemiology of the infection is also complex since it progresses to cervical cancer in only a small percentage of infected women and most infections are temporary.

Given that HPV-associated diseases are a public health priority, the development of vaccines against HPV has been long-awaited by clinicians, epidemiologists, civil society, and national and international public health authorities.

Currently, there are three vaccines available that differ in the number and type of genotypes they include. The bivalent vaccine includes two oncogenic genotypes, 16 and 18; the quadrivalent vaccine includes two low-risk genotypes, 6 and 11 and two high-risk genotypes, 16 and 18; and more recently the 9-valent vaccine has added five new genotypes to the ones included in the quadrivalent vaccine: HPV-31/33/45/52/58.

The recommended age of HPV vaccination in most immunization programs is in girls aged 9–13 years, since the immune response obtained in this age group is several folds higher than the response obtained in women over 15 years, which is potentially due to lack of exposure to the virus and higher memory B cell induction capacity in the former group.

Regarding dosing, scientific evidence has demonstrated that the antibody levels expressed as GMC in the two-dose schedule were non-inferior to the ones attained with the three-dose schedule in individuals less than 15 years. These findings have led to the recommendation for a two-dose schedule. Regarding the interval between the first and the second dose, scientific evidence has demonstrated that the response at 6 months and up to 12–15 months is higher in individuals less than 15 years as compared to schedules with a one or two month

interval. The three-dose schedule continues to be recommended for women aged 15 years and older, as well as immunocompromised or HIV-infected patients.

A number of developed countries have included the HPV vaccine in their routine immunization programs, and the effectiveness of the intervention has already been assessed. For instance, as the first country to adopt the vaccine, Australia has reported that between 2007 and 2011, prevalence of condyloma acuminatum has diminished from 11.7% to 0.85%. The United States has reported a reduction in the prevalence of CIN2+ caused by HPV-16 and 18 from 53.6% to 28.4%.

In late September 2015, the WHO reported that more than 65 countries had adopted the HPV vaccine into their immunization programs and more than 200 million doses had been distributed exhibiting a safety profile. In the Americas Region, the TAG reported that 29 countries had included the vaccine and approximately 80% of adolescent girls had access to it as of June 2017.

Since there is still a long way to go, countries need to implement epidemiological surveillance and permanent monitoring prior to the introduction of HPV vaccines into their immunization programs.

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Seasonal Influenza Vaccination

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Introduction

Influenza is an acute viral infection caused by an influenza virus typically occurring during colder winter months. Influenza viruses circulate worldwide and are classified into three seasonal types—A, B, and C. Influenza A and B viruses are further separated into subtypes (for A viruses) and lineages (for B viruses) on the basis of antigenic differences. Influenza A(H1N1) viruses, influenza A(H3N2) viruses, and influenza B viruses currently co-circulate globally. Only influenza A and B viruses are included in the annual "seasonal" influenza vaccines, because type C virus infections are much less common and only result in mild illness. Influenza viruses are transmitted primarily by droplets or respiratory secretions of infected persons.[1]

Influenza A and B viruses cause yearly seasonal influenza outbreaks and epidemics worldwide, with an annual global attack rate estimated at 5 – 10% in adults and 20 – 30% in children. Illnesses range from mild to severe and even death. Worldwide, these annual epidemics are estimated to result in about three to five million cases of severe illness, and about 250,000 to 500,000 deaths.[1] Children are efficient transmitters of influenza viruses, those younger than five years of age and particularly younger than two years have a high burden of respiratory illnesses associated with influenza. However, severe morbidity and mortality are more common among elderly people and in individuals with specific chronic medical conditions such as HIV/AIDS, asthma, and chronic heart or lung diseases. Secondary bacterial pneumonia is a frequent complication of influenza infection among these subpopulations. Influenza is associated with considerable economic burden arising from health-care costs, lost days of work or education, and general social disruption across all age groups.

Influenza A viruses may also cause worldwide pandemics characterized by rapid dissemination of new influenza A subtypes (or strains of subtypes) that have the capacity for human-to-human transmission and are sufficiently different antigenically from recently circulating influenza viruses to evade immunity in the population. Historically, major pandemics have occurred every 10 to 40 years. The most severe was the 1918 pandemic of the "Spanish flu", which caused an estimated 20–40 million or more deaths globally. Later, less severe pandemics occurred in 1957 ("Asian flu") and 1968 ("Hong Kong flu"). In 2009, the A(H1N1) pandemic later evolved into a seasonal pattern in 2010.[1, 2]

Vaccination is the primary means of preventing and reducing the burden of influenza illness. In 2003, the World Health Assembly resolved to increase the use of seasonal influenza vaccines to protect individuals at high risk for influenza and related complications.[3] In 2012, the World Health Organization (WHO) and its Strategic Advisory Group of Experts (SAGE) on immunization recommended that countries considering the initiation or expansion of programs for seasonal influenza vaccination should include pregnant women as the highest priority group.

The following high risk groups, in no particular order of priority, were also recommended for vaccination: children aged 6–59 months (especially 6–23-month-olds), the elderly, individuals with underlying health conditions such as HIV/AIDS, asthma, and chronic heart or lung diseases, and health care workers.[4] Vaccination is recommended for healthcare workers because they are at increased risk of exposure to influenza virus while the remaining groups are at particular risk of developing severe disease, i.e., disease resulting in hospitalization or death. Prevention of influenza among health workers is important because infected health workers tend to amplify transmission of influenza among care-seeking patients. Pregnant women are particularly vulnerable to respiratory illnesses compared to their non-pregnant counterparts, because pregnancy involves physiological changes in the cardiopulmonary and immunological systems. Influenza illness in pregnant women can result in fetal death, premature onset of labor, decreased birth weight, and intrauterine growth restriction (infants born small for gestational age).[4, 5]

Influenza activity is seasonal, peaking during periods that often coincide with the colder months (November–February and May–October) in the temperate regions of the northern and southern hemisphere respectively.[6, 7] Unlike in the temperate regions, influenza seasonality in the tropics is less distinct, with multiple, less pronounced peaks that frequently coincide with the rainy season. Some tropical countries even have year-round transmission. Influenza surveillance and pandemic preparedness has improved in many countries in the tropics and subtropics in recent years, which have allowed ascertaining transmission patterns in countries with less distinct seasonality.[6, 7]

Available Influenza Vaccines

The constantly evolving nature of influenza viruses requires continuous global monitoring and frequent reformulation of influenza vaccines. The WHO convenes technical consultations in February and September of each year to recommend viruses' strains for inclusion in seasonal influenza vaccines for the northern and southern hemispheres, respectively. These recommendations are based on information provided by the WHO Global Influenza Surveillance Network (GISN), now the WHO Global Influenza Surveillance and Response System (GISRS). Vaccine production takes about 6–7 months. The Northern hemisphere formulation is available by October whereas the Southern hemisphere formulation is available by April of the following year.[1, 8] The currently available vaccines are mostly trivalent, i.e. containing three influenza virus strains: an influenza A(H1N1) strain, an influenza A(H3N2) strain, and an influenza B strain. Nevertheless, quadrivalent vaccines including an additional strain of influenza B virus thus covering the two currently circulating lineages (Yamagata and Victoria lineages) are also available.[8]

Two types of influenza vaccine are available: an inactivated (killed) preparation administered as an injection (IIV) and an attenuated influenza virus vaccine normally delivered intranasally. There are three types of inactivated vaccines: split virus vaccines, subunit vaccines, and whole virus vaccines. In split virus vaccines, the virus has been disrupted by a detergent in order to reduce vaccine reactogenicity. In subunit vaccines, hemagglutinin and neuraminidase, the two glycoproteins of the influenza virus membrane have been further purified by removal of other viral components. Some formulations include adjuvants and most multidose vials contain the preservative thiomersal. Live, attenuated influenza vaccines have been based on a temperature-sensitive variant vaccine virus strains that replicate well in the nasopharynx but poorly in the lower respiratory tract.[8] Trivalent inactivated influenza virus vaccines (TIV) are available as standard or high dose vaccines for the elderly. Live attenuated virus vaccines (LAIV) are available for use in healthy individuals only.[1, 8, 9] Quadrivalent vaccines (QIV) became available in 2012 (as either IIV or LAIV vaccines).[1, 9] Table 1 summarizes the types of influenza vaccines that are available for use globally.

Table 1. Types of seasonal influenza vaccines available for use globally as of 2016

Vaccine Type	Dose	Route	Age Indications
INACTIVATED INFLUENZA VIRUS (IIV) VACCINES			
Trivalent, egg-based (adjuvanted or unadjuvanted)	Standard	Intramuscular	≥6 months
Trivalent, egg-based	High	Intramuscular	≥65 years
Trivalent, cell culture-based	Standard	Intramuscular	≥18 years
Trivalent, recombinant hemagglutinin influenza vaccine	Standard	Intramuscular	≥18 years
Quadrivalent, egg-based (unadjuvanted)	Standard	Intramuscular	≥6 months
Quadrivalent, cell culture-based (unadjuvanted)	Standard	Intramuscular	≥4 years
Quadrivalent, egg-based	Standard	Intradermal	18–64 years
LIVE-ATTENUATED INFLUENZA VIRUS (LAIV) VACCINES			
Quadrivalent since 2013-14 (previously trivalent)	Standard	Intranasal	2–49 years

Vaccine Contraindications

Inactivated influenza vaccines (IIV) should not be administered to the following individuals:

- Infants <6 months of age.
- People who have experienced a severe (life threatening) allergy to a prior dose of a seasonal influenza vaccine (IIV or LAIV).
- People who have a severe allergy to a component of the IIV vaccine. Health care providers should always consult the package inserts for vaccine components.

Recommendations for vaccinating patients who are allergic to eggs are available. A recombinant hemagglutinin influenza vaccine (RIV) is approved in individuals aged 18 years and older, and people in this age group who are allergic to eggs may receive RIV.

If RIV is not available or the recipient is not 18 years or older, most egg-allergic patients can safely receive IIV. Those who have only experienced hives as a reaction to egg may receive IIV, with some additional safety precautions. Individuals with a history of severe (life threatening) allergy to eating eggs may receive IIV if it is administered by a physician experienced in the recognition and management of severe allergic conditions.

Live-attenuated influenza virus vaccines (LAIV) should not be administered to the following individuals:

- Children <2 years of age.
- Adults ≥50 years of age.
- Pregnant women.
- Individuals with a history of severe allergic reaction to any component of the vaccine or to a previous dose of any influenza vaccine.
- Individuals with known or suspected immunodeficiency diseases or immunosuppressed states (including those caused by HIV) or asthma or certain chronic treatments such as long term aspirin therapy.

Cell Culture-based Inactivated Influenza Vaccine (ccIIV3) should not be administered to the following:

- Individuals who have had a severe allergic reaction to any component of the vaccine or after previous dose of any influenza vaccine.

Concurrent Administration of Influenza Vaccine with Other Vaccines

Inactivated vaccines do not interfere with the immune response to other inactivated vaccines or to live vaccines. Although inactivated or live vaccines can be administered simultaneously with LAIV, after administration of a live vaccine (such as LAIV), at least 4 weeks should pass before another live vaccine is administered.[9]

Vaccine Safety and Adverse Events Following Immunization

Influenza vaccines are among the safest vaccines available as demonstrated by the evidence accumulated over decades of administration of hundred millions of doses among people of all ages. Influenza vaccines are generally well tolerated.[2, 5, 9]

Inactivated Influenza Virus Vaccines

Studies support the safety of annual inactivated influenza virus vaccines (IIV) vaccination in children and adults.[10] IIV is administered as an injection and may cause pain, redness, and swelling at the injection site, and may also cause fever, malaise and myalgias, which are usually mild and go away on their own. IIV contains inactivated virus and cannot cause influenza. In one of the largest safety studies published to date, 251,600 children aged less than 18 years were screened at five managed care organizations during 1993-99 in the United States and results did not show evidence of important medically attended events associated with pediatric influenza vaccination.[11, 12] A recent study carried out among healthy adults ≥18 years of age showed that QIV, containing two B strains (one of each B lineage), was as safe and immunogenic as licensed TIV.[13] With regards to pregnant women, the WHO Global Advisory Committee on Vaccine Safety (GACVS) concluded in 2014, upon review of all safety data available globally that inactivated influenza vaccines were safe for use at any stage of pregnancy.[14] In the United States during 1990-2009, an estimated 11.8 million pregnant women received non-adjuvanted inactivated influenza vaccine and the national Vaccine Adverse Event Reporting System (VAERS) database received only 20 notifications of serious adverse events and 128 reports of non-serious adverse events following administration of trivalent IIV during that period. Multiple studies have not found new, unusual, or unexpected patterns of serious acute events, adverse pregnancy outcomes, or congenital anomalies confirming that IIV do not cause fetal harm when administered to pregnant women. Nevertheless, further active surveillance is warranted to continue expanding and solidifying the evidence base on the safety of vaccinating pregnant women.[13]

Pain and other injection site reactions are frequently reported after IIV vaccination in both children and adults. In IIV clinical trials, up to 65% of people vaccinated with IIV experienced pain at the injection site during the first week after vaccination which usually did not interfere with activity. Fever, malaise, myalgia, and other systemic symptoms can occur after vaccination with IIV, most often affecting individuals who have had no previous exposure to the influenza virus antigens in the vaccine (e.g., young children). In adults, the rate of these symptoms is similar after IIV and after a placebo injection. Vaccine components can on rare occasions cause allergic reactions (immediate hypersensitivity). Manifestations of immediate hypersensitivity range from mild urticaria (hives) and angioedema (swelling beneath the skin) to anaphylaxis. In some seasons, IIV has been associated with febrile seizures in young children, particularly when given together with 13-valent pneumococcal conjugate vaccine (PCV13) and diphtheria, tetanus and pertussis (DTaP) vaccines. Guillain–Barré Syndrome (GBS) following IIV occurs rarely. The cause of GBS, a serious neurological condition that can cause paralysis, is unknown, however, gastrointestinal and upper respiratory infections are known risk factors. Safety monitoring of seasonal IIV over the course of many years has not detected a clear link to GBS. However, if there is a risk of GBS from IIV, it would be no more than 1 or 2 cases per million people vaccinated. Each year, about 3,000 to 6,000 people in the United States develop GBS whether or not they received a vaccination —1 to 2 people per 100,000. Like other injections, IIV can also cause syncope (fainting).[2, 10, 14]

Trivalent IIV manufactured using cell culture technology, which are indicated for use in individuals 18 years of age and older, are administered as an injection and the most common (≥10%) local and systemic reactions in adults 18-64 years of age have been injection site pain, injection site erythema, headache, fatigue, muscle pain and malaise.[14]

RIV does not contain any egg protein and is approved for use in individuals 18 years of age and older. RIV is administered as an injection and similarly to the remaining IIV, may cause pain, redness, and swelling at the injection site, and may also cause fever, malaise and myalgia which are usually mild and self-limited.

Table 2. Summary of Mild and Severe Adverse Events After Administration of Inactivated Influenza Vaccine, WHO 2012

Nature of Adverse Event	Description	Rate/Doses
Mild	Local reactions:	
	Injection site reactions	10-64 per 100
	Generalized reactions:	
	Fever in children 1-5 years old	12 per 100
	Fever in children 6-15 years old	5 per 100
Severe	Anaphylaxis	0.7 per 10^6
	Guillain-Barré	1-2 per 10^6
	Oculo-respiratory syndrome (events of moderate severity)	76 per 10^6

Source: http://www.who.int/vaccine_safety/initiative/tools/vaccinfosheets/en/

Live-Attenuated Influenza Virus Vaccines

Trivalent live-attenuated influenza virus vaccines (LAIV) and closely related formulations have been well tolerated in adults, even among those with low levels of pre-vaccination antibodies. Nasal symptoms (runny nose, nasal congestion, or coryza) and sore throat were the most frequently identified adverse symptoms attributable to vaccination in conducted studies. LAIV contains attenuated viruses and cannot cause influenza. Trivalent LAIV have also been shown to be safe and well tolerated in children.[10, 14] For further information on safety and adverse events following immunization with LAIV, please visit: http://www.who.int/vaccine_safety/initiative/tools/vaccinfosheets/en/.

Vaccine Effectiveness and Impact

Seasonal influenza epidemics can be very heterogeneous due to a population's level of immunity and antigenic changes of influenza viruses. Epidemics may differ in their timing, incidence, and severity, as well as in the match between circulating influenza virus strains and the strains included in the vaccine. In addition to age, health status, and prior immunity to influenza viruses among other factors, this match between influenza vaccine strains and circulating strains will partly dictate how well a vaccine will work, i.e. what the vaccine effectiveness will be for that particular influenza season. Countries that use influenza vaccines annually have developed efficient and practical methods to gain insight into a season's vaccine performance. Such annual evaluations of influenza vaccine effectiveness aim to guide risk communication messages to the public and health professionals, reinforce the use of complementary public health measures, such as the administration of antivirals among high risk groups, and implement measures of social distancing in seasons of poor match between circulating viruses and vaccine strains. Such information is also crucial to maintain the investments in vaccination programs, and to orient public health policies. The most popular design used to systematically measure vaccine effectiveness is the test-negative design, which compares the rates of vaccination in a group of patients that seek medical care for acute respiratory illness and that are tested for influenza virus infection. Data are collected from a network of outpatient clinics or sentinel hospitals on patients that have sought medical care for acute respiratory illness. Data on vaccination status and the laboratory findings are used to calculate an estimate of how well the seasonal vaccine prevented patients from suffering from influenza illness or its complications.[15-21] Since 2004, such studies have shown that during seasons when most circulating influenza viruses are similar to the viruses in the influenza vaccine, the vaccine can reduce the risk of illness caused by influenza virus infection by about 50-60% among the overall population.[2, 9, 22] A recent meta-analysis of 56 published test-negative design studies showed that influenza vaccines provided substantial protection against H1N1pdm09 (61%), H1N1 (pre-2009) (67%), and type B (54%), and reduced protection against H3N2 (33%).[23]

This monitoring of influenza vaccine effectiveness has evolved to provide timely interim effectiveness estimates that are reviewed at the bi-annual WHO vaccine strains selection consultations.[24] A recent systematic review has shown the concordance of interim and final estimates of influenza vaccine effectiveness.[25]

Monitoring influenza vaccine effectiveness over the years can also provide valuable data to revise vaccination policies in place if necessary. For example in June 2016, the U.S. Centers for Disease Control and Prevention (CDC)'s Advisory Committee on Immunization Practices (ACIP), a panel of immunization experts that advises the CDC, recommended against the use of LAIV for the 2016-2017 influenza season. This decision was based on a thorough review of vaccine effectiveness data generated by the U.S. Influenza Vaccine Effectiveness Network. The data showed poor or relatively lower effectiveness of LAIV from 2013 through 2016. Other (non-CDC)

studies supported the conclusion that LAIV worked less well than IIV during the 2015-2016 season. Therefore, the LAIV vaccine is currently not recommended in the U.S.[9]

Among pregnant women, studies to date have shown that the effectiveness of seasonal inactivated influenza vaccination in preventing influenza infection in the vaccinated mother was moderate while the potential for maternal vaccination to protect infants through transplacental transfer of antibodies ranged from 41% to 91%.[26-29] In a randomized controlled trial conducted in Bangladesh, IIV reduced proven influenza illness by 63% in infants up to 6 months of age and averted approximately a third of all febrile respiratory illnesses in mothers and young infants.[26] A study in the United States conducted during 2000–2009 estimated the effectiveness of influenza vaccine given to mothers during pregnancy in preventing hospitalization among their infants at 91.5% (for infants aged less than six months).[30] This is particularly important for infants younger than 6 months old for whom seasonal influenza vaccines are not recommended.

Evaluating the overall impact of influenza vaccination is complicated due to the heterogeneity between seasons, the varying effectiveness of influenza vaccines, and the frequent lack of influenza surveillance data pre-vaccine introduction. Instead, health authorities typically need to combine data from multiple sources to estimate vaccine impact. Thus, influenza disease burden data are combined with recurrent influenza vaccine effectiveness estimates and vaccination coverage data to provide estimates of cases, hospitalizations, and deaths averted by vaccination.[21] For example, for the 2013–14 influenza season, using updated estimates of vaccination coverage, vaccine effectiveness, and influenza hospitalizations, the US CDC estimated that influenza vaccination prevented approximately 7.2 million illnesses, 3.1 million medically attended illnesses, and 90,000 hospitalizations associated with influenza.[30] Similar to prior seasons, fewer than half of persons aged ≥6 months were estimated to have been vaccinated. If influenza vaccination levels had reached 70%, an estimated additional 5.9 million illnesses, 2.3 million medically attended illnesses, and 42,000 hospitalizations associated with influenza might have been averted.[31]

Vaccination Timing and Strategies

The influenza vaccines currently in use globally need to be administered every year due to the frequent updates in the vaccine strains, but also due to their short duration of protection.[2] Thus, every year, influenza vaccination activities are organized shortly before the influenza season and typically start with an intensive vaccination campaign. Optimally, vaccination should occur before the onset of influenza activity in the community taking into account the average two weeks that are necessary to mount an adequate immunological response.[32] Therefore, it is recommended that campaigns reach the highest possible coverage of the targeted populations prior to the peak influenza activity in a country.[6, 33] Sometimes vaccination campaigns may benefit from piggy-backing on broader vaccination campaigns. Such is the case of the vaccination week of the Americas for countries that vaccinate against influenza in April using the Southern hemisphere vaccine.[33] Vaccination should continue to be offered through the routine health services as long as influenza viruses are circulating and unexpired vaccine is available.

All individuals targeted for vaccination should receive one dose of vaccine except children aged six months through 8 years who have never received influenza vaccine before, they should receive two doses of vaccine at least 4 weeks apart to ensure their optimal protection.[4]

Determining the best timing of vaccination is easy in temperate regions where the period of the seasonal outbreaks is well defined. It is more difficult in tropical and subtropical regions, where peaks of influenza activity are less marked. In an attempt to simplify operational guidance to countries regarding when to vaccinate and which formulation to use, a recent global review of the available evidence proposed geographical groupings of countries into vaccination zones with similar recommendations for vaccine timing and formulation.[7, 34] The optimal timing for the annual seasonal influenza vaccination campaign based on the start of the main influenza activity period could be identified for most countries in the tropics and subtropics. Once the local seasonality is defined, countries should always use the formulation that corresponds to the most recent WHO influenza virus vaccine recommendation in order to maximize its efficacy, independent of the geographic location of the country. Countries where influenza virus circulate year-round should consider strategies to increase vaccination coverage using the most appropriate formulation instead of conducting several interventions per year.[33]

Influenza vaccination is recommended at any stage of pregnancy to protect both the mother and infant. During the prenatal care period, every opportunity should be used to ensure the pregnant mother has been vaccinated.

Influenza Vaccination Promotion and Communication

Communication is critical to increase the acceptability and uptake of influenza vaccines. Messages should be adapted and tailored-made for the different audiences and local cultures. Among countries that focus on targeting high risk groups, boosting vaccination coverage will depend largely on effective communication strategies, the engagement of the scientific community and the proactive role of the healthcare personnel. Obtaining endorsements from professional societies, such as associations of obstetricians/gynecologists, infectious disease specialists, midwives, and national immunization technical advisory groups have proven to increase adherence to influenza vaccination.[35]

Conclusion

In conclusion, influenza vaccines are safe and effective in preventing disease and reducing economic burden. Current efforts to measure vaccine performance and impact, complemented by disease burden and economic studies may help health authorities sustain investments in influenza vaccines. In addition to preventing disease burden, comprehensive seasonal influenza vaccination activities (including anticipating vaccine procurement needs, targeting the array of high-risk groups, effective communication, and planning the technical and operational aspects in advance) constitute the best way to prepare for a future influenza pandemic.

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Prevention of Meningococcal Disease

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Introduction

Meningococcal disease (MD) is a major public health problem and remains a leading cause of meningitis and sepsis in several Latin American countries. Few diseases have as much power to cause panic among the population as MD, primarily because of its potential epidemic nature, the rapid onset of illness and its high case fatality rates (10% – 20%) and substantial morbidity. Up to 20% of survivors of meningococcal disease develop long-term sequelae, including deafness, neurological deficit, seizures, and/or limb amputation.[1,2]

Etiology and Pathogenesis

The causative agent of meningococcal disease (MD), *Neisseria meningitidis*, is a gram-negative, aerobic, encapsulated, non-mobile diplococcus, belonging to the *Neisseriaceae* family. The antigenic composition of the polysaccharide capsule enables the classification of *N. meningitidis* into 12 different serogroups: A, B, C, H, I, K, L, W, X, Y, Z and E.[3] Currently, serogroups A, B, C, Y, W and X are responsible for nearly all cases of disease reported worldwide, infecting only humans.[1-3] Meningococci are also classified into serotypes and serosubtypes according to the antigenic composition of the outer membrane proteins PorB and PorA, respectively. Meningococci have demonstrated the ability to exchange the genetic material that is responsible for producing the capsule, and thereby, to change the serogroup. Genetic multilocus sequence typing (MLST), polymerase chain reaction (PCR), and whole-genome sequencing (WGS) are currently the most specific methods to detect and characterize meningococcal strains.[4,5]

Meningococci are transmitted from person to person, by aerosolization of or contact with, respiratory secretions or saliva. Acquisition of meningococci can be transient, lead to colonization (carriage) or result in invasive disease. A majority of individuals will harbor *N. meningitidis* in the throat asymptomatically throughout their lives. Although meningococcal carriage is common in many or most human populations, invasive disease is a relatively rare outcome of meningococcal infection. For the majority of people, carriage is an immunizing process that results in protective antibodies.[6,7]

In non-epidemic settings, carriage studies performed around the world showed that approximately 5–10% of the population carries meningococci. Carriage rates were found to be very low in the first years of life, increasing in teenagers and young adults and then declining in adulthood.[6,7] Carriage rates of meningococci can

be considerably higher in outbreak situations, household contacts of people with the disease and in institutions, particularly in military personnel or other closed or semi-enclosed communities.[6,7]

When invasive disease develops, it usually occurs within 1–14 days of acquisition. In households where a case of meningococcal disease has occurred, the risk for invasive disease in family members is increased by a factor of 500–800.[8]

Epidemiology

MD affects individuals of all age groups, but the highest incidence is observed in children under 5 years and especially among infants. In some populations, incidence peaks can also be observed among two other age groups: adolescents and young adults, as well as adults aged 65 years of age and older. During outbreaks and epidemics, a shift in the age-distribution of MD is observed, with increased number of cases among adolescents and young adults. Most cases of meningococcal disease are sporadic with a larger number of cases during the winter.[2,3]

MD occurs all over the world, but there are marked geographical differences in incidence and the distribution of the different serogroups that cause disease. In North America, serogroups B, C and Y are the main serogroups causing MD, whereas in Africa, epidemic disease is most commonly associated with serogroup A, and more recently by serogroups C, W and X.[3,8-9] In European countries, serogroups B, W and Y are important causes of endemic invasive meningococcal disease (IMD), while serogroup C is still prevalent in countries without MenC vaccination programs.[3]

In Latin America, during the last decade, incidence rates of MD varied widely, from less than 0.1 cases per 100,000 in Mexico, Peru, Paraguay and Bolivia to 2 cases per 100,000 in Brazil, with the highest incidence generally observed in infants.[10] Regarding serogroup distribution, serogroups B and C are responsible for the majority of cases reported in the region. However, an increased number of serogroup W disease cases, associated with the ST-11 complex, was recently reported in Argentina and Chile.[10,11]

The availability and quality of published data for MD in Latin America are not uniform across the countries, with limited data available and exceedingly low rates of meningococcal disease reported by some countries. Brazil, Uruguay, Argentina and Chile are the countries with the highest burden of MD in Latin America, probably reflecting more robust surveillance systems and well-established laboratory infrastructure for MD.[10]

Clinical Manifestations

Invasive infections due to *N. meningitidis* result in a wide clinical spectrum characterized by one or more clinical syndromes, including: meningitis, bacteremia or sepsis, with meningitis being the most common clinical presentation. Pneumonia, pericarditis, myocarditis, conjunctivitis or arthritis are less common manifestations of *N. meningitidis* infection. Against this background, the term "meningococcal disease" is appropriate and has been adopted internationally. In less than 10% of the patients with MD, a self-limiting post-infectious inflammatory syndrome, most commonly characterized by fever, arthritis or vasculitis, can occur.[2]

Diagnosis

Diagnosis of invasive meningococcal disease is based on clinical presentation, as well as a variety of laboratory tests. The gold standard for the laboratory diagnosis of MD is the isolation of *N. meningitidis* by culture from a usually sterile body fluid, such as blood, cerebrospinal fluid (CSF), or, less commonly, synovial, pericardial or pleural fluid, and petechial or purpuric lesion scraping. A Gram stain of a petechial or purpuric scraping, CSF, and buffy coat smear of blood can be helpful. Latex agglutination tests utilize latex beads coated with antibodies to meningococcal capsular antigens in body fluids such as CSF, blood and urine. These kits can detect agglutination of five capsular groups: A, B, C, Y, and W.[2]

The polymerase chain reaction (PCR) is a rapid and sensitive test for diagnosing meningococcal infection. A major advantage of PCR over culture methods is that it allows for detection of *N. meningitidis* from clinical samples even when the organisms are nonviable after antimicrobial treatment.[2]

Treatment

Early diagnosis and initiation of antibiotic treatment, transfer to a hospital with an intensive care unit and aggressive management of shock are critical to reduce the case fatality rates associated with meningococcal disease. There are several acceptable antibiotic options for the treatment of MD, including penicillin G (250,000–400,000 U/kg/day divided q4–6 hours i.v.), or third generation cephalosporins like cefotaxime (200 mg/kg/day i.v.) or ceftriaxone (100 mg/kg/day i.v.). For patients with history of serious allergy to b-lactam antibiotics, chloramphenicol (75–100 mg/kg/day divided q6 hours i.v.) is the recommended antibiotic treatment.[2] Although isolates of *N. meningitidis* with relative resistance to penicillin (minimal inhibitory concentration of penicillin of 0.1– 1.0 mg/ml) have been reported in Latin American countries,[12] this degree of penicillin resistance (attributed to a genetic mutation that causes alteration in penicillin-binding protein 2) does not appear to impact response to therapy. Guidelines recommend 5–7 days of treatment. Optimal supportive care is critical. However, the use of steroids in children with shock caused by *N. meningitidis* is controversial since no pediatric studies have documented its benefit. Pediatric intensive care specialists may treat children with MD who have refractory shock and inadequate adrenal gland function with steroids.[13] There is available evidence to support the use of dexamethasone therapy given before antibiotics to reduce morbidity in children with Hib meningitis.[2] However, routine use of dexamethasone cannot be recommended for treatment of meningococcal meningitis based on current data. Adjuvant therapies have been used in children, but no beneficial effects on survival rates were observed.

Prevention

Chemoprophylaxis

Chemoprophylaxis should be offered to all household contacts of an index case of MD, people living and/or sleeping in the same household, childcare and nursery school contacts, and people who have been directly exposed to a patient's oral secretions through close contact, such as kissing or sharing of toothbrushes and others, during the 10 days before onset of symptoms of disease in the index case. Routine prophylaxis is not recommended for health care professionals, except in cases when mouth to mouth resuscitation, endotracheal intubation, or aspiration of secretions were made without respiratory precautions. To eradicate nasopharyngeal carriage of *N. meningitidis*, the index case should also receive chemoprophylaxis before hospital discharge, unless ceftriaxone or cefotaxime are the antimicrobial agents used for treatment of MD. Rifampin is the drug of choice for chemoprophylaxis of children and adults. Ceftriaxone given in a single intramuscular injection and ciprofloxacin in a single oral dose proved to be effective options to eradicate pharyngeal carriage of meningococci. Ciprofloxacin should only be used for people older than 18 years of age.[2]

Vaccines

Table 1. Meningococcal Vaccines Available Globally in 2016

Polysaccharide Vaccines	**Polysaccharide**	
Meningo A+C®	MenAC	
Mencevax®	MenACWY	
Menomune®	MenACWY	
Conjugate Vaccines		**Carrier Protein**
Menjugate®	MenC	CRM_{197}
Meningitec®	MenC	CRM_{197}
NeisVac-C®	MenC	TT
Menitorix®	MenC-Hib	TT
MenHibrix®	MenC-Y-Hib	TT
MenAfriVac®	MenA	TT
Menactra®	MenACYW	DT
Menveo®	MenACYW	CRM_{197}
Nimenrix®	MenACYW	TT
Protein Subunit Vaccines	**Antigenic Components**	
Bexsero®	fHbp, NadA, NHBA, and PorA Serosubtype P1.4	
Trumenba®	FHbp Subfamilies 1 and 2	

Source: Compiled by author.

Polysaccharide Vaccines

The polysaccharide vaccines currently available offer protection against serogroups A, C, W and Y. These vaccines, in common with other unconjugated polysaccharide vaccines, do not generate adequate immune response in children under 2 years of age, because of the lack of response to T-independent antigens at this age. Another characteristic of these vaccines is that, even in patients over 2 years of age, the protection offered is of limited duration; since they are unable to induce immune memory. Furthermore, they are capable of inducing hyporesponsiveness after subsequent doses. These features, combined with the fact that these vaccines have only transitory and incomplete effect in reducing the colonization and the transmission of the meningococci in the vaccinated population, have limited the use of polysaccharide vaccines.[14-17]

Polysaccharide Conjugate Vaccines

The conjugation of polysaccharides to protein carriers (non-toxic diphtheria mutant toxin [CRM_{197}] or tetanus toxoid) alters the nature of the antipolysaccharide response to include a T-dependent response. When B cells recognize the polysaccharide they process the conjugated carrier protein and present peptide epitopes to T-CD4+ cells. This antigenic complex induces the production of elevated antibody levels, including in young infants, higher antibody avidity and increases serum bactericidal activity. They also induce the formation of long-lasting memory B lymphocyte populations, providing an amnestic response (booster effect) on re-exposure. Furthermore, these vaccines have the capacity to prevent acquisition of nasopharyngeal colonization, reducing the number of carriers among those vaccinated and thus interrupting transmission of the pathogen within the population ("herd protection").[15-17]

Pharmaceutical companies initially developed, in the late 1990s, monovalent meningococcal conjugate vaccines against meningococcus C, containing one polysaccharide, conjugated to the mutant diphtheria toxin (MCC-CRM_{197}) or to the tetanus toxid (MCC-TT). These vaccines have proven to be immunogenic in infants, toddlers, older children, adolescents and adults. Later, it was also licensed as a combined *Haemophilus influenzae* type b (Hib)-MenC vaccine conjugated to the tetanus toxoid.[16-18] Randomized, controlled, Phase III trials, which assess the efficacy of a vaccine in a determined population, are not feasible due to low incidence of the serogroup C meningococcal disease. Thus, the serologic markers of immunity against infection by meningococcal C are used to infer the effectiveness of these vaccines and served as a basis for their licensing.[16] The correlate of protection accepted (i.e., the lowest antibody titer necessary to consider the vaccinated individual protected) is the presence of serum bactericidal antibody (SBA) $\geq$ 4 using human complement or SBA titers $\geq$ 8 when using complement obtained from baby rabbits.[16-17] During the pre- and post-licensure trials, good immunogenicity in the short-term and presence of immunologic memory associated with available conjugate vaccines were demonstrated, plus adequate tolerability and reactogenicity profiles.[14-17] In 1999, the vaccines were initially licensed in Europe with three doses for primary immunization of infants from 2 months of age. However, later immunogenicity trials showed that the scheme of primary immunization could be reduced to two or only one dose in this age group.[19]

Experience with Mass Immunization of the Population with Meningococcal Conjugate Vaccine

In 1999, the United Kingdom (U.K.) was first to introduce the MCC vaccine into routine childhood vaccination, vaccinating more than 15,000,000 individuals younger than 17 years in less than one year. The initial results were encouraging, with an 81% reduction of serogroup C incidence from the period of 1998–1999 compared to the period of 2000–2001. The one-dose effectiveness of the vaccine in reducing MD under routine field conditions was of up to 97% in adolescents and 92% in toddlers. Effectiveness was found to be 91% in infants who received three doses of the vaccine at ages 2, 3 and 4 months of age. The number of deaths attributed to serogroup C meningococcal disease dropped from 67 in 1999 to five in 2001.[18] There was a significant reduction in the incidence of meningococcal disease even in unvaccinated age groups, demonstrating that conjugate vaccines protect not only vaccinated individuals, but also the general population, most likely due to the reduction of the number of carriers of the bacteria in nasopharynx.[18-21] The success of the mass immunization program was attributed to both the high effectiveness of the vaccine (direct protection) and to the herd effect (indirect protection).

However, a few years after the introduction of the vaccine in the U.K., in 2004, a decline in effectiveness for all age groups was observed, especially in the group of infants vaccinated at 2, 3 and 4 months.[23] Between 2000 and 2003, 53 cases of MenC disease were registered in vaccinated children, and the investigation of these cases demonstrated no evidence of immunodeficiency. A similar phenomenon was observed in Spain, with a loss of protection in children that were vaccinated at 2, 4 and 6 months of age.[24-25]

Monitoring the incidence of disease caused by serogroup C, suggested waning efficacy of the vaccine after a few years, occurring mainly in children immunized in the first year of age, with two or three doses of the vaccine. As a result, the U.K. added a booster dose after 1 year of age, to ensure longer protection for infants immunized in the first year of life.[19]

In the U.K., a study on the effect of mass vaccination on rates of carriage, in 16,000 adolescents from 15 to 17 years, showed a 66% reduction in rates of meningococcal serogroup C nasopharyngeal carriage, compared to rates before the introduction of the meningococcal conjugate vaccines.[21] In this study, other serogroups' carriage rates among the vaccinated population remained relatively unchanged. One hypothetical concern is that after the dramatic reduction in the incidence of serogroup C MD in countries that adopted mass vaccination, other serogroups might "replace" the disease incidence gap left by serogroup C disease.[26] To date, surveillance data in the U.K. have not demonstrated a replacement effect.[26-27]

In 2002, the Netherlands started a routine immunization program with only one dose of the MCC vaccine conjugated to tetanus toxoid at 14 months of age. Additionally, a catchup campaign was introduced with the aim of immunizing all children and adolescents from 1 to 18 years with the same vaccine. The data from the Netherlands showed a rapid and dramatic reduction in the incidence of meningococcal disease both in vaccinated and unvaccinated age groups, with the greatest reduction (99%) verified in vaccinated age groups.[28] Other European countries obtained significant reductions in the incidence of serogroup C meningococcal disease after the introduction of the MCC vaccines in immunization programs.[29-32] These vaccines have also successfully controlled outbreaks of MenC disease. In Quebec, Canada, health authorities vaccinated all individuals from 2 months to 20 years of age with MCC vaccine. The vaccine effectiveness, verified more than one year after the outbreak, was greater than 96%, demonstrating again its potential use in controlling epidemics.[33]

Studies in the U.K. which assessed the persistence of protective antibody titers among children and adolescents vaccinated in different ages and schemes,[34] showed that only 25% of the children vaccinated between 2 months and 6 years old had protective antibody titers six to seven years after immunization. In contrast, children that had been vaccinated at older ages, between 6–15 years, maintained high rates of persistence of protective antibody titers. Four to five years after receiving the vaccine, 79% of the immunized children between 6–9 years and 88% of the immunized children between 10–15 years maintained rSBA $\geq$ 8.[35] These data confirm that the immune response provided by the MCC vaccines is age-dependent. Subjects vaccinated at older ages present more consistent and longer lasting responses. This recent evidence of rapid loss of protective antibody titers for children immunized in the first 6 years of life suggests that approximately 75% of these children are susceptible to the risk of carriage and to developing the disease when they enter adolescence.

The key to maintaining the success of the immunization program appears to be the prevention of carriage acquisition by maintaining high antibody levels in adolescents. Hence, several countries, including the U.K. and Canada, have introduced booster vaccinations in adolescents. The U.K. recently decided to replace their MenC adolescent booster with a MenACWY booster and to perform a catch-up campaign for students to prevent carriage and induce herd protection.[36]

In 2010, Brazil was the first Latin American country to introduce the MCC vaccine into the routine immunization schedule for infants, as a 2-dose schedule at 3 and 5 months of age, with a booster dose at 12 months of age. Toddlers between 12 and 23 months received one dose of the vaccine, with no catch-up campaign for older age groups. The introduction of MCC vaccine into the routine program in Brazil reduced incidence rates of disease in the age groups targeted for the vaccine. However, despite the dramatic decrease in the incidence rates of MD among the age groups that were vaccinated, no early impact was observed in other age groups, probably reflecting the lack of a catchup campaign in adolescents, usually the age group responsible for carriage and transmission.[11] Brazil is now considering the introduction of an adolescent dose of MCC to optimize the impact of the vaccination program. Currently, Brazil is the only country in Latin America that introduced the MCC vaccine routinely.

New Meningococcal Conjugate Vaccines

Currently, three quadrivalent (A, C, W and Y) meningococcal conjugate vaccines that use different protein carriers [tetanus toxoid (TT), diphtheria toxoid (DT) and non-toxic mutant diphtheria toxoid (CRM)] are licensed based on safety and immunogenicity data. In Latin America, the MenACWY-DT vaccine is licensed for children above 9 months of age, adolescents, and adults up to 55 years of age. The MenACWY-CRM_{197} vaccine is licensed for children above 2 months of age, adolescents and adults. The MenACWY-TT vaccine is licensed for children above 1 year of age, adolescents and adults.[37]

Effectiveness data regarding MenACWY vaccines are limited. In a study performed in the U.S., the effectiveness of MenACWY-DT against MenC and MenY disease in adolescents was approximately 80%–85% in the first year after immunization, with data suggesting decreasing effectiveness when evaluated after 3–5 years.[8]

In Chile, after the sustained increase in the number and proportion of serogroup W cases reported in 2012, the Ministry of Health decided to implement an immunization campaign in response, using two different quadrivalent conjugate vaccines (Men ACWY-DT and Men ACWY-CRM_{197}) aimed at children aged between 9 months and 5 years. In 2014, a vaccination program using the MenACWY-TT conjugate vaccine was included in the national immunization program for all children at 12 months of age.[11] The immunization campaign started

in October 2012 and rolled out nationwide during the first months of 2013. Coverage for the first dose of the vaccine was almost 100% for the targeted age group. A preliminary analysis of the data in Chile showed that after the Men ACWY immunization campaign, protection was observed only in the age groups targeted with the vaccine. There were no early indirect effects. The overall incidence rates of serogroup W MD in 2013, 2014 and 2015 were similar to 2012.[38] Consequently, new potential strategies, including immunization of young infants and a catch-up campaign targeting adolescents and young adults, are being discussed to optimize the impact of the vaccination program in Chile.

In the U.S., the Advisory Committee on Immunization Practices (ACIP) currently recommends the quadrivalent ACWY meningococcal conjugate vaccine to all adolescents from 11 to 12 years of age, with a booster dose after 5 years. Adolescents from 13 to 18 years, not previously vaccinated, should also be vaccinated.

In the U.S., vaccination with an age- and formulation-appropriate meningococcal conjugate vaccine is recommended for infants, children, adolescents and adults at increased risk of MD:[2]

- Individuals with persistent complement component deficiencies (C3, C5–C9, properdin, factor D, and factor H)
- Individuals with functional or anatomic asplenia (including sickle cell disease)
- Children above 2 years, adolescents and adults who have HIV, if another indication for immunization exists
- Individuals in communities with a meningococcal disease outbreak for which vaccination is recommended
- Individuals traveling to or residing in areas where meningococcal disease is hyperendemic or epidemic

Children who remain at risk should receive a booster dose of MenACWY conjugate vaccine three years after the primary series if they received their primary series before seven years of age, then every five years thereafter. If their primary series was given after seven years of age, then the booster dose should be given five years later and then every five years thereafter.[2] These vaccines are also recommended for use during epidemics or for controlling outbreaks.

In response to the continuing high levels of MenA disease in the meningitis belt in Africa, a MenA-TT conjugate vaccine was introduced through a mass immunization campaign targeting more than 150 million people of 1–29 years of age in African countries with the highest burden of disease. The incidence of MenA has dramatically decreased in the vaccinated countries and the vaccine has also had a profound impact in reducing carriage.[39-40]

Hib-MenCY-TT, a vaccine that contains meningococcal C and Y capsular polysaccharides conjugated to tetanus toxoid and *Haemophilus influenzae* type b capsular polysaccharide also conjugated to tetanus toxoid, was licensed by the U.S. Food and Drug Administration (FDA) in June 2012. Hib-MenCY-TT is approved by the FDA as a 4-dose series for children aged 6 weeks through 18 months and currently used only in U.S.

Vaccination with meningococcal conjugate vaccines is contraindicated among persons known to have a severe allergic reaction to any component of the vaccines, including diphtheria or tetanus toxoid. ACIP does not consider a history of Guillain-Barré syndrome (GBS) to be a contraindication or precaution for meningococcal vaccination. Pregnant women, if considered at risk of disease, can be vaccinated with meningococcal conjugate vaccines.[2,16] Premature infants may receive immunizations at the appropriate chronological age, according to the infant immunization schedule.

All meningococcal conjugate vaccines are inactivated vaccines, so they can be administered to persons who a re immunosuppressed as a result of disease or medications. However, response to the vaccine might be less than optimal.[2,16]

In general, the safety and reactogenicity profiles of meningococcal vaccines are adequate. The most commonly reported adverse events include pain, erythema and induration at the injection site, headache, fever and fatigue. In adolescents, syncope immediately after vaccination can occur. Anaphylactic reactions after vaccination are rare.[2,16]

Protein Subunit Vaccines

The capsular polysaccharide of meningococcus B has an antigenic structure (acetylneuraminic a-2-8-N acid) similar to that found in embryonic neural tissues. This peculiar characteristic, in addition to making it impossible for polysaccharide vaccines containing serogroup B to be immunogenic, also results in a risk of autoimmune reactions.[14,16] As a result, no polysaccharide conjugate vaccines developed for meningococcus B have been shown to be immunogenic and risk free. One attempt to overcome this problem was to develop vaccines that used non-capsular components of meningococcus B. Vaccines based on outer membrane proteins (OMV), developed in Cuba and Norway, were used successfully to control outbreaks. However, the immune response to these vaccines is specific to the serosubtypes of meningococcus B included in the vaccine. Protection was not provided to other meningococcus B serosubtypes not included in the vaccine.[14,16]

Recently, two protein subunit vaccines targeting MenB were licensed. The 4CMenB vaccine (*Bexsero*® by GSK) is composed of one variant of the factor H binding protein (FHbp), NadA, Neisseria heparin binding antigen (NHBA), and outer membrane vesicles that contain the New Zealand outbreak strain PorA serosubtype P1.4. The vaccine is licensed in the U.S. as a two dose schedule in adolescents and young adults, aged 10 to 25 years. This vaccine is also licensed in Europe, Australia, Canada, and some countries in South America beginning at two months of age.[41,42] For infants who start vaccination between two and five months of age, three doses are recommended, with the first dose administered at two months and with at least two months apart between doses. A booster dose may be administered at 12 months of age. For infants who start vaccination between six and 11 months, two doses of vaccine are recommended, with two months between them, with a booster after 12 months. For children who start vaccination between one and 10 years, two doses are recommended, with an interval of at least two months. Finally, for teenagers and adults up to 50 years of age, two doses are recommended, with at least a one month interval.

In adolescents and adults, the most common local and systemic adverse reactions observed after vaccination with 4CMenB were pain and erythema at the injection site, malaise and headache. In infants, injection site reactions, fever and irritability were frequently seen.[41]

The other protein subunit vaccine is rLP2086 (*Trumenba*® by Pfizer) which utilizes one variant of lipidated FHbp from each of the two FHbp subfamilies. The vaccine is currently licensed only in the U.S., either as a two (0 and 6 months) or three dose (0, 2 and 6 months) schedule in adolescents and young adults, aged 10 to 25 years.[41]

Both of these MenB vaccines induce SBA against selected MenB strains in adolescent populations. No robust effectiveness data for either vaccine are currently available. One study conducted in university students showed no effect of 4CMenB on MenB carriage, although 30% reductions in other groups of serogroups (CWY) were observed.[43] A Meningococcal Antigen Typing System (MATS) has been developed to predict the level of protection against a determined strain. Preliminary data for 4CMenB in Canada, US, several European countries and Brazil estimate coverage among MenB strains ranging from 66–91%.[44] For both vaccines, vaccine effectiveness against group B strains and non-B strains, as well as the duration of protection are still unknown.

In the U.S., persons aged ≥10 years who are at increased risk for meningococcal disease should receive MenB vaccine. Both MenB vaccines are approved for use in persons aged 10–25 years. However, ACIP supported routine use of MenB vaccines in persons aged ≥10 years who are at increased risk for serogroup B meningococcal disease, because there are no theoretical differences in safety for persons aged >25 years compared with those aged 10–25 years.[41] Persons at risk include those with persistent complement component deficiencies, persons with anatomic or functional asplenia, microbiologists routinely exposed to isolates of *N. meningitidis*, and persons identified as having an increased risk due to a serogroup B meningococcal disease outbreak.

The MenB vaccine series may also be administered to adolescents and young adults aged 16–23 years to provide short-term protection against most strains of serogroup B meningococcal disease. The preferred age for MenB vaccination is 16–18 years.

The U.K. was the first country to incorporate the MenB recombinant vaccine for routine immunization of infants, at a reduced schedule: two doses in the first year of life, at the age of two and four months with a booster dose at 12 months of age.[45]

Conclusions

Meningococcal disease is a major public health problem and remains a leading cause of meningitis and sepsis in countries around the world. Its high case fatality rate often causes public panic when outbreaks occur. MD affects persons of all ages, but the highest incidence is among children less than 5 years of age, especially among infants. In contrast to the rarity of MD, carriage of *N. meningitidis* in the human nasopharynx is frequent, especially among adolescents and young adults. These age groups proved to be crucial in the transmission of meningococci. Vaccination is considered the best strategy for disease prevention. The older polysaccharide unconjugated vaccines have several limitations, including the risk of hypo-responsiveness in repeated doses, the short duration of protection, and the lack of effect in preventing acquisition of carriage in vaccinated individuals. Therefore, these vaccines should be replaced with meningococcal conjugate vaccines when possible. Experience with Meningococcal C conjugate vaccines and more recently with Meningococcal A conjugate vaccine, has proven that these vaccines are safe, immunogenic and effective, inducing herd protection if used in immunization programs targeting those who are responsible for the highest rates of meningococci carriage. Experience with the use of quadrivalent conjugate vaccines is promising, yet there is limited evidence that they provide the same magnitude of indirect effects.

Available evidence suggests that the greatest impact of meningococcal conjugate vaccines occurs when introduced in the routine immunization program for infants, with a single expanded-age group vaccination campaign that includes adolescents and young adults, the age groups usually responsible for carriage and transmission. Booster doses in adolescence is crucial to maintaining protection among the population.

Finally, the recent licensure and availability of two multicomponent meningococcal B vaccines, containing surface exposed recombinant proteins, increases the possibility of broader protection against MD. Experiences with the implementation of these MenB vaccines in immunization programs in the coming years will be of paramount importance for a better understanding on the effectiveness against MenB as well as non-MenB disease, duration of protection and effect on carriage.

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Norovirus: The Disease and Vaccine Development

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Introduction

The viruses of the Norovirus genera belong to the *Caliciviridae* family, which also includes genetically-different viruses of similar structure, and composition from four other genera: Sapovirus, Lagovirus, Vesivirus, and Nebovirus. Norovirus, and sapovirus only infect human-beings, the others infect various animal species. The "Norwalk Virus" was the first virus to be identified from this family, in 1972, by immune electron microscopy in stools derived from an outbreak of gastroenteritis in Norwalk, Ohio, in 1968.[1] This outbreak affected a significant number of students and professors who experienced vomiting and acute diarrhea. Since then, researchers from different regions of the world have identified several viruses of comparable structure which cause similar outbreaks and were originally assigned nomenclature related to the location of the occurrence (Virus Snow Mountain, South Hampton, Mexico, etc.).[2] Years later these viruses were reclassified and grouped within the Norovirus genera. Currently, noroviruses cause the greatest number of human infections due to *Caliciviridae* followed by sapoviruses with prevalence rates only moderately lower to norovirus in some recent studies.[3,4]

Noroviruses are relatively small with a diameter of ~40nm and consist of a simple structure, a major capsid (VP1) protein and a capsid minor (VP2) protein where the single-stranded RNA is located. The virus has no lipid envelope.[5]

There is significant genetic diversity among noroviruses given specific mutations and genetic recombination.[2,6] Based on major and minor differences in the genetic sequences, seven genogroups (GI to GVII) are described with human viruses in the GI, GII, GIV genogroups. Within each genogroup there are minor sequence variations that determine the genotypes. The Norwalk virus, for example, is a GI.1 virus (serotype 1, belonging to the genogroup I); the viruses most frequently detected at present belong to genogroup II genotype 4 (GII.4). Moreover, there can be minimal variations within each genotype leading to "variants" of relevance as mentioned below.[2]

The potential relevance of this genetic diversity lies in its role with regard to certain viral virulence variability and/or antigenicity that may impact its susceptibility to infection. Most of the human infections have been caused by noroviruses belonging to serotype GII.4, possibly associated with higher hospitalization rates and death, as compared to other serotypes.[7,8] This epidemiological behavior may be due to the fact that when a genetic variation leads to a significantly relevant antigenic variation, the "new" virus avoids the existing herd immunity at a specific point in time and there is an increase of new cases, including more severe cases. For example, two GII.4 variants caused gastroenteritis outbreaks in Australia and New Zealand between 2005 and 2006 and one of the strains was associated with approximately 25% of the outbreaks reported in the United Kingdom in that period.[9] In 2012, the prevalent strain in the United States changed from the GII.4 "New Orleans" strain variant

to the "Sydney" variant.[10] In 2014, the GII.17 serotype emerged in Japan, spreading to the rest of the world.[11] In addition to the increase of cases in the population, genetic/antigenic variability may have individual relevance in connection with the likelihood for reinfection (with a circulating strain which is sufficiently different from an earlier infectious strain) and, thus, have impact on vaccine protection as will be addressed below. Recently, the concept of "static" versus "evolving" norovirus genotypes has been postulated, where GII.4 strains represent the genotype with the highest number of variants leading to periodic variant replacement (the predominantly "evolving" genotype).[12]

Sapoviruses are also divided into genogroups: GI to GV. To date, all of the genogroups, except for GIII, have been causes of human disease.[13]

Epidemiological Relevance and Disease Impact

In the 70's, the first nosological characterization associated with this virus family was as a "winter vomiting disease". It referred to outbreaks of various significance (from a few household cases to hundreds of cases in schools and communities) characterized by abrupt onset of vomiting, followed by watery diarrhea, with low-grade fever or no fever, lasting a few days. The most common infectious source was contaminated water or food. We currently know that noroviruses infect persons of all ages, from young infants to older adults. Most individuals, if not everyone globally, are infected at least once and typically several times throughout their lives. Most of the episodes of infection were moderate or asymptomatic, and even though only a fraction would develop moderate-to-severe clinical symptoms, with dehydration risk, the high frequency of the infection meant that this fraction is epidemiologically significant.[2,14,15] As seen later on, studies in cohorts of children are indicative of the low possibility of suffering from a second symptomatic infection from the same serogroup as the previous infection (this observation is not conclusive), creating the possibility of "natural" protection.

Acute norovirus-induced diarrhea is characterized by 4 to 8 instances of watery or semi-formed, non-bloody bowel movements. Acute onset vomiting before diarrhea is frequent, even as the only relevant symptom of the infection. Adults frequently report generalized myalgia, decreased energy and intense headaches. Fever is reported in almost half of the cases as mild, moderate or high (only in a low number of cases).[2,14,16]

Children <12 months, persons with some level of compromised immune system or who acquire the infection while hospitalized, and older adults comprise the highest risk groups for severe norovirus disease.[2,16-18] Post-infection sequelae include dyspepsia, constipation and/or gastroesophageal reflux.[19] In infants, seizures associated with the infection as well as encephalitis (less frequently) have been described.[20-22]

Noroviruses are associated with two typical clinical/epidemiological situations: Outbreaks caused by the consumption of contaminated water and/or food and acute endemic gastroenteritis in children.

Outbreaks have been described in several locations, including cruise liners and warships, schools, restaurants, nursing homes, summer camps, resorts, and communities, among many others where a contaminated product may infect several persons, and in a few hours may infect other persons through fecal-oral or oral-oral transmission.[2] Despite bivalve mollusks being specifically implicated in many of these outbreaks, vegetable and animal products, as well as water may be sources of contamination and induce outbreaks. Based on epidemiological studies conducted in countries with good surveillance systems, norovirus has been established

as the cause in approximately 50% or more of outbreaks due to water and/or food consumption in the middle-to-high income stratum.[23,24] A recent estimate concluded that in 2010 norovirus caused approximately 125 million cases of gastroenteritis associated with water and/or food consumption and resulted in 35,000 associated deaths.[25] In a norovirus outbreak, the individuals with greater exposure to the contaminated product, adults mostly but not exclusively, are the most affected. Outbreaks at hospitals and nursing homes are particularly harmful since the risk of suffering severe consequences (dehydration, hydroelectrolytic imbalance, and death) is higher given the vulnerability of the population.[26-28] In immunocompromised patients, there is significantly prolonged excretion of noroviruses in bowel movements, likely to last years, and occasionally difficult-to-manage cases of severe and/or prolonged diarrhea.[29] Among individuals with solid organ transplants, norovirus infection can cause significant morbidity due to prolonged diarrhea.[30,31]

In children <5 years of age who live in middle and upper-middle income countries, norovirus is currently the second most common cause of endemic acute gastroenteritis after rotavirus. In high-income countries that have implemented systemic rotavirus vaccination, such as the United States and Finland, norovirus has become the most common cause of hospitalizations and medical consultations due to acute diarrhea in children.[32,33] In low-income countries, such as Nicaragua, norovirus seems to be equally relevant, in particular after the introduction of the rotavirus vaccine.[4] In heavily-deprived countries in Africa and Asia, the Global Enteric Multicenter Study (GEMS) case-control study identified rotavirus, enterotoxigenic *Escherichia coli*, *Shigella spp,* and *Cryptosporidium* as the microorganisms most commonly associated with moderate-to-severe diarrhea,[34] and norovirus was relevant in only a few sites. The methodology of the GEMS study could have an impact on the outcome and findings of other studies conducted in similar regions that have previously suggested norovirus is a relevant pathogen.[35,36]

In a recent review of studies intended to measure the impact of norovirus infection in Latin America, it was concluded that 14–16% of acute gastroenteritis episodes in children (requiring hospitalization and/or consultation at a walk-in clinic) were caused by norovirus.[37] In other words, norovirus is associated with one out of six episodes of acute diarrhea in Latin American children.

In spite of the fact that norovirus-induced gastroenteritis is typically less severe than rotavirus, clinical manifestations of both may be undifferentiated, given that both lead to dehydration due to intense vomiting and frequent watery diarrhea.[14]

Prevention

The possibility of suffering from a norovirus infection emerges shortly after birth. Cohort studies demonstrate multiple exposures throughout the life span.[15,38,39] The mode of transmission is mainly fecal-oral, in particular for endemic gastroenteritis when a symptomatic person, or quite frequently, an asymptomatic excretor transmits the infection to a susceptible individual. Oral-oral transmission for individuals suffering from vomiting is also viable.[40,41] Contaminated food and water are the primary sources in outbreaks, followed by person-to-person transmission. The spread of the infection is facilitated by its low-infecting dose (between 20 and 1,000 viral particles to infect a person), prolonged excretion in stools (up to 2–4 weeks) and relative stability in the environment, food and water, as compared to other viruses.[2,42]

The possibility of vaccine prevention has long been considered a remote possibility for several reasons:

- Genotype diversity, which may or may not have cross-immunity.
- Repeated infections throughout life, which supports the concept that the virus may not develop long-lasting protective immunity.
- Studies conducted in adult volunteers in the 1970's–1990's, which concluded that immunity obtained through exposure to the viral inoculum (against similar viruses only) could be short lived (6–14 weeks).[43-45]

However, more recent studies are suggestive of the following:

- Immunity after natural infection may last several years.[46]
- In children, reinfection is frequent but symptomatic reinfection is not that frequent and exposure to a virus from a genogroup may confer a high level of protection against re-exposure to an intragroup virus.[15]

Moreover, it should be noted that, as is the case with other enteric microorganisms, some norovirus genogroups bind to the A, B, H, and Lewis antigens (of the histo-blood group oligosaccharides) on the intestinal surface and there are individuals who lack the above mentioned antigens (known as non-secreting antigens) and are thus naturally immune to the infection. Receptor binding is seemingly common intragenogroup and intergenogroup for the different "variants", since recent studies indicate that there would be cross-immunity among the variants when studying antibodies that block molecular binding of the viruses.[47,48] This finding is indicative of the possibility of cross immunity for viral binding across the intragenogroup and intergenogroup variants.

In 2011, a proof-of-concept study was designed to maximize the likelihood of developing effective vaccines with a GI.1 genotype intranasal prototype that conferred protection to adult volunteers exposed experimentally to the GI.1 virus.[49]

Vaccine Development: Where Do We Stand?

To date noroviruses are uncultivable, i.e. no system has been found to reproduce the virus, yet this situation may change in the near future as two culture systems for human noroviruses have shown promising results.[50,51] Uncultivability has hindered the development of live attenuated vaccines, such as the rotavirus vaccine. Currently available candidate vaccines are described in the table below (Table 1).

Table 1. Norovirus Candidate Vaccines

Antigens Included in the Vaccine	Current Clinical or Pre-Clinical Phase	References
VLPs GI.1, and GII.4	Phase I study in adults completed, progressed to Phase II studies in adults and children.	49,60,61
VLPs GI.3, and GII.4 plus rotavirus rVP6	Immunogenic in the murine BALB/c model; clinical studies in human-beings have not been started.	62
P Particle	Immunogenic in the murine BALB/c and gnotobiotic model; clinical studies in human-beings have not been started.	53,63,64
Particles replicating in vital vector	Immunogenic in the murine BALB/c model; one phase I clinical study has been reported although data presentation is pending.	65,66,67

The development of antigens against norovirus has been based mainly on the use of molecular tools, in particular, the expression of viral proteins through genetic expression systems, such as insect cells susceptible to infection with a so-called baculovirus that may recombine with norovirus genes.[52] In addition, other eukaryotic cells have also been used as expression systems, including yeast. These systems allow for the addition of specific norovirus genes, in particular the viral capsid genes for vaccine purposes. These genes are translated into self-assembly proteins, spontaneously creating a large amount of empty capsids resembling the viral capsid of the native virus, without the viral RNA. Therefore, these are non-infectious particles denominated "virus like particles" (VLPs).

Particles corresponding to a portion of the capsid, the P particle from the VP1 protein P domain, the protruding part of the capsid most exposed to the environment, have also been synthesized.[53] These particles may be synthesized in bacterial cells such as *Escherichia coli*, and simplify large-scale production. In addition, these particles may recombine with other antigens (rotavirus, influenza, hepatitis E, HIV) and create a platform to confer further protection against more than one virus.[54,55]

A third strategy has been the inclusion of norovirus capsid genes into viral vectors to use these recombinant plasmid-type structures to create human immunity. To date experiments have been conducted with vesicular stomatitis virus,[56] Newcastle virus,[57] adenovirus,[58] and Venezuelan equine encephalitis,[59] to induce "in vivo" (i.e. in the person rather than in an expression system as described above) VLP synthesis and protect vaccinated individuals. Out of the three strategies presented: VLPs, P particle, and viral vectors, only the first one is part of a clinical study phase and, therefore, a viable vaccine may be envisioned within the next 5 years. The use of viral vectors will have to overcome a series of perceptions on safety before human testing can take place. A recent press release reported completion of a Phase I study with a non-replicating adenovirus recombinant containing norovirus P particle in adults, but data remains to be presented.

To date, the most advanced vaccine candidate tested in three clinical studies is based on VLPs (see Table 2). The first study, a proof-of-concept study, demonstrated that adult volunteers vaccinated intranasally with the monovalent GI.1 vaccine adjuvanted with monophosphoryl lipid A (MPL) in addition to chitosan, had a 47% (95% CI: 15–67%) reduction of gastroenteritis occurrence after experimental infection with the GI.1 virus in adult volunteers. Local and systemic adverse events were reported in approximately 70% of participants vaccinated, not differing between vaccine or placebo recepients.[49] The vaccine development strategy shifted from an intranasal to an intramuscular vaccine because the latter demonstrated more efficient antigen delivery and better antibody responses in adults at a lower dosage level and with fewer doses than intranasal formulations. Low intergenogroup cross-reactogenicity and high frequency of GII norovirus infection led to the formulation of a bivalent GI.1 and GII.4 vaccine. The first study concluded that the 50ug (MPL-adjuvanted) dose per antigen conferred optimal immune response with an acceptable level of mostly mild adverse events in the injection site.[60] The second study with the GI.1/GII.4 vaccine in doses of 50ug (MPL-adjuvanted) dose per antigen, administered to adult volunteers had a protective effect against an experimental GII.4 norovirus infection. The study was not optimal because the level of infection and disease attained in the volunteers in general was below the expected level; nevertheless, protection was demonstrated.[61]

Table 2. Main Outcome of Human Clinical Studies Using VLP-Based Vaccines

Candidate Vaccine	Main Outcome	Reference
Intranasal adjuvanted VLP GI.1	Pivotal two-dose placebo-controlled study in 77 adults exposed to GI.1 norovirus demonstrated a reduction of norovirus-induced gastroenteritis by 47%.	49
Intramuscular adjuvanted VLP GI.1, and GII.4	Two-dose placebo-controlled study with various concentrations to determine safety and immunogenicity demonstrated good tolerance at various concentrations and rapid antibody response after the first dose.	60
Intramuscular adjuvanted VLP GI.1, and GII.4	Two-dose placebo-controlled study in 98 adults exposed afterwards to GII.4 norovirus demonstrated a reduction of severe (0% vs 8.3%, p=0.054), moderate-to-severe (6% vs 18.8%, p=0.68), mild-to-severe (20% vs 37.5%, p=0.74) norovirus-induced gastroenteritis and a reduction of severity (Score Vesikari 4.5 vs 7.3, p=0.002).	61
Intramuscular adjuvanted VLP GI.1, and GII.4	Two formulations including 15 or 50 µg of GI.1 combined with 50 µg of GII.2 were evaluated in 442 healthy adults aged 18–49 years. Reactions were mainly mild to moderate in 64% and 73% of vaccinated children, respectively, compared to 8% placebo controls. Immune responses to vaccination peaked by days 7–10 and persisted through day 28. GI.1 responses were highest with the 50/50 formulation, but GII.4 responses were higher with the 15/50 formulation. Authors conclude that the 15/50 formulation displayed the best balance of tolerability and immunogenicity.	68

How Will Norovirus Vaccines Be Used?

The first generation of norovirus vaccines for intramuscular administration in infants and adults will likely be available toward 2020–2022 absent any major setback. The use of the vaccine will depend on several factors to be understood from Phase II and III studies, and later trials. The protective efficacy against prevalent serogroups, in particular from the genogroup GII, as well as the interserogroup and intraserogroup cross-immunity spectrum, will be key for vaccine acceptance. A protective vaccine against 70–80% or more of the circulating noroviruses affecting individuals will likely interest decision makers in the field of vaccination. Duration of immunity will be equally important for administration in adults and infants. For adults, revaccination could be required within a few years based on the robustness of protection, which could be accepted by specific groups such as travelers or military personnel. On the contrary, the use of the vaccine during the first months of life to protect children, similarly to rotavirus vaccination, should not require revaccination at a later time so as not to diminish enthusiasm surrounding its application. Contrary to rotavirus, this is a norovirus candidate vaccine with non-replicating antigens. Therefore, although not ideal, two or more doses are likely to be required to attain a good level of protective immunity. Age at vaccination will be an important topic to define in infants considering the need to provide protection before 12 months of age since the vaccination schedule is quite prolific at 12 months. As new injectable vaccines to decrease morbidity and mortality in children, such as the norovirus vaccine, enter the market, the likelihood of vaccination at additional ages in addition to the current schedule (3, 5, and 7 months, for example) should be considered. A future significant challenge will be the development of new-generation combination vaccines that allow for a decrease of challenges while maintaining immunogenicity of the various components included in the combination vaccines. Combination of recombinant norovirus and rotavirus antigens seems to be an interesting alternative as well as the use of the P particle as platforms for other antigens. Nevertheless, these strategies require several years of analysis.

Conclusion

During the past 40 years much has been learned about norovirus structure, animal and human infectious mechanisms, immune responses, epidemiology and molecular epidemiology. Protective immunity in children and adults, in light of significant circulating virus variability, remains partially understood at the moment. Nevertheless vaccine efforts are underway and candidates are currently based on synthesized outer capsid particles, "virus-like" (VLP) or the protruding P particle. Proof of concept of vaccine protection has been obtained for an intranasal VLP in adults. The most advanced candidate is a GI.1/GII.4 bivalent VLP candidate for intramuscular use which has proven to be immunogenic in adults. Future Phase II and III studies in adults and children are projected for the near future.

Conflict of Interest Statement

Dr. Miguel O'Ryan has received research funding to conduct epidemiological studies on norovirus from Takeda Vaccines.

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Pertussis and Vaccine Prevention

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Introduction

Pertussis is an acute respiratory disease caused by a Gram-negative coccobacillus, called *Bordetella pertussis*, which is difficult to develop in culture media (fastidious) and proper identification requires non culture techniques such as PCR or serology. The disease can be serious in children less than six months. Recent outbreaks of pertussis cases are a challenge in many countries using cellular and acellular vaccines, even those countries with high vaccination coverage. Different strategies and improved vaccines are required to attain adequate global control of the disease.

Pathogenesis and Immunity

The infection is transmitted person to person via respiratory secretions of sick individuals. If exposure is extensive and within a close range, 80 to 100% of susceptible individuals may get infected. Seventeen susceptible individuals will be infected per every case (reproduction rate).[1] The infectious period may last three or more weeks absent an antimicrobial treatment.

The bacteria spreads through respiratory droplets to the ciliated epithelium in the upper respiratory tract and adheres via adhesins such as filamentous haemagglutinin, fimbriae, and pertactin. The pertussis toxin, on the other hand, penetrates the epithelium and significantly modifies the non-specific immune response such as chemotaxis, the complement system, phagocytosis, and specific immunity, by suppressing B and T cells. The pathogenesis of the encephalopathy is not completely clear and may be secondary to hypoxia, microhemorrhages or direct action of the pertussis toxin.[1]

The immune response to the natural infection or the use of vaccines is not completely characterized and seems to involve a humoral immune response and a cellular response. There is no defined serological correlate of protection. The natural infection produces an immune cellular response with interferon gamma secretion but no interleukin 5 (IL5), a classic response pattern of the T helper type 1 cells (Th1), which generates a more prolonged, but undefined, immunity than the vaccine-generated immunity.[1,2]

The Disease

The spectrum of the infection may involve from an asymptomatic manifestation up to the classic manifestation characterized by three periods: catarrhal, paroxysmal, and convalescent. The incubation period is 7 to 10 days.

The catarrhal period is the most infectious but the disease is rarely diagnosed in this phase. It lasts 1 to 2 weeks and it is indistinguishable from any upper respiratory tract infection.

The paroxysmal period is the defining one for the disease and it is characterized by nonproductive cough fits, followed by inspiratory stridor and posttussive emesis. It does not produce fever unless a superinfection occurs; the patient appears normal between coughing episodes. Cough may last up to three months and it may be evoked later on by other respiratory infections for years.

Pertussis affects individuals of any age but its manifestation is more severe in children under one year of age. In particular, infants younger than 3 months, who develop complications such as apnea, pneumonia, seizures or encephalopathy, have a fatality rate of 1.3% during the first month of life.

In adolescents and adults the infection may be asymptomatic; however, it may manifest as a prolonged, nonspecific cough but also as more classic episodes of paroxysmal cough with emesis and posttussive stridor. Studies in these populations show that 25% of adolescents and 40% of adults over 60 suffer from complications[3] such as sleep disorders, rib fractures, urinary incontinence and cough-induced syncope. The disease is also associated with work absenteeism, since between 1% and 4% of adult cases require hospitalization.[3,4]

The clinical manifestation of the disease is not only modified by age but also by vaccination: cases in vaccinated individuals are milder and less infectious than cases in non-vaccinated individuals.[5] The infectiousness of the disease is reduced with the use of antimicrobials as macrolides, but the symptoms are not modified unless the therapy is started early on in the catarrhal period.

The immunity conferred by the natural infection is more prolonged than the one conferred by the vaccines. A study of home contacts in Germany showed symptomatic reinfections only 15 to 20 years after suffering from pertussis.[6]

Diagnosis

The World Health Organization (WHO) has defined a confirmed clinical case as a person with a cough lasting at a minimum of 14 days with at least one of the classic symptoms (paroxysms of coughing, posttussive vomiting, inspiratory whooping) and, in newborns, as respiratory syndrome with apneas.[7] It is considered a laboratory-confirmed case if there is a culture, polymerase chain reaction (PCR), or positive-paired serology. Some countries have adapted the definition and added the epidemiologic link to a laboratory-confirmed case.[8,9]

Ordinary laboratory tests are not helpful except in unvaccinated children who exhibit leukocytosis with marked lymphocytosis. Confirmation of the infection requires detection of DNA, the agent itself, or the immune response. Therefore, PCR is considered the gold standard because it has high sensitivity and specificity. It is very useful in the catarrhal and paroxysmal phases but *Bordetella* DNA becomes undetectable after the third week of

the disease. It is very useful in adolescents and adults but serology (Ig A) performs better in this population since the diagnostic suspicion occurs later on. The culture is very specific but slightly sensitive and rarely available for clinical use. Immunofluorescence is not an advisable test given its variable sensitivity and specificity.[8]

Global Epidemiology

Pertussis continues to be a public health problem, including in countries with high coverages of vaccination. It follows an endemic pattern with outbreaks every 2 to 5 years. When analyzing incidence figures and the ethereal distribution of cases it is important to consider that despite being a notifiable disease, there is very significant underreporting amongst school-aged children, adolescents, and adults who are not suspected even with the classic manifestation. Studies with active case searches suggest between 10 and 1,000 undiagnosed cases per confirmed case.[9,10] In Latin America underreporting of pertussis is significant. In 2012, WHO reported 23,489 cases in the Americas, yet the CDC confirmed 49,000 cases only in the United States.[11]

Over the last thirty years there has been a progressive increase of reporting, in particular amongst school-aged children in developed countries. Starting in 2011-2012, a significant reemergence of infection has been observed in Canada, the United States, the United Kingdom, Switzerland, Germany, Australia, and Japan in spite of high vaccine coverages, with a high rate of cases in adolescents and adults.[11] This epidemiologic shift has been related to the fact that the protection conferred by the acellular vaccines used by developed countries is shorter (5 - 7 years), and there is greater suspicion and confirmation of cases through improved laboratory tests. The emergence of mutant *B. pertussis strains*, against which the vaccine confers no protection, is controversial since a recent study shows the presence of a high rate of strains lacking pertactin. This would not impact the effectiveness of the vaccine.[12]

In developing countries where cellular vaccines are used, the outbreaks have been less evident and the reported cases affect children under one year of age.[11] Argentina, Chile, and Colombia have described outbreaks in infants and do not report cases in other age groups probably due to a lack of clinical suspicion rather than a lack of cases.[8]

The source of infection in infants younger than one year of age is identifiable in 31 to 70% of the cases, including the parents, usually the mother, but also older siblings and grandparents.[13,14] The role of health-care providers in the transmission of *B. pertussis* to patients has also been properly documented, given community outbreaks.[15]

Vaccines

In the era prior to the introduction of pertussis vaccines, practically all children became infected with *B. pertussis* and presented the classic manifestation of pertussis. Countries with high vaccination coverages have managed to decrease the incidence and mortality[1] associated with pertussis by more than 90 percent. However, pertussis is still currently a public health problem since the inter-epidemic period that is maintained between 2 and 5 years has not been extended, similarly to what happened prevaccination. This is mainly due to the fact that the immunity conferred by the vaccines has a limited duration which does not exceed 4 to 12 years.[16] Also, circulation of this agent has not been significantly reduced in spite of the use of vaccines given the limited effect on colonization and infection as suggested by experimental evidence in primates.[17]

There are two types of vaccines available: whole-cell and acellular vaccines. Whole-cell vaccines (wP), available since 1940, are manufactured based on the whole cells of the inactivated (killed) bacteria and have about 3,000 antigens. They are only used in children younger than 7 years of age given the higher frequency of adverse reactions at a later age. They are used in combination with the tetanus (T) and diphtheria (D) toxoids, hepatitis B (Hep B) surface antigen and *H. influenzae* B (Hib) conjugate.

The other group of vaccines comprises acellular vaccines (aP and ap based on the antigen content level) developed in the 70s due to the fear of association of the whole-cell vaccines with neurological disorders in children, many of which were disproved later on. As of 1981, acellular vaccines are marketed in Japan and, as of 1991, in the United States. These vaccines have had the endotoxin removed and they only have between 3 and 5 highly-purified antigens such as pertussis toxin, filamentous haemagglutinin, pertactin and fimbriae. They were associated with a lower frequency of local and general reactions. There are acellular vaccines for use in children and others for use in adolescents and adults with a lower antigen content that decrease adverse events.

Whole-cell vaccines and acellular vaccines have similar efficacy, inducing high levels of antibodies that inhibit adhesion to the respiratory epithelium and neutralize toxins; however, humoral immunity is insufficient since *B. pertussis* is not only an extracellular agent but it can also invade cells as pulmonary macrophages and stay there for months. Proper protection requires cell-mediated immunity. Whole-cell vaccines induce an immune response dependent on T helper type 1 cells (Th1) and, thus, memory immunity that generates longer protection. Therefore, the Pan American Health Organization (PAHO) recommends the use of this vaccine in infants. Acellular vaccines, in turn, induce Th2-type immunity with little memory.[18,19]

Children who only received acellular vaccines in their first year of life have a six-fold risk of suffering from pertussis during the school years or adolescence, as compared to those who received at least one dose of the whole-cell vaccine.[20] Recent information on the use of the tetanus, diphtheria, and acellular pertussis (Tdap) vaccine among students 11 to 19 years shows that its effectiveness would not exceed 65 to 70 percent.[21]

In Latin America whole-cell vaccines are used in the primary schedule at 2, 4 and 6 months, with boosters during the second year of life and also between 4 and 6 years. Only some countries use an additional dose of the acellular vaccine in adolescence.[8]

The impact of the vaccine depends on high coverage and is particularly critical for this infection, whose reproductive number is very high and similar to the measles number. Therefore, coverage rates of 95% are needed for proper control. In Latin America, pertussis vaccine coverage was maintained at close to 90% in the 2005-2013 period, but it decreased to 88% in 2014. This decrease follows a declining trend over the last four years, but with great variability based on the countries and their districts. In 2014, only 20 of 40 countries in Latin America had coverages ranging between 80 and 95% and three of them only reached 50 to 84%.[22]

Other Control Strategies

The main control measure is to maintain high vaccine coverages, ideally over 95% for the third DTP (diphtheria-tetanus-pertussis) dose in the first year. This is not easy to achieve in other age groups so the agent continues to circulate and generate outbreaks. Consequently, other strategies have been used such as the method known as cocooning. This strategy intends to protect the newborn and young infant through immunization with acellular vaccines for the mother, and other family members in close contact with the child. This approach

has been used in countries such as Australia, France, Germany, and Costa Rica. The impact of these measures is limited, costly and quite burdensome. In Chile, it was used in some regions where 91% of the mothers were vaccinated in the postpartum period but only 60% of the other family members received the vaccine. Together with other measures, disease fatality was eliminated amongst children in these regions while using cocooning.[23] Considering that the immune response in vaccinated individuals takes about two weeks, this strategy would maintain the child unprotected during the first 2 to 3 weeks of life.

Ideally, the vaccine should be administered during pregnancy. The Tdap vaccine is not registered for use in pregnant women but the strategy is quite promising, since it allows earlier protection for the child by avoiding infection in the mother and attaining passive protection of the child through the transfer of prenatal antibodies.

Additionally, recent data shows that mothers vaccinated during pregnancy have significantly higher levels of anti-pertussis antibodies in their breast milk[24] and such impact should be assessed. In a recent study conducted in the United States with the Tdap vaccine during pregnancy,[25] levels of antibodies were compared in newly postpartum women with or without the vaccine and a significant increase of antibodies was observed against diphtheria, tetanus, pertussis toxin, FHA, pertactin and fimbriae in the vaccinated group. Additionally, observational studies and case-control studies in England — one country that uses this approach — show that the efficacy to prevent pertussis in children under 2 months would reach 90%.[26,27] The best age for vaccination would be between 27 and 30 weeks of gestation. In the United States, the recommendation is to revaccinate the mother with every new pregnancy. The vaccine is properly tolerated and it is not associated with complications for the mother or the fetus.[28] There is no interference with either pediatric vaccines of the infant or impact on the growth or development of the child.[28-30] This approach is used in the United States, New Zealand, Belgium, Israel, and the United Kingdom, and in several Latin American countries including Argentina, Brazil, Chile, Colombia, Costa Rica, Mexico, Paraguay, Panamá and Uruguay. This strategy has proven to be the most cost-effective and it is currently recommended by the Global Pertussis Initiative.[31]

The acellular booster in adolescence is another strategy used in Argentina, Panama, Uruguay, and Chile.[11] Vaccination of health workers in contact with children is another option used in Argentina, Panama, and also in Chile during the last 2011–2012 outbreak. The duration of the protection of acellular vaccines is short but this strategy may be useful in outbreak situations. Finally, countries such as Canada, France, and the United States have opted for recommending universal vaccination of adults every 10 years with Tdap; however, such coverage would require special effort since observance of vaccination in adults is very low and the cost of this strategy is very high.

Conclusions

None of the strategies proposed will be able to adequately control pertussis, in particular with the progressive introduction of acellular vaccines that reduce the severity of the disease and mortality, but have minimal impact on colonization and transmission. Therefore, the development of improved vaccines should be a priority. Such vaccines must require less doses, must generate long-lasting and better quality immunity, and ideally be used from the newborn period onwards. Some of the vaccine candidates include products with a higher number of antigens,[32] as well as encapsulated vaccines with adjuvants that favor Th1 and Th17 response,[32-34] and also live attenuated vaccines administered nasally in the neonatal period. The latter vaccine candidates have successfully completed Phase I trials in humans.[32]

As we wait for improved vaccines to enter the market, the strategies recommended for Latin America are:

- To improve surveillance systems and use of PCR diagnostic confirmation of *B. pertussis* infection.
- To continue the use of cellular vaccines in countries currently following a whole-cell vaccination schedule while the duration of immunity provided by acellular vaccines is evaluated.
- To provide timely vaccination and maintain standardized DTP coverages above 95% throughout the region and the districts.
- To use the Tdap vaccine in pregnant women during the second and third trimesters.

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Efforts and Progress Towards Polio Elimination in the Americas and the World

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Introduction

Polio Disease

Poliomyelitis is a life-threatening communicable disease, resulting in Acute Flaccid Paralysis (AFP), difficulty breathing and sometimes death. It is caused by the poliomyelitis virus, an enterovirus from the Picornaviridae virus family, which is subdivided into three serotypes: 1, 2, and 3.[1]

Humans are the only reservoir of polio disease. The predominant transmission mode of this disease in developing countries is the fecal/oral route. The virus replicates in the intestines and is excreted in feces. From the gastrointestinal system, the virus enters the bloodstream, eventually finding its way to the central nervous system. One week after onset there is less virus in the throat, but virus continues to be excreted in the stool for several weeks. If sanitation conditions and personal hygiene are inadequate, others can be infected from improper hygiene or contaminated water and food. Intestinal immunity is important in order to reduce or eliminate polio virus replication and excretion and thus prevent transmission.

Polio disease can strike at any age, but it mainly affects children under five years old who have not been vaccinated. Up to 72% of all polio infections in children are asymptomatic, but such individuals still shed poliovirus in the stool which can be transmitted to others for weeks. Approximately 24% of polio infections in children consist of a minor, nonspecific illness without neurological manifestations, with complete recovery within a week. Less than 1% of polio infections in children result in flaccid paralysis. Paralytic symptoms generally begin 1 to 18 days after prodromal symptoms and progress for 2 to 3 days. The illness progresses to flaccid paralysis, usually asymmetrical, with diminished deep tendon reflexes. Patients do not experience sensory losses or changes in cognition. Most people with paralytic poliomyelitis never recover completely, having residual paralysis of varying severity for the rest of their lives. Weakness or paralysis still present 12 months after onset is usually permanent.

Poliovirus may be isolated from the stool, less likely from the pharynx, and only rarely from cerebrospinal fluid (CSF) or blood. After virus isolation, further tests need to be conducted using polymerase chain reaction (PCR) or genomic sequencing, to determine if the virus is wild type, vaccine-derived poliovirus (VDPV) or Sabin (see below).

Until 1988, the global burden of paralytic poliomyelitis was estimated to be over 350,000 cases per year, with wild poliovirus (WPV) transmission reported in more than 125 countries. Since 1988, sustained use of polio vaccines worldwide has led to a precipitous drop in the global incidence of poliomyelitis by over 99%. The number of countries with endemic polio dropped from 125 to just three in 2016, when only 37 cases were reported, as of 13 December 2016.

Polio Vaccines Available

To date, two types of polio vaccines are available on the international market: oral polio vaccine (OPV) and inactivated polio vaccine (IPV). Both have been used extensively worldwide for decades.

IPV, first developed and licensed in 1955, is an inactivated vaccine given by injection and is available only in trivalent form. IPV stimulates a good humoral response. Polioviruses can be transmitted through oral secretions, and IPV is as effective as OPV in blocking this type of transmission. However, on its own it does not induce the same level of intestinal immunity as OPV, which means that it does comparably less well in preventing the wild virus from being excreted in feces and spreading in the environment.

OPV is a live attenuated vaccine licensed in 1961 as a monovalent (mOPV) vaccine and was followed by a trivalent version (tOPV) licensed for use in 1963. In 2009, a bivalent formulation (bOPV) was developed as part of the global polio eradication efforts. Thanks to its elevated intestinal immunogenic characteristic and its ease of administration, OPV use made it possible to eradicate polio in the Americas and other regions. Those vaccinated with OPV excrete the vaccine virus in feces, spreading it into the environment, which can then immunize others who have not been vaccinated. However, though very infrequently, OPV can cause some undesirable events such as vaccine-associated paralytic polio (VAPP) and polio vaccine-derived disease.

Polio Vaccine-Derived Disease

Vaccines containing live attenuated viruses (OPV) are very effective against the wild virus, but on very rare occasions they can cause Acute Flaccid Paralysis (AFP) by means of two mechanisms: re-acquiring neurovirulence and mutation toward neurovirulence.

In the **re-acquiring neurovirulence mechanism**, live attenuated viruses in OPV can, through prolonged replication in immuno-compromised persons or in a community with low vaccination coverage, reacquire the neurovirulence and transmissibility characteristic of the wild poliovirus. These vaccine-derived viruses can cause cases or outbreaks of paralytic poliomyelitis. Vaccine-derived polioviruses are subdivided into three categories:

1. **Circulating Vaccine-Derived Poliovirus (cVDPV):** A cVDPV is associated with sustained person-to-person transmission and is circulating in the environment. First recognized in 2000 during an outbreak on the island of Hispaniola (Haiti and the Dominican Republic), recent experience indicates that low vaccination coverage is a major risk factor for cVDPV outbreaks. cVDPV outbreaks can be stopped with 2 to 3 rounds of high-quality, large-scale supplementary immunization activities.
2. **Immunodeficiency-associated vaccine-derived viruses (iVDPV):** excretion of the virus is prolonged in people with immune system disorders; excretion has been reported to persist in some cases for 10 years or more.
3. **Ambiguous vaccine-derived viruses (aVDPV):** clinical isolates from people with no known immunodeficiency or sewage isolates of unknown source.

The **mutation toward neurovirulence mechanism** causes vaccine-associated paralytic poliomyelitis (VAPP). VAPP is a rare adverse event following OPV. IPV does not contain live virus, so it cannot cause VAPP. The mechanism of VAPP is likely to be a mutation, or reversion, of the vaccine virus to a more neurotropic form. Reversion is believed to occur in almost all vaccine recipients, but it only rarely results in paralytic disease. The paralysis that results is identical to that caused by wild virus, and it is permanent. VAPP does not spread to other people, so there are no outbreaks associated with VAPP. There are an estimated 250 to 500 cases of VAPP per year worldwide; of which, nearly 40% are due to the type 2 component of tOPV.[2] In Latin America and the Caribbean, one study evaluated the period between 1992 and 2011 and identified 191 cases of VAPP. The results showed an overall estimated risk of VAPP in LAC of 1 case per 1.19 million newborns or 1 case per 7.68 million doses administered.[3] An early study evaluating data from 1989 to 1991 estimated a higher risk of VAPP, showing 1 case per 1.5 to 2.2 million doses of OPV administered.[4]

History of Efforts Toward Polio Eradication

In light of the dramatic success of the Pan American Health Organization's (PAHO) mass polio campaigns in Brazil, Cuba, and Mexico in the early 80s, in September 1985, PAHO Member States unanimously adopted a resolution at the XXXI Meeting of the PAHO Directing Council, establishing the goal to eradicate the indigenous transmission of wild polio in the Americas by 1990. To that end, a Regional Polio Vaccination Strategy was adopted in the Americas.[5, 6] The strategy consisted of four components[7]:

1. Achievement and maintenance of high immunization levels with OPV, from the smallest geopolitical unit to the national level,
2. Effective surveillance and accurate diagnosis of all cases of AFP among persons under 15 years of age,
3. Area-wide vaccination around all new cases, and
4. Operation "Mop-up": special house-to-house campaigns to vaccinate all children under 5 in high-risk areas.

The high vaccination coverage reached with tOPV managed to interrupt transmission of the wild poliovirus in the Americas. The polio strategies helped countries continue to strengthen their routine immunization programs overall.

The last case of polio caused by wild poliovirus in the Region was detected in 1991 in Peru. In 1994, the International Commission for the Certification of the Eradication of Polio reviewed the data available in each country and territory and concluded that indigenous circulation of the wild virus had been interrupted in the continent, making the Americas the first region in the world to achieve this target.

Following polio control in the Region of the Americas, the 41st World Health Assembly (WHA) adopted in 1988 the resolution on global polio eradication that marked the commitment to eradicate polio from the world by the year 2000, and the creation of the Global Polio Eradication Initiative (GPEI) spearheaded by the World Health Organization (WHO), UNICEF, the Centers for Disease Control and Prevention (CDC), and Rotary International.

In the following years, three more regions received the certification of polio eradication: the Region of the Western Pacific in 2000; the Region of Europe in June 2002; and the Region of Southeastern Asia (including India) recently, in March 2014. Two Regions (Eastern Mediterranean and Africa) still have yet to be certified.

By 2011, all Regions of the world except for the Americas had suffered the reintroduction of poliovirus. The Independent Monitoring Board (IMB) of the GPEI stated in a report in October 2011 that the world was not on track to interrupt poliovirus transmission, and expressed concern about the real threat for failure of the GPEI, which would have had disastrous consequences, both in terms of lives lost and disabilities caused, and also as the most expensive public health failure in history.[8, 9, 10] For this reason, in May 2012, the 65th World Health Assembly adopted a landmark resolution declaring the completion of poliovirus eradication a "programmatic emergency for global public health" and requested that the WHO develop a comprehensive strategic plan for polio eradication. In response, the WHO Executive Committee approved the Polio Eradication and Endgame Strategic Plan 2013–2018 (the Endgame Plan), which provides a detailed approach and concrete timeline for complete polio eradication (see next section).

Also in 2012, WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recommended suspending use of the type 2 component of tOPV in all national vaccination programs and switch to bivalent OPV (bOPV), which includes only type 1 and 3. SAGE recommended the switch because WPV type 2 had not been detected since 1999, and the continued use of tOPV in areas where coverage is inadequate was contributing to the emergence of cVDPV cases and undermining global polio eradication. Around 90% of polio cases due to cVDPV and a third of all vaccine-associated paralytic poliomyelitis (VAPP) cases were being caused by poliovirus type 2.[11]

SAGE also recommended that all countries introduce at least one dose of the inactivated polio vaccine (IPV) into their infant vaccination schedules before switching from tOPV to bOPV, as a risk mitigation measure to provide immunity in the event of a possible VDPV type 2 emergence or reintroduction of wild poliovirus due to failures in lab containment. This measure was later not implemented exactly as recommended due to supply constraints and other logistical issues. [11]

In January 2013, the WHO Executive Board approved the goals, targets, and timelines of the Endgame Plan 2013–2018.

The Endgame Plan

The Polio Eradication & Endgame Strategic Plan 2013–2018 was developed by the GPEI (Global Polio Eradication Initiative) in extensive consultation with national health authorities, global health initiatives, scientific experts, donors and other stakeholders. Its goal is the complete eradication of poliomyelitis and the elimination or containment of all wild and vaccine-derived polioviruses, while taking advantage of the backbone of the polio effort and plan to use it for delivering other health services to the world's most vulnerable children (Polio Legacy).[12]

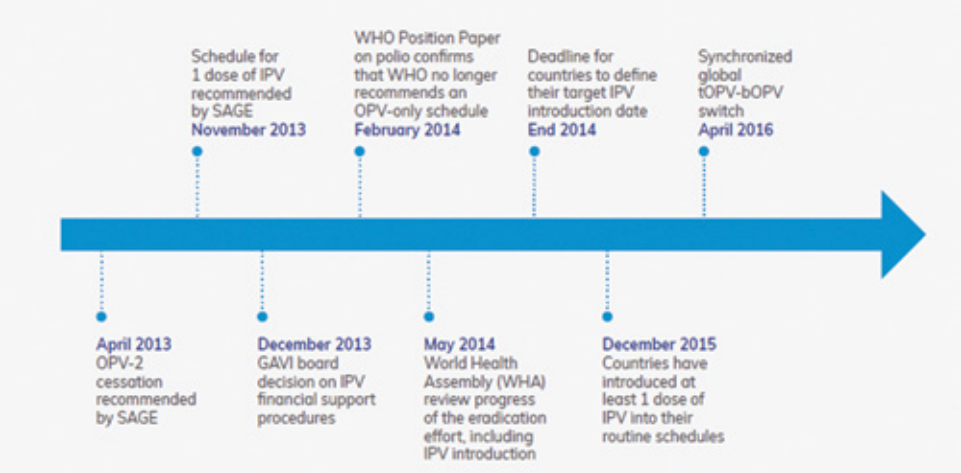
The Plan has four main objectives:

1. **Detect and interrupt all poliovirus transmission:** to stop all WPV transmission and any new outbreaks due to a cVDPV within 120 days of confirmation of the index case, through enhancing global poliovirus surveillance, improving OPV campaign quality to reach children in the remaining endemic and persistent cVDPV countries and ensuring rapid outbreak response.
2. **Strengthening of routine immunization systems, introduction of IPV and withdrawal of type 2 OPV:** to eventually withdraw all OPV, beginning with the withdrawal of the type 2 component of tOPV. The withdrawal of this type 2 component (OPV2) entails strengthening immunization systems, introducing at least one dose of affordable IPV into the routine immunization schedule globally.

3. **Certification of eradication and containment of residual live polioviruses:** to certify all regions of the world are polio-free and ensure that all poliovirus stocks are safely contained by 2018, including finalizing international consensus on long-term biocontainment requirements for polioviruses.
4. **Planning for post-polio eradication transition (originally termed 'legacy planning'):** to ensure that the world remains permanently polio-free and that the investment in polio eradication provides public health benefits in the future.

The introduction of IPV to reduce the risks associated with the withdrawal of OPV is a key element of this strategic plan. SAGE called for the withdrawal of tOPV from the world market in 2016, and once global eradication is achieved—envisioned for 2018—, bOPV use will also cease.[13] As a risk mitigation measure, prior to switching from tOPV to bOPV, SAGE recommended that all countries that were using only tOPV in their vaccination programs introduce at least one IPV dose into their routine vaccination schedules before the end of 2015. In October 2015, SAGE determined April 17 to May 1, 2016 as the two-week window for the global switch from tOPV to bOPV. [14] Figure 1 shows the projected timeline for IPV introduction, the switch, and OPV cessation.

Figure 1. Timeline for IPV Introduction and the Switch



Source: Lessons Learned on IPV Introduction and the Switch from tOPV to bOPV in the Americas. PAHO, Washington DC, 2017, page 13.

Regarding polio containment, in December 2014 the WHO developed the third edition of a global action plan to minimize poliovirus facility-associated risk after polio eradication that includes the containment of all polioviruses: wild, VDPV and Sabin. This containment plan is sequential and began with the containment of WPV-type 2 and VDPV2 in December 2015, followed by the containment of Sabin poliovirus type 2 by July 2016. The final containment of all wild poliovirus is planned for 2019 before bOPV cessation. All Sabin polioviruses type 1 and 3 will be contained after the interruption of bOPV. In the Americas, this first phase of containment has already included the containment of all WPV and VDPV types 1, 2, and 3.

The Endgame Plan in the Americas

In response to the creation of the Endgame Plan, in July 2013 the PAHO Technical Advisory Group of Immunization (TAG) recommend that PAHO convene a Polio Working Group (WG) to develop an adapted strategic plan for the Americas. The WG was tasked with analyzing the current polio epidemiology and immunization strategies in the Region as well as the different vaccination policy scenarios available in the context of the global push towards polio eradication. Based on this assessment, the WG made recommendations to the TAG on how to adapt the polio endgame to the Americas, particularly focusing on the introduction of IPV.[15]

The January 2014 WHO position paper had recommended a schedule consisting of a primary series of 3 OPV doses and at least 1 IPV dose, with an additional dose of OPV at birth for endemic countries or countries with high risk of importations. It also stated that, "if 1 dose of IPV is used, it should be given from 14 weeks of age (when maternal antibodies have diminished and immunogenicity is significantly higher) and can be co-administered with an OPV dose. Countries may consider alternative schedules based on local epidemiology, including the documented risk of VAPP prior to 4 months of age." It also stated the following: "In countries with high immunization coverage (e.g. 90%–95%) and low importation risk (neighboring countries and connections with similarly high immunization coverage) an IPV–OPV sequential schedule can be used when VAPP is a significant concern." [16]

Based on this and the regional epidemiology, the TAG Polio Working Group decided the evidence led to recommending IPV as first dose, which would be most beneficial particularly given the fact that around 50% of VAPP cases in the Region are due to the first OPV dose.[17, 3] As a consequence, the TAG recommended PAHO countries a sequential schedule as follows: "countries should consider two IPV doses followed by two OPV doses. However, if a country is considering only one IPV dose, this should be administered with the first DTP dose and followed by three OPV doses." [18]

In 2015, a non-inferiority study of an IPV-bOPV schedule compared to an all-IPV schedule was published. The study, which was conducted in Chile, assessed the immunogenicity of two different IPV-bOPV schedules compared with an all-IPV schedule in infants. The study concluded that seroconversion rates against polioviruses types 1 and 3 were non-inferior in the sequential IPV-bOPV schedules compared with an all-IPV schedule, and that the proportion of infants with protective antibodies was high after all three schedules. Furthermore, one or two doses of bOPV after IPV boosted intestinal immunity for poliovirus type 2, suggesting possible cross protection. Finally, the study showed evidence of humoral priming for type 2 from one dose of IPV.[19]

Another noteworthy difference in the implementation of the Endgame Plan of the Americas compared to the rest of the world is the fact that the Americas had from the onset utilized the polio elimination strategy to strengthen the national immunization programs by means of a complete integration with the Expanded Program on Immunization (EPI). In fact, the EPI program was given responsibility and ownership over the polio elimination plan. The Endgame Plan's objective related to ensuring that the investment in polio eradication provides public health benefits in the future ("legacy planning") had already been implemented in the PAHO Region thanks to the seamless integration of the polio elimination strategy and the EPI.

Finally, the PAHO Revolving Fund for Vaccine Procurement (RF) facilitated the purchase and licensing process of bOPV in most countries (98%), who readily accepted the vaccines without having to go through a special registration in the country.

Progress and Challenges

Strong progress towards global polio eradication has been made in the past few years, with more and more children in the remaining endemic countries now fully protected. The Endgame Plan was developed to capitalize on this progress to end all polio disease.

The Region of the Americas reported the last case of polio in 1991 and was certified as a polio-free Region in 1994. In the last 25 years since the certification of eradication, the Region has had only one outbreak of polio, which occurred in Haiti and the Dominican Republic between 2000 and 2001 caused by cVDPV.

The South-East Asia Region, which includes India, was certified a polio-free Region in March 2014. With this achievement, 80% of the world's population now lives in polio-free regions. The number of countries with endemic polio dropped from 125 in 1988 to just three (Afghanistan, Pakistan, and Nigeria), when only 37 cases were reported as of December of 2016.[20]

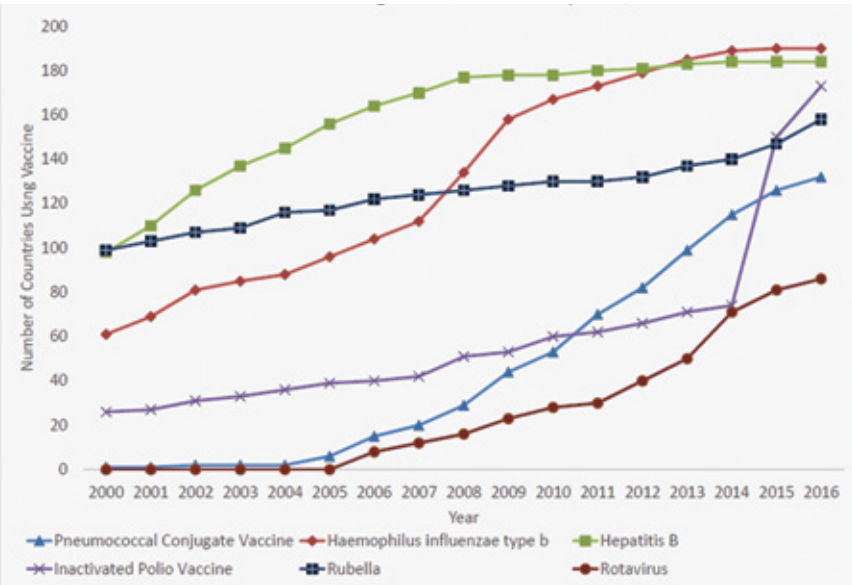
Nonetheless, coverage levels are still not optimal, especially in insecure and politically unstable areas. And since polio is an epidemic-prone disease, ongoing endemic transmission in a few countries will continue to threaten polio-free areas everywhere, unless it is eradicated completely.

For this reason, to meet the global polio eradication goal of eliminating all wild and vaccine related viruses, the use of OPV must eventually be stopped. However, until all wild polio viruses are eradicated, most countries will continue to use OPV because it is still considered the most effective vaccine at fighting wild poliovirus. The eventual withdrawal of OPV will be phased, and has already begun with the type 2 component of tOPV. Type 2 withdrawal from OPV is possible because no cases of WPV type 2 have been detected since 1999 and the continued use of the type 2 oral polio virus presents more risks than benefits, and actually undermines global eradication initiatives. Between 17 April and 1 May 2016, 155 countries around the world, 36 of which being from the Region of the Americas, simultaneously switched from the trivalent oral polio vaccine (tOPV), containing all 3 types of poliovirus, to the bivalent oral polio vaccine (bOPV), containing only types 1 and 3.

In order to ensure that populations have continued immunity against poliovirus type 2 after the switch to bOPV, all countries needed to introduce at least one dose of the inactivated poliovirus vaccine (IPV), which contains killed viruses from all 3 serotypes, and presents no risk of vaccine derived polio.

Prior to this recommendation in 2013, 126 countries globally including 32 countries in the Americas, did not use IPV.[21] This means that within a 2-year timeframe 126 countries needed to introduce a new vaccine into their routine immunization programs. Some new vaccines can take more than 10 years to be introduced on a global scale.[22] This was the fastest and largest global vaccine introduction in the history of vaccines, see Figure 1 on comparative time table for vaccine introductions.

Figure 2: Number of Countries Using Select Vaccines by Year, 2000–2016



Source: World Health Organization Immunization Repository and Year of Vaccine Introduction Database.

Unfortunately, due to unforeseen global shortages of IPV, 21 countries in other regions of the world (AFRO, EMRO, EURO and WPRO), did not meet the intended deadline of IPV introduction.[20] Additionally, at least 29 countries will face stock outs. All 50 of these countries are low risk countries for VDPV emergence; it is expected that these countries will not receive IPV until the end of 2017. However, 32 countries in the Americas that had previously not used IPV were able to introduce the vaccine in between early 2015 and early 2016.

Lessons Learned From the IPV Introduction and Switch in the Americas

Global Coordination Efforts

Many international and regional partners were of paramount importance for the success of the introduction of IPV and the switch in the Americas. The support received from partners such as WHO Headquarters, UNICEF, Centers for Disease Control and Prevention (CDC), the Task Force for Global Health, Rotary International and Gavi was critical throughout the entire process. The UNICEF Regional Office for Latin America and the Caribbean played a role in advocacy, social mobilization, and switch preparation and validation. Gavi and GPEI channeled financial support from multiple international donors to some countries to support gaps in the national budget for IPV introduction and the switch. Rotary played an important role advocating for IPV introduction and participating in the independent monitoring of the switch in some countries. Finally, the Bill and Melinda Gates Foundation (BMGF) conducted an immunological study of one dose of IPV in Chile, and studies of OPV-IPV in Cuba, which was a key piece of evidence to support the decision-making process in the Region.

PAHO's Regional Support to Countries

After the aforementioned Polio Working Group (WG) was convened in March and April 2014 to adapt the Endgame Plan to the regional situation in the Americas, the PAHO TAG held a special meeting and recommended supporting the renewed polio eradication efforts and the endgame eradication goals, including the permanent withdrawal of OPV from routine vaccination programs, and the use of sequential schedules.

Based on TAG's recommendation and the urgency of the IPV introduction and the switch, PAHO developed a comprehensive technical cooperation strategy that included several virtual and face-to-face meetings and the development and adaptation of support documents to maximize chances of a successful regional IPV introduction.

By the first quarter of 2015, PAHO had received the formal commitment from all LAC countries for the introduction of IPV. PAHO formed a new Regional Certification Committee (RCC), which met for the first time in June of 2015.

Part of the countries' success depended on the availability of technical and communication materials to support any vaccine introduction process. Countries frequently find it a challenge to develop their own materials due to time and financial constraints, and sometimes also a lack of technical capacity on specific technical issues. To help countries overcome this challenge, and also to promote the use of uniform materials and communication messages across the Region, PAHO developed the PAHO IPV Introduction Practical Guide and adapted and expanded on several materials developed by the Immunization Management Group (IMG) of the GPEI, to support countries in the introduction of the IPV vaccine. The IMG is made up of partners from WHO, UNICEF, GAVI, CDC, Rotary, and BMGF. The adaptation of materials was necessary largely due to the fact that the Region had opted for a different vaccination schedule with IPV as the first dose. The materials included technical documents, training and communication information and tools. These materials were shared with countries in editable formats (Word documents or Power Point slides) so that countries could adapt them as needed. The PAHO communication focal points were also involved together with their PAHO immunization counterparts in country, to allow for an integrated approach for the use of these materials in country.

WHO sent guidelines for the switch, which PAHO translated and shared with countries. PAHO requested countries to share their switch plans by mid-2015. By September 2015, PAHO had received the switch plans from all countries, and reviewed the plans against an adaptation of a checklist that had been provided by GPEI. PAHO provided significant direct technical cooperation, including some regional visits to selected countries prior to the switch, to ensure preparedness and avoid any delays in the Region. Additionally, to have an overall picture of the situation across the Region, PAHO developed a dashboard to monitor the implementation of key activities. The switch dashboard contained 41 activities selected according to the optimal period for their implementation to guarantee a safe switch. The dashboard allowed for a quick identification of activities that were falling behind schedule or required greater attention. Of the 41 activities, 18 were marked as "milestones". Carrying out the activities helped ensure a successful switch, while failure to meet the milestones compromised the safety of the switch in the country and, consequently, in the Region. This tool was useful for Regional Certification Commission (RCC) for the Polio Endgame in the Region of the Americas and National Certification Committees (NCC) members, immunization program managers and personnel, and PAHO to follow up with the progress and detect difficulties or delays.

Countries' Perspectives on IPV Introduction

In the PAHO Region, 19 countries, representing 70% of the birth cohort in the Americas, were already using the IPV vaccine in their national schedule prior to 2015. The remaining 32 countries, representing 30% of the birth cohort in the Americas (4,606,700) introduced IPV as part of the Endgame Plan, between 2015 (22 countries) and the first half of 2016 (10 countries).

In March 2016, PAHO sent out a survey to the 32 countries from Latin America and the Caribbean that had introduced IPV in 2015 or 2016 as part of the Endgame Plan. Overall, 31 out of the 32 countries replied to the survey. It is noteworthy that over half the countries took less than 3 months to make the decision to introduce IPV, and the main facilitators were the global and national commitment to polio elimination. Regarding the IPV introduction process itself, PAHO's technical support and staff training were the predominant facilitators, and the negative perception of change from "drop to shot" was perceived as the main challenge. A summary of the survey results is presented in Table 1.

Table 1. Main Findings from the IPV Introduction Survey to Countries

Key Findings IPV Introduction Survey (N=31)				
			Number	**Percent %**
Decision to Introduce IPV	**Time to Decide**	Countries that took 6 months or less to make the decision	26	86%
		Countries that took 1 to 3 months to make the decision	17	56%
	Main Facilitators	Global commitment	9	29%
		National political support and commitment	6	19%
		Presence of a regional TAG recommendation	5	16%
		Availability of supporting evidence around the rationale for the introduction	4	13%
	Main Barriers	No difficulties in the decision-making process	21	68%
		Financial issues	4	13%
IPV Introduction Process Itself	**Nationwide or Phased Introduction**	Countries that introduced IPV simultaneously nationwide.	25	81%
		Countries with phased introduction	6	19%
	Main Facilitators	PAHO support (technical cooperation and guidelines)	23	74%
		Staff training	19	61%
		Political will and support	17	55%
		Commitment of staff	17	55%
		International commitment to the need for global IPV introduction to achieve polio eradication	14	45%
		Experience, preparedness and planning of the EPI	13	42%
	Main Barriers	Negative perception of change from drop to shot	19	61%
		Insufficient or delayed training	12	39%
		Financial constraints	8	26%
		Insufficient monitoring or supervision in the field	8	26%

Source: PAHO IPV Introduction Survey, 2016.

Countries' Perspectives on the Switch From tOPV to bOPV

Thirty-six countries of the Americas switched from tOPV to bOPV in the Americas in April 2016. In July 2016, PAHO administered a survey to these 36 countries and all countries replied. Again, PAHO's support and staff training were the main facilitators to plan the switch. Commitment of healthcare workers was the main facilitator for successfully implementing the switch, and support of stakeholders involved in the validation process was the main positive factor in the validation of the switch. Table 2 provides a summary of main findings from the survey.

Table 2. Main Findings From the Survey to Countries on the Switch

Key Findings Switch Survey (N=36)				
			Number	**Percent %**
Planning the Switch	**Main Facilitators**	Staff training	11	31%
		Counting on PAHO technical support and documents	11	31%
		Commitment of healthcare workers	9	25%
		Involvement of healthcare workers and key national players	9	25%
		Political will	7	19%
	Main Barriers	Countries that did not encounter any obstacles in the planning process	15	42%
		Concomitant events as a factor that made the planning more difficult	11	31%
Implementing the Switch	**Main Facilitators**	Commitment of healthcare workers	10	28%
		Monitoring and supervision activities	5	14%
		Staff training	4	11%
	Main Barriers	Countries with no implementation obstacles for the switch	14	39%
		Vaccine transportation-related issues	7	19%
Validating the Switch	**Main Facilitators**	Commitment/support of stakeholders involved in the validation process	12	33%
		External support (technical or financial)	10	28%
	Main Barriers	Countries with no obstacles in the validation process	11	31%
		Insufficient financial resources for the switch	5	14%
		Delays in receiving the validation forms from the lower level	5	14%

Source: PAHO tOPV to bOPV Switch Survey, 2016.

Conclusions

One of the most critical points for success of IPV introduction and the switch to bOPV was the global structure to support the regions. There were many international organizations working together to support the 126 countries across the globe that needed to introduce IPV and make a synchronized switch. The WHO, UNICEF, CDC, Task Force for Global Health, Rotary International, and Bill and Melinda Gates Foundation, all worked together in the Immunization Systems Management Group (IMG), with permanent and substantial exchange with the regions. The issues with global vaccine supply and vaccine delays were major obstacles that had to be dealt with at international, regional and national levels. Pan Americanism played an important role when the global vaccine shortage did not allow for countries to introduce more than one dose of IPV so PAHO had to recommend all countries who were not already using IPV, to introduce a single dose. This experience, the largest scale up of a vaccine ever undertaken worldwide, and its lessons learned conform an important documentation of the Polio Legacy in the Americas and worldwide.

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Rotavirus

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Introduction

Rotavirus is the leading cause of severe, dehydrating diarrhea among children aged <5 years globally.[1] Since 2006, two rotavirus vaccines are available and were introduced into 93 countries worldwide by January 2018.[2] Several countries that have implemented routine childhood vaccination against rotavirus have documented a tremendous impact on severe diarrhea and rotavirus disease requiring hospitalization. Additionally, some countries in the Region of the Americas, including Mexico, Brazil, and Panama, have documented substantial decreases of 22%–50% in diarrhea mortality among children <5 years of age following vaccine introduction.[1]

Etiological Agent

Rotaviruses belong to the *Reoviridae* family, *Rotavirus* genus. The viral particles were first identified by Bishop et al. by direct visualization on electron microscopy in 1973, in a duodenal mucosa biopsy[3,4] and stools[4,5] from children with acute diarrhea. Viral particles have typical morphology, similar to a cartwheel (based on which researchers suggested the "rotavirus" denomination).[6-8] They are 80nm to 100nm in diameter, icosahedral structure, three-layer protein capsid and no viral envelope, which increases their resistance to soluble lipids and other adverse environmental conditions. They are very stable and may remain viable in the environment during weeks or months absent disinfestation.[4,9]

The viral genome contains 11 double-stranded RNA segments, contained in a nuclear capsid. The viral genome segments encode 6 structural proteins: VP1, VP2, VP3, VP4, VP6, VP7; and 6 non-structural proteins: NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6, with the exception of segment 11 which codes for two proteins (NSP5 and NSP6).[4,8]

The intermediate capsid comprises the VP6 protein, encoded by segment 6. VP6 is the most abundant protein in the virion and the basis for classification of rotaviruses into serotype groups, ranging from A to H, and for serologic identification through ELISA test.[4,10] Groups A, B, C, and H have been described in human-beings and animals, while the others (D-G) have been found only in animals (mammals and birds).[4,11] Group A has been identified as the most important one for public health since it is a significant cause globally of severe diarrhea in young children.[4,11,12]

Every serogroup may be classified into several genotypes as determined by the VP4 (P protein) and VP7 (G protein) proteins located in the external capsid. These proteins have multiple epitopes inducing the synthesis of neutralizing antibodies and, therefore, they may impact on the efficacy of the rotavirus vaccines.[4,8,9] Twenty-seven genotypes for the G protein, and 37 genotypes for the P protein have been described, with the G1P[8], G2P[4], G3P[8], G4P[8], and

G9P[8] combinations accounting for the highest number of cases. Most of the genotypes circulate concomitantly during a season and facilitate viral reassortment, which combined with genome mutations and rearrangements, are considered to be the main mechanisms for the evolution of the rotavirus genetic diversity.[13-15]

Pathogenesis

The rotavirus transmission model is not well known even though the disease is communicated through personal contact (oral-fecal transmission), contaminated fomites or air sprays.[16-18] Viral replication takes place in the small intestinal villi, advancing from the proximal to the distal areas.[9,17]

There are two principal mechanisms for diarrhea to occur: osmotic imbalance and secretion. Rotavirus infection produces an extensive necrosis of the enteric epithelium, which impairs intestinal absorption of sodium, glucose, water, lactose, and sucrose, thus inducing isotonic diarrhea.[9,11,19] This is followed by reactive crypt hyperplasia with increased intestinal secretion, which also contributes to the severity of diarrhea.[4,19]

The secretory mechanism is produced from the release of the NSP4 protein acting as a viral enterotoxin in addition to its role in viral replication and intracellular morphogenesis. This enterotoxic effect is produced on uninfected cells when interfering with the Ca2+ ion metabolism, increasing its intracellular concentration and altering the electrolytic homeostasis, which accounts for the acute diarrhea observed even before histopathological changes in the epithelium and even absent very extensive damage.[4,11,19]

The enteric nervous system may also be involved in rotavirus-induced diarrhea, since the substances blocking this system mitigate diarrheal symptoms. Likewise, although viremia is apparently frequent, systemic disease is rare, suggestive of rotavirus spread to other organs concurrently with systemic disease caused by other organisms.[4,19] In sum, rotavirus-induced diarrhea is a complex mechanism involving poor absorption, hypersecretion, and alteration of intestinal permeability and motility. The severity of the disease is dependent on the characteristics of the virus and the host.[4,11,19]

Rotavirus infection triggers an intestinal and systemic local immune response, despite being an infection that mainly affects the intestinal mucosa.[20] The primary rotavirus infection produces specific homotypic humoral immunity, typically not permanent. After the first natural infection, 38% of infected children have been observed to have protection for an ensuing infection, 77% were protected against diarrhea and 87% against acute diarrhea. The ensuing infections produce homotypic and heterotypic immunity, offer greater protection and are usually less severe than the primary infection.[9,21-23]

Incubation and Communicable Period

Transmission is mainly fecal-oral.[9,24,25] Incubation is relatively short, usually less than 48 hours and the disease has a sudden onset.[9,11,19] Transmission is high since it requires a low infectious inoculum and the number of virus particles excreted in diarrhea is very high, and may reach 10^11 viral particles/mL feces before and after onset of symptoms, during the acute phase of the disease.[19,24] Virus excretion starts before the onset of symptoms and continues even upon conclusion of diarrhea; based on studies with immunoassays it varies from 4 to 29 days, with a 7-day median, while molecular tests (PCR) have detected virus excretion in a 4–57 day range with a 10 day median.[4] The viral particle is very resistant to the environment, and it may persist for up to 10 days on dry surfaces and 4 hours on human hands.[25]

Clinical Characteristics

The rotavirus infection is more frequent and more severe in children 3 to 36 months of age.[26] The clinical manifestations vary depending on whether it is the first infection or a reinfection. The infection may be asymptomatic, induce self-limiting watery diarrhea or produce severe diarrhea accompanied by fever and vomiting. Other symptoms are quite rare, such as central nervous system impairment, hepatitis, and chronic infections. The first infection, after the first three months of life, is usually the most severe.[9,16,23,26] Diarrhea usually lasts 3 to 8 days with 10 to 20 daily episodes. Fever and vomiting are more frequent at the outset. Fever is usually low but up to a third of the children may have temperatures higher than 38.5°C–39.0°C with the risk of suffering from febrile seizures. Vomits occur in 80% to 90% of the cases of severe diarrhea; they are usually severe and last less than a day.[9,16,23,27] Rotavirus-induced diarrhea is usually more associated with dehydration and hospitalization than diarrhea induced by other agents.[4,27] Infection in immunocompromised individuals due to bone marrow transplant or another type of transplant may present extended viral excretion, as well as severe symptoms and higher risk of death.[4,9]

Rotavirus-induced diarrhea is clinically similar to diarrhea induced by other agents. Case confirmation requires laboratory tests, including immunoassays such as ELISA or rapid tests from agglutination tests, usually in stool samples.[9,19] Treatment is mainly channeled to rehydration of the patient, by mouth or parenterally, with the recommended addition of zinc since it has been proven to reduce the diarrhea duration. For rehydration by mouth, the use of oral rehydration salts with low-osmolarity is recommended.[18,19]

Epidemiology

Rotavirus is globally the leading cause of diarrhea in children aged <5 years, both in developing and in developed countries, which is suggestive of the fact that the infection cannot be prevented by solely improving sanitation services since viruses equally affect different geographic areas, social or ethnic groups.[7,9,11,28] In low income countries, the median age at the primary rotavirus infection ranges from 6 to 9 months (80% occur among infants <1 year old). Whereas in high income countries, although the majority still occur in infancy (65% occur among infants <1 year old), the first episode may occasionally be delayed until the age of 2–5 years.[18,29]

As of April 2016, the World Health Organization (WHO) estimates that globally 215,000 (197,000–233,000) child deaths occurred during 2013 due to rotavirus infection compared to 528,000 (465,000–591,000) in 2000, but it is still the most important cause of diarrhea-related death.[1] About 90% of these fatalities occurred in low-income countries, in particular in Africa and Asia.[18] National estimates of rotavirus attributable deaths among children under five years of age ranged from 47,100 (India) to fewer than 5 deaths (79 countries). Twenty-two percent of all rotavirus deaths under five years of age occurred in India. Four countries (India, Nigeria, Pakistan and the Democratic Republic of the Congo) accounted approximately half (49%) of all rotavirus deaths under age five in 2013. Globally these 215,000 child rotavirus deaths accounted for approximately 3.4% of all child deaths and the cause-specific mortality rate (rotavirus deaths under age five per 100,000 population under age five) was 33.[1]

Before the vaccine was available, it was estimated that 1 out of every 5 children received medical care and that 1 out of every 50 to 70 was hospitalized in the first 5 years of life due to rotavirus-induced infection.[30] This accounted for a total of 114 million episodes of gastroenteritis that required home treatment, 24 million clinic

visits and 2.4 million hospitalizations in children <5 years globally.[31] Likewise, the disease has been determined to have a seasonal pattern occurring more frequently during winter months.[9]

Globally, prevalent genotypes are G1P[8], G2P[4], G3P[8], and G9P[8]. Genotype distribution may be different for various seasons or years.[32] G1P[8] is the most frequently isolated genotype subject to seasonal and regional variation.[32]

Before the introduction of the rotavirus vaccine in Latin America and the Caribbean, there were 15,000 deaths, 75,000 hospitalizations, and 10 million cases of rotavirus-caused diarrhea annually. Data from epidemiological surveillance in 11 countries and territories showed that the median percentage of positive specimens for rotavirus in hospitalized cases was 31.5% and 39.0% in 2006 and 2007, respectively.[33] A meta-analysis conducted with studies published between 1990 and 2009 showed that the percentage of positive specimens for rotavirus in hospitalized patients was 29.7%.[12] In 2011, epidemiological surveillance showed a median percentage of positive specimens in 15 countries of 19.0%, after the introduction of the vaccine in several countries of the region.[34]

Rotavirus-induced mortality estimated for the 2005–2007 period for 10 countries in the Latin American region showed that 1 out of every 2,874 children <5 years died due to rotavirus, for a total of 3,492 deaths and a 34.8 per 100,000 rate among children <5 years, which was consistent with estimates published by the World Health Organization (WHO) in 2004.[28,33] The mortality rate estimated in a meta-analysis with data from 22 countries for the 1977–2009 period was 88.2 (79.3–97.1) per 100,000 children <5 years.[12]

As observed in other countries, a seasonal pattern has been observed, with a higher number of cases and percentage of positive specimens in the months from November to March in Northern-Hemisphere countries while in Southern-Hemisphere countries cases occur with higher frequency is in the months of May to September.[33]

The more frequently circulating genotypes in Latin America and the Caribbean for the 2005–2007 period were G1P[8] (32.0%), G9P[8] (20.9%), and G2P[4] (18.3%), based on information from the surveillance system.[50] Similar information published in a 2011 meta-analysis for the period prior to 2010 showed G1P[8], 17.9% (12.2%–24.4%), G2P[4], 9.1% (4.9%–14.5%), and G9P[8] 8.8% (4.1%–15.0%) as the most frequent genotypes.[12]

Availability of Vaccines

Efforts to find a vaccine against rotavirus started in the 1970's, driven by the recognition, in 1979, of rotavirus as a significant cause of infant morbidity and mortality by WHO.[11,35] However, it was not until February 1998 that the first tetravalent vaccine was licensed in the United States (G1-G4) in a three-dose schedule (2, 4, and 6 months) to prevent rotavirus-induced diarrhea.[35] However, the vaccine was interrupted in July 1999 by recommendation of the U.S. Centers for Disease Control and Prevention (CDC) due to the detection of intussusception after vaccination. There were no new cases after administration of the vaccine was suspended.[36] Years later, two new vaccines have been licensed, with an excellent safety profile.[18]

Rotavirus Specie A (RVA) Vaccine

The two vaccines currently available in the international market against RVA are: 1) the live, attenuated monovalent (G1P[8]) vaccine Rotarix™ (GlaxoSmithKline), and 2) the reassortant pentavalent (G1-4P[8]) vaccine Rotateq™ (Merck). Two other vaccines (by the Lanzhou Institute of Biomedical Products, China) and Rotavin-M1 (manufactured by Polyvac, Vietnam) are not available in the international market.[18]

The Monovalent Vaccine (RV1)

The RV1 is a live single-strain vaccine of genotype G1P[8] derived from a human rotavirus strain. This strain has undergone 43 passages of tissue culture and the resulting attenuated vaccine strain, RIX4414, is propagated in Vero cells. The vaccine is administered orally in a two-dose schedule. The first vaccine should be administered to infants at 6–14 weeks of age, and the second dose should be administered at a 4-week interval. According to the manufacturer, the second dose should be administered prior to 24 weeks of age.[18,37]

The first clinical studies were conducted in Finland and enrolled 63,225 children. They showed a 42% efficacy (29.0%–53.0%) for the reduction of hospitalizations due to all-cause diarrhea. In Latin America and Asia, the efficacy was 70.0% to 85.0% for RVA-induced diarrhea and 85.0% to 93.0% for acute RVA diarrhea. The vaccine proved to be safe with no excessive risk of causing intussusception in vaccinated children. It was first licensed in Mexico in 2004 and then in other countries of Europe and the Americas.[9,37,38]

The Pentavalent Vaccine (RV5)

The RV5 vaccine was developed from an attenuated bovine virus WC3, of genotype GXPY. This genotype was reassorted at the laboratory and the genotypes G1-G4 and P[8] from the human strains were added. Four strains express one of the VP7 proteins G1-G4 from a human strain and the VP4 protein P7[5] from a bovine strain. The fifth strain expresses a VP4 protein P1A[8] from a human strain and the VP7 protein G6 from a bovine strain. They are propagated in Vero cells using culture techniques.[18,39]

The vaccine is administered orally in a three-dose schedule. Based on the manufacturer's recommendation, the first dose should be administered at 6–12 weeks of age and subsequent doses should be administered at intervals of 4–10 weeks. The first three doses should be administered by 32 weeks of age.[18,40]

This vaccine was approved in clinical trials conducted in more than 70,000 children, mainly in the United States and Finland, although studies were also conducted in South America, Europe, and Asia. The clinical studies showed 94.5% (92.2%–96.6%) efficacy in the reduction of hospitalizations and emergency department visits related to RVA-induced diarrhea. Other studies had efficacies of 74.0% (66.8%–79.9%) for all-cause diarrhea due to RVA and 98.0% (88.3%–100.0%) for acute diarrhea due to RVA. The risk of intussusception was similar among the vaccinated and unvaccinated children.[40] It was first licensed in the United States in February 2006.[41]

Both vaccines, RV1 y RV5, have high efficacy and an excellent safety profile,[42] and they were prequalified by WHO in January 2007 and August 2008, respectively.[43,44]

Vaccine Recommendations

WHO recommends the administration of either vaccine against RVA starting at 6 weeks of age, before 24 months of age, concomitantly with the other vaccines in the national vaccination schedules. The goal is for a greater number of children, in particular in low-income countries, to have access to vaccination. An incremental mortality study demonstrated that an additional 21% to 28% death could be prevented by moving from a restricted vaccination schedule to an unrestricted schedule for date of initiation. RV1 should be administered in a 2-dose schedule, with a 4 week interval between doses. RV5 should be administered at the time of DTP1, DTP2, and DTP3, with an interval of 4 weeks between doses.[18]

In October 2012, the Pan American Health Organization (PAHO) though its Technical Advisory Group (TAG), also recommended starting vaccination after the dates established in the WHO recommendations in children who live in areas difficult to access with a high mortality risk. In all cases, the vaccine should be administered as early as possible.[45] Countries introducing the RVA vaccine should monitor for the occurrence of intussusception to guarantee the safety of the vaccine in the immunization programs, and the baseline incidence of this disease should be estimated prior to vaccine introduction.[18,46]

Rotavirus Vaccine Introduction in Latin America and the Caribbean

Six countries in the region (Brazil, El Salvador, Mexico, Nicaragua, Panama, and Venezuela) introduced the rotavirus vaccine into their national immunization schedules in 2006, the same year the vaccine was licensed. For the first time in history, developing countries introduced a vaccine at the same time as developed countries.[47] However, the introduction took place before the implementation of rotavirus surveillance, contrary to the recommendation by PAHO/WHO.[34,48] Other factors must have impacted the decision, such as local publications on rotavirus.[49]

As of December 2016, 21 countries and one territory in Latin America and the Caribbean had included a rotavirus vaccine, where 96% of the target population is estimated to live. The most widely-used vaccine is the monovalent, which is not used in only Mexico and the Cayman Islands.

Impact of the Rotavirus Vaccine in Latin America

Vaccine Effectiveness

Both vaccines have demonstrated high levels of effectiveness in the studies published. A meta-analysis published in 2012 reviewed this data.[51] This study included 29 clinical trials (101,671 participants) to study the RV1 and 12 clinical trials (84,592 participants) to study the RV5. The results for the RV1 study are shown in Table 1 and the results for the RV5 study are shown in Table 2.

Table 1. RV1 Effectiveness for Diarrhea Prevention

Age/Scope	Countries With a Low Mortality Rate	Countries With a High Mortality Rate
Infants <1 Year	RV1 prevents 86% cases of acute diarrhea (RR=0.14, 95% CI: 0.07–0.26) and 40% episodes of all-type diarrhea (RR=0.60, 95% CI: 0.50–0.72).	RV1 prevents 63% of acute diarrhea cases (RR=0.37, 95% CI: 0.18–0.75) and 34% episodes of all-type diarrhea (RR=0.66, 95% CI: 0.44–0.98).
Children Up to 2 Years of Age	RV1 prevents 85% cases of acute diarrhea (RR=0.15, 95% IC: 0.12–0.20) and 37% episodes of all-type diarrhea (RR=0.63, 95% CI: 0.56–0.71).	RV1 prevents 42% cases of acute diarrhea (RR=0.58, 95% CI: 0.42–0.79) and 18% episodes of all-type diarrhea (RR=0.82; 95% CI: 0.71–0.95).

Table 2. RV5 Effectiveness for Diarrhea Prevention

Age/Scope	Countries With a Low Mortality Rate	Countries With a High Mortality Rate
Infants <1 Year	RV5 prevents 87% cases of acute diarrhea (RR=0.13, 95% CI: 0.04–0.45) and 72% episodes of all-type diarrhea (RR=0.28, 95% CI: 0.16–0.48).	RV5 prevents 57% of acute diarrhea cases (RR=0.43, 95% CI: 0.29–0.62). Data was insufficient to assess episodes of all-type diarrhea.
Children Up to 2 Years of Age	RV5 prevents 82% cases of acute diarrhea (RR=0.18, 95% CI: 0.07–0.50) and 96% episodes of all-type diarrhea (RR=0.04, 95% CI: 0.00–0.70).	RV1 prevents 41% cases of acute diarrhea (RR=0.59, 95% CI: 0.43–0.82) and 15% episodes of all-type diarrhea (RR=0.85; 95% CI: 0.75–0.98).

The study groups had no differences as to adverse events or frequency of intussusception in particular. Efficacy was similar for both vaccines and it was higher for acute diarrhea, in children <1 year of age and in countries with low-mortality rate.[51]

Effectiveness in LAC

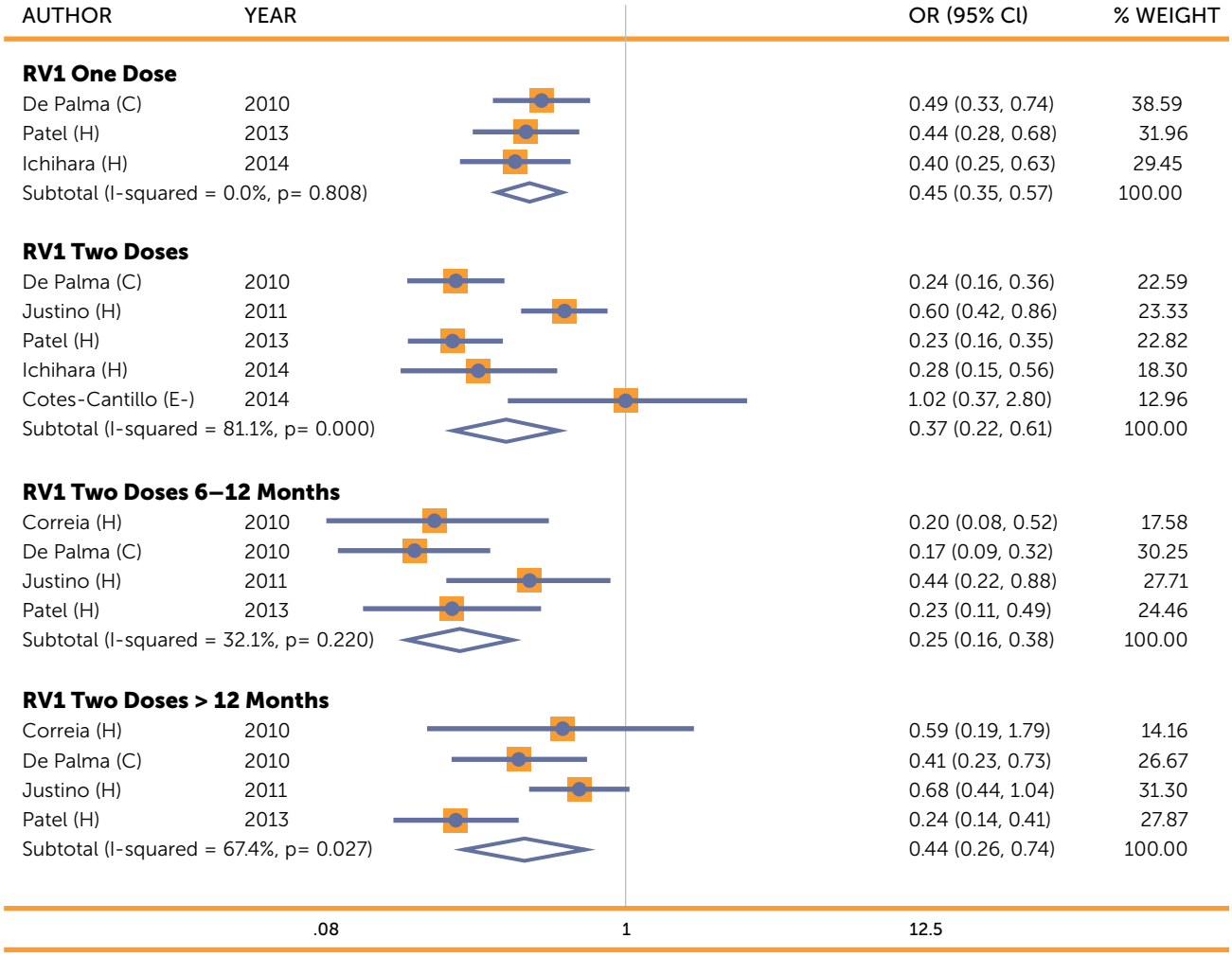
A meta-analysis[52] published in 2015 of studies with data from the region demonstrated both vaccines are effective in preventing hospitalizations due to rotavirus-induced diarrhea. This research included 8 case-control studies, with a total of 6,265 cases and 21,448 controls. The estimates were based on different control types, which led to the identification of different levels of effectiveness.

The results found for RV1 were:

- Effectiveness of two doses to prevent RVA-induced hospitalizations ranged between 63.5% (95% CI: 39.2%–78.0%) and 72.2% (95% CI: 60.9%–80.2%).
- Effectiveness of two doses in children <1 year to prevent hospitalizations ranged between 75.4% (95% CI: 64.6%–82.9%) and 81.8% (95% CI: 72.3%–88.1%).
- Effectiveness of two doses in children >1 year to prevent hospitalizations ranged between 56.5% (95% CI: 26.2%–74.3%) and 66.4% (95% CI: 54.1%–75.5%).

Figure 1 shows the odds ratio (OR) results in each RV1 study selected for the meta-analysis.

Figure 1. Effectiveness of the Monovalent Rotavirus Vaccine, Based on Dose and Age



NOTE: Weights are from random effects analysis **RV1: Monovalent Vaccine** **C: Community Control** **H: Hospital Control** **E-: EIA Test Negative**

Source: De Oliveira et al., 2015.[52]

RV5 results were:

- Effectiveness to prevent diarrhea with a Vesikari score >11 in infants at 6–11 months of age ranged between 76.1% (95% IC: 57.6%–86.6%) and 88.8% (95% CI: 78.3%–94.3%).
- The effectiveness to prevent hospitalized diarrhea cases caused by G2P[4] was 63.5% (95% CI: 29.4%–82.6%).

In sum, RVA vaccines offered consistent protection against diarrhea-induced hospitalization in LAC. The effectiveness was significant for hospital and community controls but higher in the latter group. Likewise, effectiveness was higher in children <12 months of age.

Finally, PAHO estimates that as of 2013 RVA vaccination has prevented between 6,903 and 8,621 deaths in children <5 years of age.

Conclusion

To conclude, it is important to consider the following:

- RVA vaccination should be administered and completed within the schedule as soon as possible. RV1 requires two separate doses with at least a 4-week interval and RV5 requires three doses, with a 4-week interval also.
- Efficacy and effectiveness studies have demonstrated the significant impact this vaccine has on morbidity caused by diarrhea in developed and developing countries.
- Epidemiological surveillance is important to monitor disease trends, study genotype distribution, and characterize RVA epidemiological profile.
- For effectiveness studies, it is important to analyze the impact for various genotypes.
- Studies to analyze trends suggest a significant reduction of morbidity and mortality, in children <5 due to the RVA vaccine.

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New Insights for the Rational Design of Human Respiratory Syncytial Virus Vaccines: From Molecular Biology to Clinical Trials

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Introduction

Human respiratory syncytial virus (HRSV) was first identified in 1956 as a virus producing rhinitis in a colony of chimpanzees, which was originally named as the Chimpanzee Coryza virus (CCV)[1]. In 1957, soon after the identification of CCV the same virus was isolated from the respiratory secretions of children suffering acute lower respiratory tract infections[2]. Given that CCV caused the formation of large syncytia in cultured cell lines, the virus was renamed human respiratory syncytial virus (HRSV)[2]. Moreover, HRSV was determined to be the major etiologic agent for bronchiolitis in young children[2,3]. Further epidemiological studies confirmed HRSV as the single most important pathogen causing bronchiolitis in infants, young children and adults suffering underlying medical conditions[4-13]. Indeed, HRSV seroprevalence is 70% in infants, suggesting that most children get infected during their first year of life. Importantly, seroprevalence reaches 100% by the age of two, and most young adults maintain circulating HRSV-specific antibodies, implying that HRSV continuously circulates in the community[12].

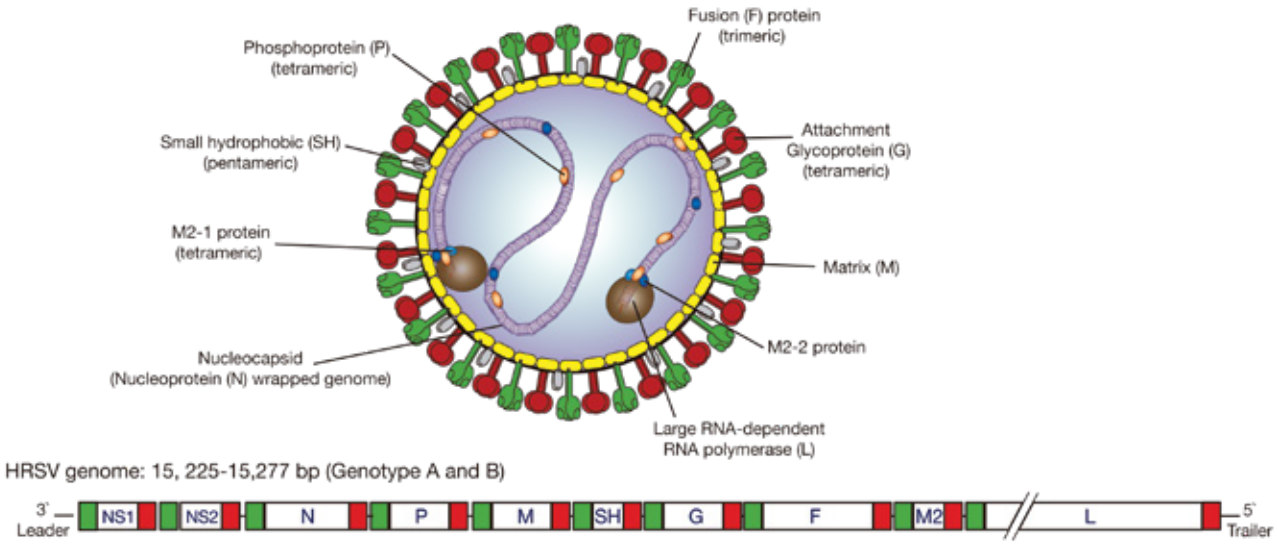
HRSV affects children from both developing and developed countries equally. The morbidity and severity of the disease does not change significantly among different socioeconomic backgrounds[14]. Furthermore, worldwide HRSV infections lead to significant increases in both governmental and private health expenditures, with still immeasurable costs related to parental absenteeism at work, bed conversion at hospitals and outpatient health expenditures[15,16]. Despite its significant burden, to date there is no cost-effective antiviral nor a single safe

licensed vaccine approved for immunization of susceptible individuals. Current research in the field of HRSV has yielded several vaccine candidates that are either under testing in animal models, or under clinical evaluation for safety and immunogenicity in humans. In this paper we summarize important aspects of HRSV biology related to virulence and the rational design of vaccines against the virus. We also discuss the two major goals of HRSV vaccine development, which are intended to 1) immunize pregnant mothers to confer passive immunity to newborns less than 6 months of age, and to 2) directly generate acquired immunity in infants by means of repertoires of memory T and B lymphocytes[17].

HRSV Proteins as Virulence Determinants and Antigens for Vaccine Development

HRSV is classified within the order *Mononegavirales*, the family *Paramyxoviridae*, and the *Pneumovirus* genus[18,19]. HRSV has a single-stranded, non-segmented RNA genome of negative polarity, and a variable length of approximately 15.2 Kb[20,21]. Ten individual genes are contained in the viral genome, which are arranged as follows: 3`-NS1-NS2-N-P-M-SH-G-F-M2-L-5`(Figure 1). After transcription of the viral genome 10 mRNAs are synthetized, which are translated by host ribosomes into 11 different viral proteins. Table 1 summarizes the function as well as the genetic variability of structural and non-structural proteins in the two known genetic lineages of HRSV (A and B). With this information at hand the reader will be able to understand the rationale behind the strategies used for the development of the newest HRSV vaccines.

Figure 1 . HRSV Virion Structure and Genome Composition



Notes: *Upper panel*- schematic cartoon of the HRSV infectious virus particle (virion) showing the interaction of different viral proteins within the viral envelop (F (dark green), SH (pale green) and G (red) glycoproteins), and inside the virus core. The nucleoprotein forms a helical structure (yellow green) with the viral RNA genome, which is associated with the L (brown), P (orange) and M2-1 (pale blue) proteins to form the replicase-transcriptase (RT) complex. The nucleocapsid anchors to the plasma membrane through interactions with the Matrix protein (M, yellow) located in the inner leaflet of the lipid bilayer forming the envelope. Upon fusion of the HRSV and host cell membranes the RT complex initiates the transcription of viral mRNAs in the cytosol of infected cells. *Lower panel*- schematic cartoon of the HRSV single stranded, non-segmented RNA genome showing each of the 10 viral genes flanked by gene-start (green boxes) and gene-end (red boxes) sequences. The genome figure was drawn not to scale.

Table 1 . Function and Genetic Variability* of HRSV Proteins

Protein	Function	Genetic Variability HRSV A[115]	Genetic Variability HRSV A and HRSV B[115,116]
Non-Structural Protein 1 (NS1)	Inhibition of the production of type I interferon[27,30,31]	6%	0-3%
Non-Structural Protein 2 (NS2)	Inhibition of the production of type I interferon, and inhibition of $CD8^+$ T cell cytotoxicity[117,118]	7-9%	0-2%
Nucleoprotein (N)	Involved in genome encapsidation and interference with immunological synapse formation[41,119]	7-8%	0-1%
Phosphoprotein (P)	Participates in the viral replicase-transcriptase (RT) complex acting as a cofactor for the large (L) RNA-dependent RNA-polymerase[31-33]	5-6%	1-3%
Matrix (M) Protein	The M protein participates in virion assembly. Also inhibits the viral transcriptase activity[120]	6%	0-3%
Small Hydrophobic (SH) Protein	Interfere with the permeability of infected cells by forming cation-selective ion channels[121]	8-10%	0-5%
Glycoprotein (G)	Transmembrane glycoprotein that facilitates the attachment of HRSV to glycosaminoglycans like heparan sulfate[122,123]	10-18%	2-12%
Fusion (F) Protein	Transmembrane glycoprotein that mediates fusion between HRSV and cytoplasmic membranes. Mediates virus penetration and syncytia formation[47]	6-9%	1%
Matrix M2-1 (M2-1) Protein	M2-1 is an anti-terminator of translation (as translation elongator factor). Participates in association with M and N proteins in HRSV assembly and budding[23-25,34,124]	5-6%	1-3%
Matrix M2-2 (M2-2) Protein	Negatively modulates viral mRNA synthesis[35]	9-20%	0-5%

Notes: **Genetic variability* (GV) refers to the degree of differences found in the genetic code (or gene sequences) of different viral strains or genetic lineages (A and B). The GV may be expressed as the percentage of amino acids (in proteins) or nucleosides (in DNA/RNA) that differ from a previously determined consensus sequence. A related concept, referred to as *identity,* is used to reflect the degree of conservation in protein and nucleic acid sequences.

Reverse genetics is the methodological approach of investigating the role of viral proteins by modifying DNA/RNA sequences within a gene to generate proteins of reduced functionality, or by deleting the entire gene to generate viruses lacking the protein of interest[22]. This technology has promoted both the understanding of the

role of HRSV proteins as well as the development of novel vaccines of acceptable safety and immunogenicity. For instance, approaches of reverse genetics vaccinology have either deleted or codon-deoptimized the genes of the non-structural proteins NS1 and NS2, which have key functions in the viral replication cycle and the modulation of the host acquired immunity and interferon response[23-30]. These highly attenuated HRSV strains produce protective acquired immunity in chimpanzees and mice[31,32]. Importantly, the simultaneous deletion of NS1 and NS2 proteins has been demonstrated to be poorly immunogenic due to an exacerbated attenuation and poor replication of HRSV in the respiratory tract[33], stressing the relevance of taking viral biology into the rational design of HRSV vaccines. As with non-structural proteins, strains lacking either the transcription anti-terminator M2-1, which promotes the synthesis of long, positive polarity RNAs[34], or the transcription regulator M2-2[35] have been also generated. These mutants confirmed the individual roles of M2 proteins in the virus replication cycle and led to the development of attenuated HRSV strains with reduced infection kinetics and reduced virulence in animal models of infection[36,37] (see also "Novel HRSV vaccines: theoretical principles for their application in humans").

An interesting feature of N, P and M2 structural proteins is their 1) essential role in the assembly of the Transcriptase-Replicase complex and the viral nucleocapsid[38-41], and 2) high degree of genetic conservation, making them interesting targets for the development of vaccines aimed at generating immunity of T cells (refer to Figure 1, upper panel; and Table 1). To that end, a good immunization strategy against HRSV, as well as against the close relative paramyxovirus human metapneumovirus (HMPV), is the use of N, P and M2 proteins as antigens[42-44]. To better achieve delivery of HRSV/HMPV antigens to professional antigen presenting cells (APCs) while inducing their activation, recombinant Bacillus Calmette-Guerin (BCG) bacteria expressing either the HRSV N, HRSV M2-1 or HMPV P antigens have been developed. Professional antigen presenting cells are phagocytes initiating acquired immunity at secondary lymphoid tissues by exhibiting antigens drained from the periphery to T and B cells. As expected, in mice these vaccines are potent inducers of IFN-γ secreting memory $CD4^+$ and $CD8^+$ T cells with the capacity of preventing both viral dissemination in the lower airways and the development of lung damage due to infections with these viruses[42-44]. As with recombinant BCG vaccines, immunization with other vaccine prototypes based on recombinant measles virus (MV) expressing the N or M2-1 proteins of HRSV also elicited a T_H1 response in the cotton rat model, as evidenced by the induction of IFN-γ-secreting $CD8^+$ T cells[45]. Importantly, the prevention of pulmonary pathology and the efficient clearance of HRSV from the lungs occurred despite poor induction of neutralizing antibodies by either recombinant approach, suggesting an instrumental role of T cells in the immunity generated by these BCG and MV vaccines[45].

Recognition of target cells by HRSV occurs through the concerted function of both the attachment glycoprotein (G) and the fusion (F) protein, which bind to glycosaminoglycans, such as heparan sulfate, located in the apical surface of epithelial cells[46-49]. The F protein also binds to nucleolin during the initial contact of virions with the surface of epithelial cells[50], and thereby is considered an attractive target for the generation of neutralizing antibodies. Furthermore, compared with the other two viral glycoproteins, G and SH, the ectodomains of HRSV F proteins have a high degree of conservation, with < 9% variability in most of their functional and antigenic sites[51]. Despite this feature, HRSV infection in mice fails in eliciting a high quality humoral response against F and G, generating antibodies with limited neutralizing activity, and no protective effect against secondary exposures to the virus. Nevertheless, a variety of vaccine prototypes have demonstrated the induction of protective neutralizing antibodies against F and G antigens in animals[45,52-63] (reviewed in detail in reference[64]). The small hydrophobic (SH) is the third HRSV surface glycoprotein and forms viroporins of unknown function in the viral replication cycle[65]. Although compared to the F and G proteins to date there is no *in vivo* evidence demonstrating the generation of neutralizing antibodies specific for SH, two recent studies have demonstrated that immunization with the ectodomain of SH (SHe) elicits a protective, non-neutralizing humoral response, which is thought to mediate protection by activating the destruction of HRSV-infected cells[66,67].

HRSV Infection Elicits a Deregulated Immune Response Leading to Pulmonary Pathology

Understanding of the pathological mechanisms leading to HRSV-induced lung disease is central for the development of safe vaccines. Studies in animal models for HRSV disease had provided compelling evidence that the major phenomenon leading to damage of the airways is the excessive inflammation of the lung parenchyma due to the sequential activation of local innate and acquired immunities[68,69]. Although the antiviral response elicited by HRSV eliminates the virus from the airways, it develops slowly and at the expense of an exacerbated inflammatory response that impairs the respiratory function of the infected lung[70,72].

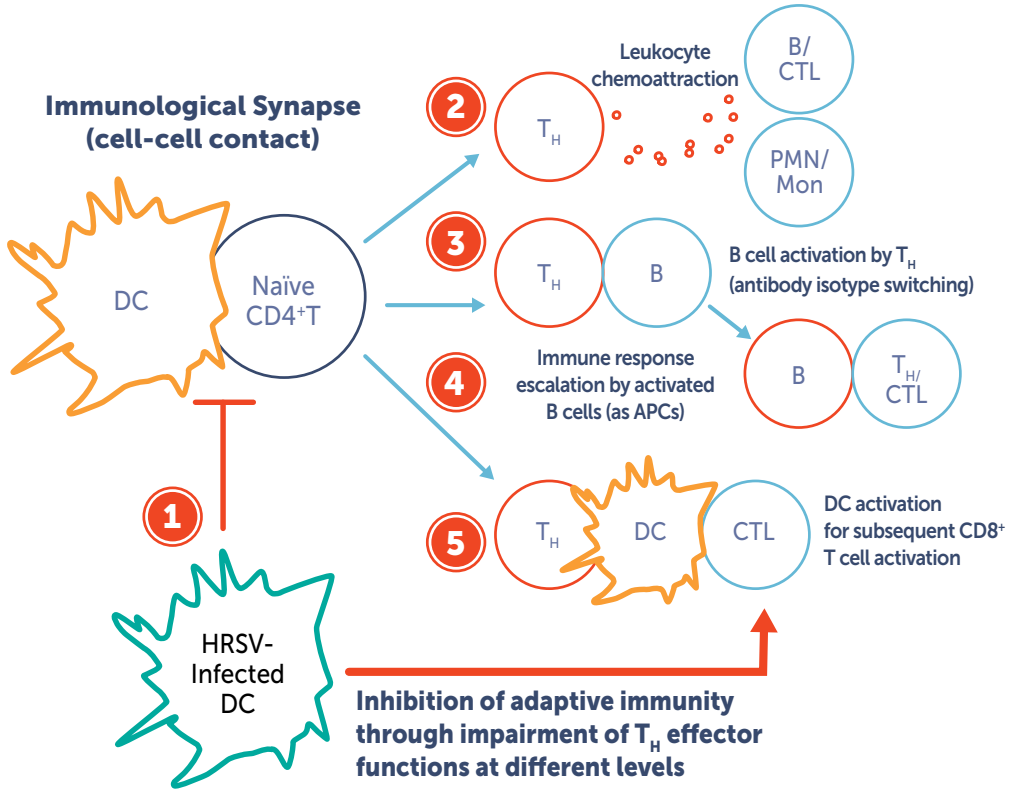
The pathogenesis of lung inflammation starts in the acute phase of infection after HRSV has infected airway epithelial cells of the lower respiratory tract. Ciliated epithelial cells inhabiting the trachea, bronchi, bronchioles and alveolar sacs are the major targets for HRSV infection. Furthermore, HRSV infects $p63^+$ basal cells impairing the proper turnover of ciliated epithelial cells, and increasing the proportion of mucus producing cells[73]. HRSV-infection of airway epithelial cells is central in the inflammatory pathogenesis of the lung due to the secretion of thymic stromal lymphopoietin (TSLP) by the epithelium[74,75]. TSLP is a cytokine that activates and changes the phenotype of respiratory DCs, which acquire a T_H2-prone priming phenotype leading to the expansion of helper T cells secreting cytokines evoking a poor antiviral, but enhanced allergic-like immunological response[74,75]. These cytokines recruit a number of leukocytes into the lung interstitium and the air spaces, including neutrophils, eosinophils, monocytes, T cells and inflammatory DCs[76-79]. Importantly, T cells have been proposed as key mediators of the pulmonary pathology, yet at the same time essential for virus elimination[79]. Similarly, lung damage caused by HRSV has been linked to the deposition (due to reduced clearance) of antibody-antigen immunocomplexes in the lung parenchyma[80], and to the generation of antibodies with low affinity, reduced half-life and poor neutralizing capacity[81,82]. These antecedents suggest that a highly regulated immune response elicited through vaccination, such that inducing antiviral T cells and highly neutralizing antibodies, would be beneficial in the prevention of HRSV-associated acute lower respiratory tract infections.

Impairment of Host Acquired Immunity as a Key Mechanism of HRSV to Avoid Herd Immunity

Importantly, memory of both B and T cell repertoires develops poorly during the resolution of experimental infections in humans[81] and animals[83]. Therefore, it is thought that humans develop a suboptimal acquired immunity to natural infections[84], limiting herd immunity and favoring reinfections throughout life. Compelling evidence suggests that HRSV virulence determinants target dendritic cells, as a more general mechanism to inhibit the acquisition of proper cellular and humoral immunities[85] (Figure 2). HRSV infection leads to impairment of the antigen presenting and T-cell priming capacities of dendritic cells, as well as to a lesser impairment in the capacity of dendritic cells to activate memory T cells[86,87]. The reduced activation of $CD4^+$ naïve T cells results in a reduction in the numbers of effector and memory helper T cells (T_H), and therefore the evidence suggest, this is a key mechanism impairing the orchestration of the acquired antiviral immunity to HRSV[41,69]. Among others, T_H escalate the antiviral immune response through the downstream activation of dendritic cells, naïve/resting $CD8^+$ T cells, naïve/resting B cells and innate phagocytes (through IFN-γ secretion). Moreover, T_H induce isotype switching in B cells sharing specificities for the same pathogen (but not necessarily for the same antigen of the pathogen), and kill infected cells through perforin secretion[88]. Altogether, these antecedents suggest that natural HRSV infections impair $CD4^+$ T cell immunity, and as a result, impair the proper activation of virus-specific B cells leading to a waning humoral response inefficient in controlling reinfections

by HRSV. In support of this evidence, a recent study surveying the frequency of circulating memory B cells producing high neutralizing antibodies against F proteins of HRSV and HMPV, concluded that the frequency of this repertoire is scarce, being present in only 7 of the 200 donors studied[89].

Figure 2. Model of How Dendritic Cell (DC) Infection by HRSV Leads to Impairment of the Host Acquired Immunity



Notes: (1) Infection of dendritic cells by HRSV leads to the impairment of naïve T cell priming and the generation of suboptimal $CD4^+$ T helper memory responses needed for key immunological processes, including: (2) the recruitment of antiviral effector T cells to sites of virus replication; (3) activation and antibody isotype switching of antiviral B cells; (4) escalation of the immune response by antiviral B cells acting as professional antigen presenting cells (APC) for T cells sharing specificity for HRSV; and (5) activation of antiviral $CD8^+$ T cells through dendritic cells previously activated by T_H cells.

The Winding Road Towards Safe and Immunogenic HRSV Vaccines: Lessons Learned From the Formalin Inactivated-HRSV Immunization Incident

In 1966, vaccine prototypes based on the chemical inactivation of HRSV with formalin (FI-HRSV) were studied in four clinical trials aimed at determining their immunogenicity in children <2 years old[90-93]. As with polio vaccines, the direct evaluation of Fl vaccines was accepted with no further ethical requirements, such as previous determination of safety in animal models of infection. Opposite to what was expected, however, immunization with the FI-HRSV vaccine failed in preventing viral pneumonia in vaccinees, most of which displayed an increased inflammatory lung disease, defined as a peribronchial monocytic inflammation with eosinophilia, compared to unvaccinated individuals upon infection with community-acquired HRSV[94]. Eighty percent of vaccinated children were hospitalized by severe lower respiratory tract infections and two died due to respiratory insufficiency[94]. This incident, among others, led to the coining of a new pathological condition promoted by immunization referred to as either vaccine-enhanced disease (VED) or enhanced RSV disease (ERD), which deep-seated concerns regarding the safety of new vaccine prototypes against HRSV, and promoted the development of new regulatory policies for the testing of HRSV vaccines in humans. Since its observation, VED led to challenging scientific questions regarding the immunological mechanisms leading to increased morbidity and exacerbated lung damage in FI-HRSV vaccinees.

Studies in mice have yielded key mechanisms mediating VED due to FI-HRSV immunization, including the generation of: 1) low-avidity and poor neutralizing antibodies that are thought to preferentially bind to formalin-modified epitopes in the F protein[95,96]; 2) deposition of HRSV-antibody immunocomplexes in the lung parenchyma due to a reduced clearance by the immune system[80]; and 3) activation of T cells with a more pathogenic phenotype leading to an allergic-like, unregulated T_H2-like inflammatory response in the lungs[97,98]. Furthermore, in mice VED can be also elicited by a mixed T_H2/T_H1 immunity, and with a reduced participation of eosinophils[99]. Importantly, VED is not limited to FI vaccine formulations, as evidenced by increased respiratory disease in animals immunized with recombinant F protein delivered either as purified protein or encoded in vaccinia Ankara virus, the later of which induced a T_H1 VED[100,101]. The later, warns about potential adverse effects for different vaccine formulations and stresses the need of characterizing vaccine safety empirically considering the functionality of effector T helper subsets, as well as their associated immunoglobulin response in terms of both affinity and virus neutralizing capacity.

Novel HRSV Vaccines: Theoretical Principles for Their Application in Humans

Understanding the immune mechanisms mediating VED has been a central paradigm in the rational design of new generation HRSV vaccines, most of which are safe in animals, and are currently being tested in numerous clinical trials at different phases[17]. As shown in Table 2, a total of 11 vaccine candidates exploiting different vaccination strategies have been developed and are under clinical evaluation. Moreover, these 11 formulations encompass different mixtures of antigens (in different formats), and adjuvants proven safe, immunogenic, protective, and efficacious in animals (Table 2). Most of these approaches aim to the direct induction of acquired immunity in vaccinees, including infants and the elderly, as the main target populations[64]. The vaccination of pregnant mothers has been developed as a secondary strategy that seeks the passive immunization of neonates via the natural transference of neutralizing antibodies transplacentally or through milk. Both strategies have been proven successful in animal models of HRSV infection, and some of the prototypes studied in the clinical setting have provided compelling evidence further supporting such approaches.

Table 2. Vaccine Candidates Against HRSV Tested in Clinical Trials, 2016

Vaccine Strategy	Vaccine Name	Company/ Manufacturer	Clinical Phase	Effects after Vaccination	ClinicalTrials.gov Identifier
Live Attenuated	ΔNS2/Δ1313/1314L	NIAID/NIH	Phase I	Generation of neutralizing antibodies	NCT01893554
	RSV cps2[125]	MedImmune/ NIAID/NIH	Phase I	Ongoing research	NCT01968083 NCT01852266
	MEDI-559	MedImmune	Phase I/IIa	Generation of neutralizing antibodies	NCT00767416
	RSV MedI ΔM2-2	MedImmune/ NIAID/NIH	Phase I	Generation of neutralizing antibodies	NA
	RSV LID ΔM2-2	NIAID/NIH	Phase I	Ongoing research	NA
Target F Protein	MEDI-7510[126]	MedImmune	Phase Ib/II	Ongoing research	NCT02115815 NCT02289820 NCT02508194
	RSV F Nanoparticle	Novavax	Phase II	Reduction of lung viral titter Generation of Palivizumab-like neutralizing antibodies	NCT01704365 NCT02266628 NCT02247726 NCT01960686
	DPX-RSV[127]	Immunovaccine	Phase I	Antigen-specific immune responses to HRSV antigen	NCT02472548
	GSK3003891A	GlaxoSmithKline	Phase II	Ongoing research	NCT01905215 NCT02360475
Adeno-Virus	Ad35.RSV.FA2	Janssen Pharmaceutical	Phase I	Ongoing research	NCT02561871 NCT02440035
	GSK3389245A	GlaxoSmithKline	Phase I	Ongoing research	NCT02491463

Regarding the direct induction of acquired immunity in vaccinees, a useful strategy widely applied in the prophylaxis of paramyxoviruses is the use of attenuated virus strains, which generally show consistent immunogenicity and acceptable safety[102]. Since attenuation holds a strong correlation with safety and immunogenicity, the identification of key proteins having little impact in virus growth, and major effects over virulence, is an essential step in the development of effective, inactivated virus vaccines. Deletion of complete genes (knockouts), substitutions of codons in order to generate proteins with non-functional amino acid residues, or the generation of viral strains with codons poorly utilized by the human ribosomal machinery, also known as codon deoptimization, are the most exploited strategies to generate novel vaccines[32]. For instance, an attenuated HRSV strain integrating the deletion of the NS2 gene, and both the deletion of residue at position 1313 and the substitution of one isoleucine residue by a leucine at position 1314 of the L gene, which is termed ΔNS2/Δ1313/1314L, was demonstrated to be safe in chimpanzees[103] and is currently under study in a Phase I trial (NCT01893554) (see Table 2). Another HRSV strain, termed rA2cp248/404/1030ΔSH is well tolerated in adults and seropositive children, yet it elicits a limited response to a booster dose inducing an increase in anti-HRSV IgG and IgA antibodies in less than 50% of infants aged 1- to 2-months[104] (Table 2). Importantly, a secondary prototype adding 39 silent amino acid substitutions to the rA2cp248/404/1030ΔSH strain, which is termed MEDI-559, has been shown to elicit a significant neutralizing humoral response while retaining attenuation in cotton rats and seronegative children (Table 2)[105]. Nevertheless, a slight increase in the presentation of acute lower respiratory tract infections in vaccinees compared to placebo-receiving infants, suggests a potential VED in MEDI-559 vaccinated children that has to be further studied[106].

Seeking the induction of adaptive immunity against HRSV others have developed vaccines based on protein subunits. For instance, to achieve this, MEDI 7510, a protein subunit vaccine using the post-fusion conformation of F, counteracts the T_H2-polarizing properties of HRSV by using a T_H1-polarizing adjuvant termed glucopyranosyl lipid A (GLA)[107], which is being tested in older adults >60 years in Phase Ib and II clinical trials (NCT02289820, NCT02508194). A clinical study has been performed with a nanoparticle vaccine using the F protein expressed by insect cells[55]. This vaccine prototype was well tolerated in healthy adults aged between 18 and 49 years old, showing no considerable side effects and inducing a consistent increase in anti-F IgGs in most vaccinees[55]. This prototype is currently being studied in seropositive children, older adults >60 years old, and women in their third trimester of pregnancy (NCT02266628, NCT01704365, NCT01709019, NCT02247726, NCT01960686 and NCT02296463 in Table 2).

Regarding prototypes focused in the induction of HRSV-specific T cell responses, the authors have been working on a recombinant BCG-based vaccine expressing the N protein of HRSV manufactured under cGMP standards. Soon it will be tested in healthy adults to determine its safety, tolerability and immunogenicity. Since it was developed using BCG as vector, this vaccine is expected to elicit a bivalent acquired T cell response able to prevent HRSV acute lower respiratory tract infections, and to elicit an anti-mycobacterial immunity comparable to that induced by conventional BCG vaccines, as recently demonstrated in the BALB/c model of infection (Céspedes, unpublished results). Because the viral antigen used in this BCG prototype is the nucleoprotein of HRSV, no interference with maternally derived antibodies, and therefore, no detrimental effects over the antibody response of the infant, are expected. Moreover, protection by this rBCG-N vaccine was achieved with a single, low dose of 3×10^5 colony-forming units per animal (Céspedes, unpublished results). This feature suggests that the rBCG-N is highly immunogenic.

Potential for Maternal Immunization

The strategy of immunizing mothers in their third trimester of pregnancy seeks to mimic the protection conferred by a humanized anti-F monoclonal antibody known as palivizumab. Currently, palivizumab (Synagis®) is the unique prophylactic tool used in high-risk infants <6 months of age to prevent severe HRSV disease[108]. However, it has several drawbacks including being highly expensive, and having a limited demonstrated protection in children aged >1 year[109]. Immunization of pregnant mothers seeks to take advantage of the naturally occurring transplacental transfer of neutralizing antibodies from the mother to the fetus to passively immunize neonates during their first 6 months of life. Maternal immunization is further supported by the good results obtained for other vaccines generating transmaternal immunization, such as those targeting influenza or *Bordetella pertussis*[110], and is expected to contribute in reducing the economic burden generated by the application of repeated palivizumab injections in high-risk infants. The protective capacity of maternally derived antibodies, is further supported by the fact that the severity of HRSV disease is inversely correlated with the amount of circulating neutralizing antibodies in infants[111]. Nevertheless, this immunization strategy has some challenges. For instance, in order to enhance the protective capacity of maternal antibodies, immunization policies should consider the seasonality of HRSV in a given country[108]. Also, high titers of F- and G-specific, transferred antibodies exert an immunosuppressive effect in infants, and therefore, may dampen the development of acquired immunity to these HRSV glycoproteins upon a community-acquired infection[112-114]. Finally, given the risk in pregnancy of systemic inflammatory reactions, vaccine candidates for maternal immunization should have limited reactogenicity[110].

Conclusion

The advances in molecular virology and the understanding of immunity to HRSV infections have yielded key technological and scientific advances paving the road towards a safe, stable and immunogenic HRSV vaccine. In the upcoming years, it is expected that novel vaccination policies aimed to develop an optimal vaccination scheme against HRSV will be developed. Likely, the best immunization scheme will arise from the integration of the two major vaccination strategies; the immunization of mothers and their offspring. Challenges will include mitigating the risk of VED. An integrated vaccination strategy will undoubtedly aid to control global HRSV morbidity, mortality, and economic burden. Importantly, several other vaccines are under development, which may also provide new tools for public health systems and prophylaxis of HRSV-associated acute lower respiratory tract infections, especially in the elderly and people with chronic pulmonary diseases, who are at high risk for severe respiratory disease.

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Vaccines to Prevent Typhoid Fever

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Introduction

Typhoid and paratyphoid fever were highly endemic in many countries in Latin America in the 20th century. This chapter reviews the disease typhoid fever and the vaccines available to prevent it.

Etiologic Agents

Typhoid fever and paratyphoid fever, the "enteric fevers", are acute generalized infections of the reticuloendothelial system, intestinal lymphoid tissue, and gallbladder. *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) is the etiologic agent of typhoid, while *Salmonella* Paratyphi A or *Salmonella* Paratyphi B (or rarely, *Salmonella* Paratyphi C) cause paratyphoid fever.

Epidemiology

Facile transmission of the agents that cause typhoid and paratyphoid fever ensues where populations have poor sanitation and lack access to potable water. Thus, these infections are endemic in many developing countries, while their transmission is rare in industrialized countries. High endemicity is observed in regions of South and Southeast Asia, the Middle East, Northeast Africa, sub-Saharan Africa and some Pacific Islands. In endemic areas typhoid generally comprises ~70–80% of enteric fever and paratyphoid the remainder,[1] but in some areas of South Asia, *S.* Paratyphi A is nearly as common as *S*. Typhi.[2,3] The burden of enteric fever has diminished markedly in Latin America since the early 1990s but endemic foci still persist in Central America, the Caribbean and some regions in South America. When enteric fever was highly endemic in South America, *S*. Paratyphi (mostly B) was responsible for ~one-third of cases.[4]

Endemic typhoid often exhibits seasonality. In Chile, Ecuador and Peru, where typhoid was highly endemic in the 1960s–1980s, there was a summer peak.[5]

Chronic gall bladder carriers constitute the long-term reservoir of *S*. Typhi and *S*. Paratyphi A and B.[6,7] In endemic areas, particularly during "typhoid season", persons with sub-clinical and clinical infection who

are short-term excretors constitute another important reservoir from which the infection is transmitted to susceptibles. Where urinary tract *Schistosoma haemotobium* or *Schistosoma mansoni* infections are co-endemic with typhoid, chronic urinary bladder carriers of *S*. Typhi serve as a reservoir.[8]

Typhoid and paratyphoid infection is almost always acquired by ingestion of food or water vehicles contaminated by human excreta that contain *S.* Typhi or *S*. Paratyphi A or B. In most large cities of North America and Europe in the late 19th and early 20th centuries, the treatment of water supplies by chlorination or sand filtration (or both) broke the cycle of endemicity and diminished the incidence of typhoid, even though the prevalence of chronic carriers in the populations remained high for decades thereafter.[9,10] A South American exception to this pattern was Santiago, Chile, where high endemicity persisted despite 96% of the population having access to potable water and 80% being connected to a sewerage system. In Santiago, sewage water was not treated and during summer (when there was no rain) it was used to irrigate crops (particularly salad vegetables) that were brought to the city's markets, sold and eaten uncooked.[11,12]

Enteric fever is transmitted by either a "short cycle" or a "long cycle" fecal-oral route. Short cycle involves an individual carrier who contaminates food vehicles consumed in proximity by family members or participants at a communal gathering (e.g., wedding), or by a food handler carrier in a restaurant.[13] Examples of short-cycle sporadic cases and outbreaks include families served by the notorious cook, "Typhoid Mary,"[14] and restaurant outbreaks in Texas,[15] Maryland,[13] and New York.[16] Examples of transmission by long-cycle include the contamination of water supplies by sewage,[17] irrigation of crops with untreated sewage,[11] contamination of widely distributed piped municipal water,[17,18] and dissemination of typhoid bacilli via contaminated processed foods transported over long distances.[19] Clinical microbiologists have increased potential exposure to *Salmonella* Typhi in the occupational setting and therefore also constitute a special high-risk group.[20,21]

The Disease

Clinical manifestations of acute typhoid fever vary somewhat depending on the host, the specific strain, inoculum size and vehicle of transmission. The older child or adult with severe clinical typhoid fever exhibits persisting high fever, malaise, abdominal discomfort, and frontal headache. In the pre-antibiotic era the clinical illness progressed over several weeks, culminating in a case fatality rate of ~10–20%.[22,23] The protracted, debilitating nature of this febrile illness in untreated (or improperly treated) cases is accompanied by mental cloudiness or stupor.

In individual patients it is impossible to differentiate on clinical grounds whether the enteric fever is caused by *S*. Typhi or *S*. Paratyphi.[24,25] Full-blown cases begin with malaise, anorexia, fever (that increases stepwise to reach 39°–40°C), abdominal discomfort, and headaches.[23,26,27] Without appropriate antimicrobials, fever persists for at least 10–14 days (and sometimes for weeks, if the patient survives). Appropriate antibiotics cause the fever to diminish stepwise over several days. During the period of sustained fever, ~20% of Caucasians manifest "rose spots", an exanthum seen on the chest, abdomen, and back consisting of subtle, salmon-colored macules, 2–4 mm in diameter, which blanch with pressure and from which *S*. Typhi can be cultured.[28] Constipation or diarrhea may be seen in older children and adults, whereas diarrhea may occur in ~20% of young children with typhoid fever. Although infants may manifest severe clinical forms of typhoid fever, bacteremic *S*. Typhi infection in children younger than 2 years of age can often be remarkably mild and not recognized clinically as enteric fever but rather as a non-descript febrile syndrome.[29,30] A bronchitic cough is common early in the illness in all ages. A particularly severe form of typhoid fever is occasionally encountered in which cerebral dysfunction, including obtundation, delirium or coma, and shock ensue, requiring adjunct corticosteroids plus appropriate antimicrobial therapy to avoid a case-fatality rate that can exceed 20%.[31]

In the preantibiotic era relapses were observed in about 8% of typhoid fever patients. The relapse rate in patients treated with the first (chloramphenicol) and second (ampicillin, amoxicillin and trimethoprim/sulfamethoxazole) generation of antibiotics used for typhoid therapy ranged from 10–25%. Typhoid bacilli can be recovered from bile and bone marrow many weeks after the patient has fully recovered from symptoms. Relapses typically occur ~3 weeks after the last febrile day or 2 weeks after cessation of antibiotics and are clinically milder and shorter than the initial illness and promptly respond to appropriate antibiotics. Following treatment of dug-sensitive acute typhoid with oral fluoroquinolones or azithromycin or after parenteral ceftriaxone, relapse is uncommon.

Two feared complications of typhoid fever, intestinal perforation and hemorrhage, occur in ~ 0.5–1.0% of cases, particularly those who have been ill for several weeks without appropriate antibiotic therapy.[32] These complications are consequent to the prominent lesions in the gut-associated lymphoid tissue. Typhoid can cause complications involving any organ system.[23] Uncommon complications include hepatitis, empyema, osteomyelitis, psychosis, septic arthritis, meningitis, myocarditis, and empyema of the gallbladder.[22,23]

Approximately 1%–5% of patients with enteric fever, depending on age and sex, become chronic gallbladder carriers of the organism (defined as excretion of the pathogen for >12 months following acute infection).[33,34]

Pathogenesis and Immunity

S. Typhi and *S*. Paratyphi A and B are invasive bacteria that efficiently pass from the intestinal lumen across the mucosa, to reach eventually the reticuloendothelial system, where, after an 8–14 day incubation, they initiate a systemic illness. *S*. Typhi and *S*. Paratyphi A and B are highly host-adapted pathogens, as humans comprise the only natural host and reservoir of infection.

In the fasting normochlorhydric stomach gastric acid kills many typhoid bacilli that are ingested, but some foods effectively buffer this acid barrier. After passing through the pylorus and reaching the small intestine, typhoid bacilli rapidly penetrate the mucosa to reach the lamina propria. *S.* Typhi targets M (microfold) cells overlying Peyer's patches and other gut-associated lymphoid tissue,[35] and are then ingested by dendritic cells and macrophages underlying the M cells. The bacilli may also invade enterocytes (absorptive cells) of the small intestine and enter endocytic vacuoles that transit the bacteria to be released into the lamina propria without destroying the enterocyte;[36] *Salmonella* may also pass paracellularly between enterocytes.[37]

Upon reaching the lamina propria in the nonimmune host, typhoid bacilli elicit an influx of macrophages and dendritic cells that ingest the organisms but are generally unable to kill them. Some bacilli apparently remain within macrophages of the small-intestinal lymphoid tissue, while others are drained into mesenteric lymph nodes where further multiplication and ingestion by macrophages take place.

Postmortem studies have documented the inflammatory responses that occur in distal ileum Peyer's patches and other organized lymphoid aggregations. Later in the disease course hemorrhage can occur from these lesions. Gross bleeding comes from eroded vessels in or near the Peyer's patches. When perforations of the bowel wall occur, it is in the same sections of the gut as the hemorrhages.

Shortly after invasion of the intestinal mucosa, a primary bacteremia ensues in which *S.* Typhi is filtered from the circulation by fixed phagocytes of the reticuloendothelial system. Having gained its intracellular haven

throughout the organs of the reticuloendothelial system, the pathogen resides therein during the incubation period (usually 8–14 days) until the onset of clinical enteric fever. Clinical illness is accompanied by a fairly sustained, albeit low level (1–10 organisms/ml), "secondary" bacteremia. During bacteremia, the Vi capsular polysaccharide protects the bacteria from the lytic effects of O antibody (if present) and complement.[38] *S.* Typhi strains lacking Vi are rare[39] and somewhat less virulent than Vi-expressing strains.[40] Typhoid fever bacteremia can persist for several weeks if antibiotic therapy is not given. Symptoms and signs of typhoid fever are not due to circulating endotoxin.

During the primary bacteremia, typhoid bacilli also reach the gallbladder, an organ for which *S.* Typhi has a remarkable predilection,[41,42] and *S.* Typhi can be readily cultured from bile or from bile-stained duodenal fluid in patients with acute typhoid fever.[43-45] In ~2–5% of patients, the gallbladder infection becomes chronic. The propensity to become a chronic carrier is greater in females and increases with age at the time of acute *S.* Typhi infection, thereby resembling the epidemiology of gallbladder disease. The infection becomes chronic in individuals who have pre-existent gallbladder pathology at the time of acute *S.* Typhi infection. Carriers shed as many as 10^9 organisms/g feces but these organisms travel the length of their gastrointestinal tract without penetrating or causing disease.[46]

Following acute *S*. Typhi infection, serum antibodies to somatic O (lipopolysaccharide) and flagellar H antigens appear but, curiously, most patients with acute typhoid fever do not manifest rises in serum anti-Vi antibody.[47,48] In contrast, serum Vi antibody is highly elevated in chronic gall bladder carriers.[47,48] Intestinal secretory IgA antibodies responses to *S*. Typhi can also be detected.

Measurements of cell-mediated immunity (CMI) in patients with wild type infection has been limited in the modern era but CMI responses have been extensively studied in subjects vaccinated with attenuated strains administered as oral vaccines, demonstrating the appearance of classical MHC I-restricted cytotoxic T cells and T cells that secrete cytokines upon exposure to *S*. Typhi antigens.[49]

Diagnosis

Confirming the diagnosis of enteric fever currently requires recovery of *S.* Typhi or *S*. Paratyphi from a suitable clinical specimen. Multiple blood cultures should be obtained from patients in whom the diagnosis is suspected clinically. The isolation rate of *S.* Typhi or *S*. Paratyphi from blood cultures depends on many factors, including the volume of blood cultured, the ratio of volume of blood to volume of culture broth (ideally, the ratio should be > 1:8), inclusion of anti-complementary substances in the broth (e.g., sodium polyanethol sulfonate or bile), and whether the patient has already received antibiotics to which the *S.* Typhi is sensitive. If three 5-ml blood cultures are obtained, *S.* Typhi can be recovered from the blood in approximately 65–70% of untreated suspect cases.

The "gold standard" of bacteriological confirmation of typhoid fever is bone marrow culture, which is positive in 85–95% of cases, even when the patient has received antibiotics.[28,43,44,50] Use of duodenal string devices to obtain bile-stained duodenal fluid for culture is also quite useful.[43] The combination of a duodenal string and two blood cultures generally provides a sensitivity of bacteriological confirmation equal to that achieved with bone marrow cultures, but without the invasiveness of the latter.[43] Culture of skin snips from rose spots also provides a high yield.[28] Stool cultures are generally positive in only 45–65% of cases (somewhat higher in children). Bacteriologic confirmation of *S*. Typhi, *S*. Paratyphi A and *S*. Paratyphi B isolates can be made by agglutination of the isolate with typing sera or by testing its DNA by multiplex polymerse chain reaction (PCR).[51]

Over the years many attempts have been made to develop tests that detect *S.* Typhi antigens in blood, urine, or body fluids, thereby providing a rapid diagnostic test for typhoid fever. With few exceptions, these tests have been disappointing and have failed to warrant the enthusiasm of initial reports. PCR methods have attempted to ampify *S*. Typhi genes from blood.[52-56] However, even these sensitive assays are limited by the fact that the level of bacteremia in typhoid is low (~1–10 organisms per ml of blood). Heretofore, these methods have been amenable only to research laboratories and are not presently available for routine use in clinical laboratories in developing or transitional countries. Significant hurdles will have to be overcome to adapt them to become practical tests for clinical care even in industrialized country settings to diagnose enteric fever in travelers.

Serodiagnosis of typhoid fever was described in 1896 by Widal and Sicard,[57] who reported that the serum from patients with typhoid fever agglutinated typhoid bacilli. Widal tests are still used today in many developing countries to measure agglutinins in serum from patient with suspected enteric fever. The test is more accurate when performed with antigen in tubes rather than on slides. By careful choice of antigen, both O and H antibodies can be selectively measured. Using *S.* Typhi strain O901 (which lacks flagellar and Vi antigens), *S.* Typhi O antibody can be selectively measured. A strain such as *Salmonella* Virginia, that possesses the identical Phase 1 flagellar antigen H:d as *S.* Typhi but shares no O somatic antigens with serovar Typhi, can be used to measure H agglutinins.[58] Most patients with typhoid fever have elevated levels of O and H antibody at the time of onset of clinical illness.[58] The prevalence of H antibodies in adults living in endemic areas is generally too elevated for the test to be useful in that age group but it can be useful as a diagnostic test in children <10 years of age in endemic areas and in persons of any age from non-endemic areas.[58,59] One study from Indonesia supported use of the slide test for O agglutinins of *S.* Typhi, even for adults in that endemic area.[60]

Treatment

The first antibiotic to treat typhoid fever, chloramphenicol, reported in 1948,[61] was successfully used for a quarter century thereafter and remains useful where strains of *S.* Typhi are routinely susceptible. However, large-scale epidemics of chloramphenicol-resistant typhoid fever abruptly occurred, first in Mexico (1972),[62,63] then in Southeast Asia (1974),[64] and then in Peru[65] (1979–1980). The antibiotic-resistance genes were encoded on plasmids of incompatibility group HI1.[62,65] After ~2 years the resistant strains in Mexico and Peru were replaced by chloramphenicol-sensitive *S.* Typhi. Beginning in the late 1980s, *S.* Typhi strains resistant to chloramphenicol, amoxicillin, and trimethoprim–sulfamethoxazole disseminated widely throughout Asia.[66-68] Initially, effective alternative antibiotics included oral ciprofloxacin and parenteral ceftriaxone but widespread use of ciprofloxacin and other fluoroquinolones, often in inadequate dosages and duration, encouraged the emergence of fluoroquinolone-resistant strains.

The management of typhoid and paratyphoid is challenging, particularly where the disease burden is high, there is a dearth of clinical microbiology facilities to confirm the diagnosis and provide antimicrobial susceptibility, and the prevalence of multi-drug resistant strains is high.[69-74] Antibiotic-susceptible, uncomplicated typhoid and paratyphoid can be managed in outpatient settings with chloramphenicol, amoxicillin, ciprofloxacin or ofloxacin. Ciprofloxacin has the advantage of more convenient dosing and lower clinical relapse rates.[70,75]

WHO recommends cefixime as an alternative [76] for treating multi-resistant typhoid but reports of high failure rates in Nepal and Vietnam are concerning.[77,78] Oral azithromycin is another increasingly used first-line therapy in areas of high multi-drug resistant typhoid.[79] Severe or complicated typhoid should, if possible, be treated in hospital with parenteral antibiotics (preferably intravenous ceftriaxone) and careful monitoring to ensure good

clinical outcomes. Switching to an oral agent to which the strain is (or is presumed) susceptible can occur once the patient is afebrile. Prompt administration of high-dose dexamethasone reduces case fatality in patients with severe typhoid fever without increasing the occurrence of complications, carriers, or relapse among survivors.[31,80]

Typhoid and Paratyphoid Vaccines

Ty21a live Oral Vaccine. Ty21a, an attenuated strain of *S.* typhi that is safe and protective as a live oral vaccine, was developed in the early 1970s by chemical mutagenesis of pathogenic strain Ty2.[81] Mutations in this strain include the inability to express Vi polysaccharide and inactivation of the *gal*E gene (encoding an enzyme involved in LPS synthesis), along with ~ two dozen additional mutations. In large-scale field trials with Ty21a involving approximately 465,000 schoolchildren in Chile and 32,000 in Egypt, and approximately 20,000 subjects from 3 years of age to adults in Indonesia, passive surveillance failed to identify vaccine-attributable adverse reactions or other safety issues.[82-87]

Controlled efficacy field trials of Ty21a emphasize that the formulation of the vaccine, number of doses administered, and spacing of the doses markedly influence the level of protection that can be achieved.[83-86,88,89] Two formulations, including enteric-coated capsules and a "liquid" formulation (in which lyophilized vaccine is reconstituted along with buffer powder into a vaccine cocktail), are licensed; however, in recent years only the enteric coated capsule formulation has been manufactured. Based on a field trial in Chile that demonstrated that three doses of Ty21a in enteric-coated capsules given on an every other day schedule conferred 67% efficacy over three years of follow-up and 62% protection over seven years of follow-up,[83,90] this formulation and schedule are used throughout the world except for the USA and Canada where a four-dose regimen is used. The four-dose North American immunization schedule is based on results of a large-scale, randomized comparative trial carried out in Santiago, Chile where recipients of four doses of Ty21a in enteric-coated capsules (every other day schedule) experienced a significantly lower incidence of typhoid than those allocated to receive two or three doses.[89] Ty21a confers significant cross protection against *S.* Paratyphi B [86] but not against *S.* Paratyphi A.[86]

In the mid-1980s, a "liquid suspension" formulation of Ty21a that was amenable to large-scale manufacture was prepared consisting of two packets, one with the lyophilized vaccine and the other with buffer,[85] to be mixed together in a cup containing 100 ml of water for ingestion. Randomized, placebo-controlled field trials in Santiago, Chile[85] and Plaju, Indonesia[86] showed the liquid formulation of Ty21a to be more protective (significantly so in the Santiago trial) than the enteric coated capsule formulation,[85,86] and to protect young children as well as older children. In a randomized controlled field trial in Area Suroriente of Santiago, the liquid formulation of Ty21a conferred 78% vaccine efficacy over five years of follow-up.[91] Disappointingly, this efficient formulation of Ty21a, which is also amenable to immunizing toddlers and pre-school children,[92] is no longer being manufactured.

Vi Polysaccharide Parenteral Vaccine. In the 1970s and early 1980s, purified Vi capsular polysaccharide was manufactured that was 99.8% free of contaminating LPS and was not denatured.[38,93-96] This was an important breakthrough because as little as 5% impurity with LPS can cause systemic adverse reactions in a few percent of recipients.[94] In contrast, highly purified Vi vaccine is well-tolerated and febrile reactions are observed in only 1–2% of subjects. In clinical trials, well-tolerated 25 mcg and 50 mcg single parenteral doses of purified Vi stimulated rises of serum Vi antibodies in the vast majority of vaccinated adults and schoolage children.[94-96] Administration

of subsequent parenteral doses did not boost antibody titers.[97] This is because Vi polysaccharide, like other unconjugated polysaccharide vaccines (e.g., pneumococcal and meningococcal), does not stimulate immunologic memory and the ability to raise antibody titers further by administering booster doses. Passive surveillance carried out during field trials showed the Vi vaccine to be as well-tolerated as the licensed (meningococcal and pneumococcal) polysaccharide vaccines that served as the control preparations in these trials.[95,96]

Two randomized, controlled, double-blind field trials were carried out in Nepal and South Africa to assess the efficacy of a single 25-mcg dose of non-denatured purified Vi vaccine. Over 17 months of surveillance in Nepal, Vi vaccine conferred 72% vaccine efficacy.[96] In South Africa, Vi vaccine provided 64% protection over 21 months of follow-up [95] and 55% protection over 3 years.[98] The Nepal trial included participants from preschool age to adulthood, whereas the South African trial was performed in school-children. A third controlled field trial was carried out in subjects 3–50 years of age in Guangxi, China that evaluated the protective efficacy of a single 30 mcg dose of a Vi polysaccharide vaccine manufactured in China.[99] The vaccine conferred 69% efficacy (95% CI, 28%–87%) over 19 months of follow-up.

Although Vi vaccine provides protection after a single dose, the anti-Vi titers cannot be boosted and the efficacy does not appear to persist beyond three years. Concern over the relatively short-lived duration of protection of Vi was heightened following epidemiologic investigation of an outbreak of typhoid fever that occurred among Vi-vaccinated French soldiers deployed to Ivory Coast.[100] Prior to the outbreak, the standard operating procedure had been to immunize French soldiers with Vi vaccine every five years. The outbreak investigation revealed that receipt of Vi more than three years earlier was associated with a significantly increased risk of developing typhoid fever during the outbreak.[100]

The epidemiologic observations that Vi efficacy endures for only ~3 years fits with a report that monitored the duration of serum Vi antibodies for three years after a single inoculation of adults in a non-endemic area. The percentage of subjects with a putative protective level (1.0 mcg/ml) of Vi antibody fell from 87% at one-month post-immunization to 46% after 2 years and to only 35% at 3 years post-immunization.[101]

In a cluster-randomized effectiveness trial in Kolkata, Vi conferred indirect protection on non-vaccinated subjects,[102] but the same Vi vaccine tested in Karachi in a trial of similar design did not provide indirect protection. In the Kolkata trial the Vi vaccine significantly protected pre-school children, whereas in Karachi the same lot of vaccine conferred no measurable protection for pre-school children. Table 1 summarizes salient characteristics of Ty21a, unconjugated Vi and two licensed (in India) Vi conjugate vaccines.

Table 1. Salient Characteristics of Licensed Live Oral Ty21a and Parenteral Vi Polysaccharide and Conjugated Vi Polysaccharide Typhoid Vaccines

Parameter of Comparison	Ty21a	Vi Polysaccharide	Vi-Protein Conjugates
Route of Administration	oral	parenteral	parenteral
No. of Doses	3 (4 in USA & Canada)	1	1–2
Interval Between Doses	~ 48 hours	–	1–2 months
Well Tolerated	yes	yes	yes
Efficacy	~ 65%	~ 65%	89–100%
Duration of Efficacy	7 yrs	up to 3 years	4 years
Herd Immunity	yes	yes	unknown
Serum Igg Anti-Vi	no	yes	yes
Boostable Immune Responses	yes	no	yes
Cmi (Including Cytotoxic Lymphocytes)	yes	no	not reported
Amenable for Infant Immunization	no[a]	no[b]	yes
Protects Against Vi-Negative Strains	presumably	no	no
Protects Against S. Paratyphi	*S.* Paratyphi B only	no[c]	no[c]
Recommended for Pregnant Women	no	yes	likely to be
Large-Scale School-Based Vaccination	yes	yes	yes
Effective in Endemic Population	yes	yes	yes
Effective in Travelers	yes	yes	not tested

[a] Enteric coated capsules cannot be administered to infants

[b] Vi polysaccharide is a T-independent antigen that is poorly immunogenic in infants

[c] *S.* Paratyphi A and B do not express Vi.

New Generation Typhoid

Vi Conjugates. Vi polysaccharide has been conjugated to carrier proteins such as recombinant exotoxin A of *Pseudomonas aeruginosa* (rEPA),[103,104] diphtheria toxin protein CRM_{197},[105,106] and tetanus toxoid,[107-109] to increase the immunogenicity of these parenteral vaccines by conferring T-cell-dependent properties upon the antigen, including the induction of immunologic memory. Pre-licensure trials have shown differences in the patterns of anti-Vi responses of the different Vi conjugate vaccine candidates, suggesting that differences among the vaccines in the carrier protein and conjugation method used, amount of polysaccharide and other factors may impact immunogenicity. Widely-spaced booster parenteral doses of some Vi conjugate vaccines given to adults and children in endemic areas have increased the titers of antibody over those elicited by a priming dose, suggesting induction of immunologic memory.[104,110,111]

Efficacy data from field evaluations are available for two Vi conjugates. A pre-licensure randomized, controlled field trial of a 2-dose regimen (6 weeks apart) of Vi-rEPA in children at 2–4 years of age in Vietnam's Mekong Delta demonstrated 91.5% vaccine efficacy (95% CI, 77.1–96.6%) over 27 months of active surveillance[110] and 82% efficacy (95% CI 22.3–99.1%) during an additional 19 months of follow-up that utilized a passive surveillance system.[111] There

has also been a post-licensure effectiveness evaluation of the Vi-TT conjugate Pedatyph™.[112] The design of the latter trial was not rigorous and there are many details that are not described. Nevertheless, in this comparison there were no cases of confirmed typhoid fever during 12 months of surveillance among 765 recipients of 2 doses of vaccine (6 weeks apart), while 11 confirmed typhoid fever cases were recorded among 860 unvaccinated schoolchildren.

Two Vi conjugates consisting of Vi linked to tetanus toxoid, Pedatyph™ and Typbar-TCV®, produced in India, have been licensed by the national regulatory authority. Immunogenicity data are available for both vaccines,[107-109] and some efficacy data are also available for Pedatyph™ and Typbar-TCV.[112] The Advisory Committee on Vaccines and Immunization Practices of the Indian Academy of Pediatrics has recommended use of the Typbar-TCV conjugate for children as young as six months of age.[113]

Extensive immunogenicity data from clinical trials with Typbar-TCV document this conjugate's immunogenicity in infants as young as six months of age, its' ability to elicit significantly higher, longer-lasting and higher avidity anti-Vi antibody titers than recorded among recipients of unconjugated Vi polysaccharide.[109] Typbar-TCV also conferred upon adult Oxford volunteers markedly higher protection (87.1% VE) against experimental challenge with virulent *S.* Typhi than protection conferred by unconjugated Vi polysaccharide (52.3% VE) in a randomly allocated, placebo-controlled trial when the readout was fever (38°C) followed by a positive blood culture.[114] An application for pre-qualification of Typbar-TCV was submitted to the World Health Organization (WHO) in 2017. Also in 2017, WHO's Scientific Advisory Group of Experts (SAGE) committee voted that Vi conjugate vaccine should be administered to infants as young as six months of age as a single dose and that accompanying community-wide "catch-up campaigns" in children > 6 months to school age should be encouraged where feasible. Table 2 summarizes target populations by age and the immunization strategies and regimens to vaccinate those sub-populations by harmonizing Vi conjugate administration with existing EPI visits or campaigns.

Table 2. Strategies for Vaccinating Sub-Populations with High Disease Burden with Vi Conjugate Vaccines and Immunization Regimens

Disease Burden and Target Population	Immunization Strategy for Delivering Vi Conjugate Vaccine	Immunization Schedule
High incidence in toddlers and pre-school children (12–59 months of age)	Expanded Program on Immunization (EPI)	**Option 1:** Two doses, the first given at age ~ 9 months in conjunction with measles containing vaccine 1 (MCV1) and the second at age 15–18 months in conjunction with MCV2 **Option 2:** Two or three doses to young infants in conjunction with pentavalent vaccine[a] **Option 3[b]:** Two doses, one given in conjunction with pentavalent-2 or pentavalent-3) and the second in conjunction with MCV1
High incidence in school age children	School-based immunization or combined with measles vaccination campaigns	Single-dose
High incidence in young adults	Mass immunization campaigns in conjunction with other vaccines[c]	Single-dose

[a]Pentavalent vaccine (or DPT where pentavalent is not used) is given at 6, 10 and 14 weeks in sub-Saharan Africa and at 2, 4 and 6 months of age in many countries in South Asia and in Latin America

[b]Although this option is plausible for certain pediatric populations where the disease incidence is high in toddlers and young pre-school children, there are no clinical trials that have been reported where this regimen has been tested

[c]For example, in conjunction with Japanese encephalitis virus vaccine campaigns in Asia or with MenAfrivac campaigns in Africa

Single-Dose Live Oral Vaccines. Engineered recombinant strains of *S.* Typhi that contain precise attenuating mutations have been shown to be well tolerated and immunogenic after ingestion of a single oral dose in Phase 1 and 2 clinical trials. Live oral vaccine candidates include strains M01ZH09,[115-117] Ty800,[118] CVD 908-*htrA*[119,120] and CVD 909.[121,122]

Prevention and Control

Safe water and food. Since enteric fever pathogens are typically acquired via the ingestion of contaminated water or food, enteric precautions should be taken when living or traveling in endemic areas. Only treated (boiled or chemically treated) water should be consumed and foods that may be fecally contaminated (e.g., uncooked salad vegetables) should be avoided.

Conclusion

Both for the prevention of disease in populations in typhoid-endemic countries and for travelers from industrialized countries to regions of the world where typhoid is endemic or epidemic, parenteral (including a new Vi conjugate vaccine) and oral vaccines currently exist to protect against typhoid fever. Widespread use of these vaccines can diminish the burden of typhoid worldwide. Additional Vi conjugates and new live oral vaccines to prevent typhoid are in clinical development. Moreover, parenteral conjugates and live oral vaccines to prevent paratyphoid fever are also in clinical development.

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Varicella and Varicella Vaccines

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Introduction

Diseases resulting from infection with the Varicella-Zoster Virus (VZV) cover a broad clinical spectrum: from typical varicella to serious VZV manifestations or bacterial superinfection. Immunosuppressed individuals, pregnant women, newborns, and the elderly may suffer from severe forms of varicella or herpes zoster. The infection is endemo-epidemic leading to outbreaks in child-care centers, schools, assisted living facilities, and hospitals.[1,2]

Efficient vaccines are available to prevent these diseases. The incidence and the hospitalization rates of varicella have changed dramatically in countries with universal varicella vaccination, as well as due to the vaccination of individuals at an increased risk of serious disease. Universal varicella vaccination significantly decreases the frequency of VZV diseases in vaccinated and unvaccinated individuals. Some vaccinated individuals acquire the disease, especially those who receive one dose of the vaccine, but the clinical manifestation is mild (fewer than 50 lesions and no fever).[1,2]

The World Health Organization (WHO) encourages countries to implement universal varicella vaccination whenever infection by VZV is considered a public health problem and/or based on the socioeconomic impact. Sustained vaccine coverage ≥80% may be attained with an affordable vaccine. In Latin America (LA), the varicella vaccine is included in the National Immunization Programs (NIP) in Argentina, Brazil, Colombia, Costa Rica, Ecuador, Panama, Paraguay, and Uruguay.[1,2]

Etiology and Pathogenesis

Together with herpes simplex types 1 and 2, the VZV belongs to the family *Herpesviridae,* within the subfamily *Alphaherpesviridae*. Varicella is the manifestation of the primary infection caused by the VZV. After primary infection with VZV, the virus remains dormant in the sensory nerve ganglia and can reactivate later in life, causing herpes zoster.[3,4]

The structure of the virus consists of a central nucleus comprising (linear double-stranded) DNA, encased within an icosahedral capsid. It is enveloped by a lipid envelope, developed upon separation from the infected cell. The envelope contains protein and glycoprotein spikes needed for attachment to the infected cell. It is heat labile at room temperature and it deactivates outside of the cell.[4]

The VZV enters the body mainly through the upper respiratory tract and via the inhalation of aerosolized droplets from respiratory tract secretions of patients with varicella. It may enter also through the conjunctiva. The vesicles comprise a large amount of the virus. Direct contact with the vesicles or aerosolized droplets from vesicular fluid may result in infection. Upon replication in the upper respiratory tract mucosa, it spreads quickly to the regional lymphatic tissues; a second round of viral replication takes place in the liver and spleen at days 4 to 6, followed by a secondary viremia at 14 to 16 days following the onset of the infection. The secondary viremia invades capillary endothelial cells and the epidermis, producing intercellular and intracellular edema leading to the formation of the vesicles. The incubation period, the time from when the virus entered the body to when vesicles appeared on the skin (exanthema) and mucosal membrane vesicles (enanthema), ranges mainly between 14 and 16 days, with a minimum of 10 days and a maximum of 21 days. The incubation period may extend up to 28 days if the individual received post-exposure prophylaxis with gammaglobulin. Affected individuals are contagious one to two days prior to exanthema and until all of the lesions have crusted over.[1–6]

The VZV remains dormant in neurons or satellite cells of the sensory ganglia, without being recognized by the immune system. Seemingly, this "immune evasion" allows them to remain intact.[3,6]

Varicella infection usually confers immunity for life to immunocompetent individuals; clinical reinfection after re-exposure to the VZV is rare but does not prevent latent infection. Cellular and humoral immunity are acquired a few days after onset; cellular immunity limits primary infection and prevents reactivation. The antibodies (immunoglobulins A, M, and G) peak at 4 to 8 weeks after varicella or herpes zoster infection and remain high for 6 months. IgG antibodies remain detectable for decades in immunocompetent individuals.[1–6]

Immune mothers confer protection to their newborns in the first few months of life through the passive transfer of antibodies in the placenta. Modification of cellular immunity predisposes individuals to herpes zoster infection but does not completely compromise immunological response to VZV. For example, older adults with reduced cellular immune response have no recurrent varicella. On the other hand, younger children may suffer from varicella even with detectable levels of prenatal antibodies and there are cases of modified or breakthrough varicella in previously vaccinated children who suffer from leukemia, in spite of having a detectable humoral or cellular immune response to VZV.[4]

Epidemiology

Varicella is a disease with worldwide distribution and variable epidemiology dependent on the climate, population density, and the risk of exposure linked with universal varicella vaccination. In temperate countries without universal varicella vaccination, most of the individuals are infected before their early adult life (10% remain susceptible); incidence is higher in individuals younger than 15 years of age, predominately between 1 and 4 years of age; incidence peaks in winter and spring. In tropical countries, acquisition of infection occurs at older ages; amongst children younger than 15 years of age, predominately in the 5 to 9 years of age. Adolescents and adults are highly susceptible. Incidence peaks over the dry months. Seroprevalence in adults is lower for populations residing on islands or in rural areas.[1,2,7]

Human beings are the exclusive VZV reservoir and the virus is highly contagious. Upon exposure, it infects individuals who have not acquired the disease or received vaccination. Individuals infected with varicella or herpes zoster transmit the disease from person to person, there is no fomite transmission.[1–4]

Pregnant women are rarely infected since they are usually seropositive due to an earlier varicella infection or vaccination. The clinical manifestation in pregnant women is more severe if they become infected during the third quarter due to the frequency of VZV-induced pneumonia and visceral dissemination. Fetal infection results from prenatal or hematogenous transmission of the virus during the viremic phase of the maternal infection, and it is more likely to develop when infection occurs before week 20 of the pregnancy.[1–4]

Varicella-infected patients are highly contagious in the family setting as well as at schools, recreational facilities, residential facilities, prisons, military units, and hospitals. The attack rate in susceptible cohabitants is between 80% and 90% and there is a higher number of vesicles in these cases. Without universal varicella vaccination, 10% of individuals remain susceptible in the early adult years; many may have an increased risk of exposure or acquisition of severe infection, such as individuals who work with children (teachers), health workers, pregnant women, individuals with severe chronic diseases or immunosuppressed individuals.[1,2,4–6]

In countries with universal varicella vaccination, there may be a shift in the infection to impact older children aged 9 to 11. Varicella in vaccinated individuals, in particular if one dose was administered, is usually mild with fewer than 50 lesions, mostly without vesicles or fever. These cases are only a third as contagious as individuals infected with typical varicella but transmission of the infection has been documented. Frequently, the index case remains unidentified. This situation may result in varicella outbreaks at schools. Mild varicella is also known as modified varicella or breakthrough varicella: it is the most frequent clinical presentation of vaccine failure, even though typical varicella is still possible.[3,4,7,8]

Burden of the Disease in Latin America

The most conservative estimates indicate that there are 1,420,000 cases worldwide of varicella every year: 4.2 million are severe cases and approximately 4,200 deaths occur.[2] In 2012, a meta-analysis to estimate the burden of disease in Latin America and the Caribbean was published. Incidence was 42.9 per 1,000 individuals annually (95% CI: 26.9–58.9) in the population aged 5 and younger. The hospitalization rate was 3.5 per 100,000 population in individuals younger than 15 years of age (95% CI: 2.9–4.1) and the hospitalization period averaged 5 to 9 days. The most frequent complications were: skin infection (3% to 61%), respiratory tract infection (0% to 15%), and neurological problems (1% to 5%).[1,9]

Recently, new data were published on the burden of disease for varicella in Latin America. When analyzing the data consideration should be given to whether the disease is notifiable, and whether the surveillance is passive or through sentinel sites.[1]

In Argentina, the National Health Surveillance System recorded between 150,000 and 180,000 annual cases of varicella during 2008–2013; the estimated rate was 250–450 cases/100,000 population. VZV is a notifiable disease but there is significant underreporting amongst outpatients. Children younger than 10 years of age are most affected: the specific incidence per age is higher between 12 and 48 months. During 1997–2012, an estimated 17 deaths per year were reported, and approximately 60% of the deaths were amongst children younger than 10 years of age. Argentina is estimated to have between 350,000 and 400,000 cases per year. In 2015, the National Immunization Program introduced the vaccine to be administered in one dose at 15 months.[1,5]

In Brazil, varicella is not a notifiable disease, so reporting is passive. There are no consistent data to estimate incidence. During 2000–2013, the number of cases reported by the Ministry of Health was 7,113, with 3,444 hospitalizations, and 1,503 deaths (39% in children aged 1 to 4 years). However, three million cases are estimated to occur every year. In 2013, Brazil introduced the tetravalent vaccine (measles-rubella-mumps-varicella) in the National Immunization Program for children between 15 months and 2 years as long as they had been previously vaccinated (at 12 months) with a dose of the triple viral vaccine (MMR). In 2017, the period to be vaccinated with the viral tetravalent vaccine was extended to 5 years of age.[10–12] In 2002, the city of Florianopolis implemented vaccination for children younger than 2 years of age in Brazil. A 75% reduction was observed in the incidence of varicella in the 1–4 years age group. In 2015, at the Meeting of the European Society of Pediatric Infectious Diseases, a case-control study performed in Goiânia and São Paulo was presented, with 74% and 78% vaccine coverage, respectively. The group of children infected with varicella had a lower rate of vaccinees (18.8%) as compared to the group that did not acquire the infection (control group) comprising 54% vaccinees. Vaccine efficacy was 86.5% (95% CI: 70.2%–94.1%) for mild/severe cases.[13]

In Chile, there are 21 sentinel sites throughout all regions of the country. During 2008–2012, the average number of reported cases was 2,135, and was 1,661 in 2013. Varicella reached rates of 16 to 39 per 10,000 population during 2007–2013; the rate was 39.4 in 2011. Children between 1 to 9 years of age were the most affected and accounted for 70% of the cases.[14]

In Colombia, an incidence of 140/100,000 population was reported during 2005–2009, and increased to 213/100,000 population during 2010–2015. The highest incidence occurred in the 1 to 9 years age group (accounting for 67.4% of the cases). During 2012–2015, 2,126 varicella cases were reported in pregnant women accounting for 0.3% to 0.8% of the cases reported yearly. During 2012–2015, 5,488 hospital admissions (averaging 1,372 cases/year) were reported, accounting for 1% to 2% of the total number of cases; children younger than 5 years of age were most affected, followed by the 15–24 year age group and those older than 60 years. In the same period, there were 114 deaths due to varicella. In July 2015, the varicella vaccine was introduced into the National Immunization Program as part of a two-dose schedule (12 months and 5 years).[1]

In Costa Rica, varicella is a notifiable disease. During 1991–2006, the annual rates ranged between 400 and 800 cases/100,000 population. In 2007, the varicella vaccine was included in the National Immunization Program for children aged 15 months. For 8 years during the pre-vaccine period, the Infectious Disease Department of the National Children's Hospital recorded 432 discharges of complicated varicella cases, including 58% amongst children younger than 2 years of age. The average hospital stay was 5 days (ranging from 1 to 44 days) and mortality was 2.8%. Eight years after the introduction of universal varicella vaccination, and upon averaging coverage at 84.3% (ranging from 76% to 95%) in the target population, the reduction of incidence was 73.8% for the total population and 79.1% for children younger than 5 years of age. The reduction of hospitalizations was 85.9% in the general population and 87% in children younger than 5 years of age. These data demonstrate an important herd effect.[1,15–16]

In Mexico, varicella is a notifiable disease but cases are believed to be underreported. The incidence of varicella is cyclical, peaking every 4 to 5 years. A total incidence that ranged between 2.33 and 3.81/100,000 population, with a 2.98 median was reported during 1995–2010. Most of the infected population was younger than 10 years of age. The National Health Surveillance System reported meningoencephalitis in 4.6% of hospitalized varicella cases, pneumonia in 2.5%, and other complications in 18%. The varicella vaccine is not included in the Mexican National Immunization Program; however, it is indicated for populations at risk: children attending day-care centers, immunocompromised individuals, pediatric cancer patients (following the safety criteria established for application), and staff at day-care centers and assisted-living facilities, who have not acquired the disease or

have seroprotection.[1] In 2017, a publication analyzed data about hospitalization for varicella from the National Information System for Epidemiological Surveillance (SUIVE) from 2000 to 2013. The average number of annual cases of varicella was 296,733, mostly in children under 9 years (57%) and mostly during March to May. From 2004 to 2012, hospital discharge of varicella included 17,398 cases, of which 4.6% had meningoencephalitis, 2.5% had pneumonia and 18% had other complications.[17]

In Paraguay, varicella is a notifiable disease. During 2007–2012 before the introduction of the vaccine, the annual average number of cases was 3,500, ranging from 2,000 to 4,200. In 2013, universal varicella vaccination was introduced with one dose at 15 months.[6]

In Peru, varicella was not a notifiable disease until 2016. Before 2016, the Regional Health Directorates (DIRESAS) reported a yearly average of 4,000 cases during 2009–2015, and 36,296 medical consultations during 2009–2014. Seventy-nine percent of the individuals who received care were younger than 11 years of age. In 2016, 9,977 cases were reported and outbreaks of severe varicella were recorded.[1,18,19] A retrospective study conducted amongst 1,073 patients hospitalized for complicated varicella at the National Institute of Children's Health (INSN) observed that 72% developed skin and soft tissue superinfection, and neurological (18%) and ocular complications (8%). Sixty-nine cases (6%) suffered from severe varicella with complications. Most of the cases were in children aged 2 and 5 years (46%). Fatality was 1.2% due to necrotizing varicella and pneumonia. Peru also has data on the health care cost for hospitalized patients.[20,21]

In Uruguay, varicella is a notifiable disease. It was the first country in Latin America to include varicella vaccination as part of the National Immunization Program in 1999, with a dose at 12 months. As of 2014, two doses are administered (12 months and 5 years). Coverages have ranged between 95% and 97%. In the pre-vaccine period, there were outbreaks every two to three years. Until 2007, those affected were not vaccinated. Starting in 2010, 70% of the cases were reported amongst vaccinated individuals due to the increase of vaccinated cohorts, mostly as outbreaks in schools. During the vaccine period, the majority of infected individuals had fewer than 50 lesions, did not require hospitalization, and no deaths were reported. In the pre-vaccine period, annual reporting reached about 5,000 cases. In 2009, 1,000 cases were reported. During 1989–1998, incidence in the general population averaged 148/100,000 population (95% CI: 136–144), decreased to 39 (95% CI: 36–40) in 2000–2012, accounting for a 73% reduction. The rate continued to decrease to 20/100,000 population in 2009, and remained nearly unchanged in the following years until the rate reached 58/100,000 in 2013. This increase was related to mild varicella outbreaks (breakthrough infections) in vaccinated children during school outbreaks. In 2014, the second dose was added. The rates in 2014 and 2015 were 40 and 41 per 100,000, respectively.[1,7,22] Hospitalizations (including intensive care) decreased significantly by 81% in children younger than 15 years of age and by 94% in children aged 1 to 4 years. There was a significant reduction in outpatient care (-87%). The association between varicella and severe infections due to *S. pyogenes* or *S. aureus* in vaccinated individuals has not been described during the vaccine period. Vaccination with one dose significantly reduced morbidity, hospitalizations and mortality. The addition of a second dose will contribute to controlling outbreaks and will increase individual protection as well as the herd effect already acquired.[1,7,8]

In Venezuela, varicella is a notifiable disease. During 2007–2014, 267,282 cases were reported. The highest incidence was in children ranging from 12 months to 14 years of age (59% of the cases). Varicella ranks ninth as the most frequent cause for medical consultation. During 1989–2011, 1,072 deaths were attributed to varicella. In 2014 and 2015, the incidence rate was 146.17/100,000 population (44,153 cases) and 146.69/100,000 population (44,922 cases at week 40), respectively. The annual average number of deaths was 30 throughout all age groups. However, during the years 1994, 2001, and 2008, 90 deaths were reported annually.[1]

Outbreaks

In the Latin American countries with universal varicella vaccination, mild outbreaks clearly prevail at schools. In countries without universal varicella vaccination, outbreaks continue to occur every 3 to 4 years with cumulative cases in cities or regions with severe clinical manifestations and fatalities.[7,8,15,17,18]

Clinical Manifestation

Varicella

The incubation period ranges between 14 and 16 days and is asymptomatic. The typical clinical picture starts with mild fever and malaise over 24 to 48 hours before the onset of exanthema and enanthema. The vesicles appear in successive rashes 6 to 24 hours apart and are significantly pruritic. Initially, the lesions are macules or erythematous papules; within hours they develop a central vesicle and evolve to crusting. The polymorphic exanthema develops from the trunk to the extremities and the scalp. The three types of lesions co-exist in the same area. Unvaccinated individuals present between 250 and 500 vesicles. Seven to 14 days after the onset of the exanthema crusting resolves mostly without scarring. Usually it is a benign and self-limiting disease; occasionally it may leave sequela or turn fatal. Asymptomatic primary infection is very rare. Symptomatic reinfection is infrequent in immunocompetent individuals.[1–5]

The most frequent complication is bacterial superinfection (impetigo or cellulitis). It may evolve into fasciitis, necrotizing cellulitis, myositis, bacterial pneumonia or sepsis. The germs involved are mostly *Streptococcus pyogenes* and *Staphylococcus aureus*. Toxic shock syndrome is a rare but very severe complication. In terms of frequency, it is followed by central nervous system impairment: acute cerebellar ataxia (1/4,000 cases) and encephalitis (1.7/100,000 cases). Ten percent of cases are left with sequela and mortality ranges between 5% and 20%.[1–4]

Other infrequent complications include: aseptic meningitis, Guillain-Barré syndrome, transverse myelitis, thrombocytopenic purpura (generally 1 to 2 weeks after the onset of the disease), Reye's syndrome, arthritis, glomerulonephritis, myocarditis, pericarditis, hepatitis, orchitis, and neutropenia.[1,2,4,5]

In general, the following persons are at high risk of developing severe symptoms: immunocompromised individuals, susceptible pregnant women, newborns whose mothers acquired varicella in the perinatal period, and healthy adolescents or adults.[2,4,5]

In immunosuppressed individuals with impaired cellular immunity (persons living with the human immunodeficiency virus/acquired immunodeficiency syndrome [HIV/AIDS], patients with leukemia or solid tumors, solid organ transplants or hematopoietic cell recipients, patients under extended corticosteroid or immunosuppressive treatment), varicella has higher morbidity and mortality rates; with numerous vesicles and persistent fever; visceral dissemination of VZV (pneumonitis, hepatitis, central nervous system impairment); hemorrhagic varicella (vesicles with hemorrhagic content) and recurring herpes zoster are the most frequent. Complications affect 40% of these cases.[4,5]

Congenital varicella syndrome (CVS) and perinatal varicella are potentially extremely severe. The incidence of CVS in pregnant women is approximately 1–5/10,000 pregnancies based on the risk of exposure. The risk of fetal infection is about 25%; 1% to 2% of infected individuals during the first 20 weeks of the pregnancy may suffer from congenital malformations. This is the highest risk period. CVS is characterized by low birth weight, hypoplasia/aplasia, and paresis in the extremities, rudimentary fingers and skin scars. Neurosensory features include: microcephaly, cortical and cerebellar atrophy, psychomotor retardation, seizures, chorioretinitis, optic atrophy, blindness, cataracts, nystagmus, and microphthalmia, and hearing loss. These extremely severe cases may result in high incidence of zoster during childhood, as well as fetal and child mortality.[1,2,4,5]

Regarding perinatal varicella, when the maternal disease occurs between 5 and 21 days before delivery, the neonatal infection manifests in the first 4 days of life; prognosis is generally good with transplacental transmission of antibodies. When the diagnosis of maternal varicella occurs within 5 days before and up to 48 hours after delivery, the newborn is at high risk of suffering from severe varicella with pneumonitis, hepatitis or encephalitis; due to the lack of passage of antibodies to the newborn and immunological immaturity. Mortality may reach 30%.[1,2,4,5]

Varicella in adolescents and adults may result in higher fever, greater general impairment and higher number of lesions. About 10% of such cases are left with scars or severe complications, including pneumonia. Their risk for hospitalization is nine times higher and the risk to suffer from encephalitis is seven times higher than in children. Fatality rates in healthy adults are 30 times higher than in children. Susceptible pregnant women have an even higher risk of severe disease and complications.[1,4,5]

Herpes Zoster

Ten to thirty percent of individuals who have acquired varicella may suffer from herpes zoster at about 50 years of age. Infected individuals have painful erythematous vesicular exanthema with grouped lesions, following sensory dermatomes. VZV is transmitted through direct contact with the vesicles and may cause varicella in susceptible contacts. About 15% of the patients experience pain or paresthesia in the affected dermatome for several weeks or months (post-herpes neuralgia). Herpes zoster in children is usually milder than in adults; it is more frequent in patients with HIV/AIDS. In immunocompromised individuals, it may affect several dermatomes, spread to the skin beyond the primary dermatomes (the pancreas, lungs, liver, and the central nervous system) and may be fatal.[2,4] In México from 2000 to 2013, 7,042 discharges due to herpes zoster were notified, mainly in patients 65 years or older, in a female-male ratio of 1.3:1. The most frequent complications were: neuralgia (11%), eye involvement (7%), meningoencephalitis (5.4%) and disseminated disease (2.8%).[17]

Diagnosis

Diagnosis is clinical and difficult to establish in vaccinated or immunocompromised individuals. The presence of the virus may be confirmed in vesicle, tissue or body fluid samples through polymerase chain reaction (PCR) techniques that detect the DNA or viral culture. The PCR may differentiate the natural virus from the vaccine virus; as it is highly sensitive. The viral culture is less sensitive but it may differentiate the VZV from the herpes simplex virus; yet it is costly and the result takes weeks. Viral antigens may be detected in material from lesions through direct immunofluorescence (marked antibodies). The observation of multinucleated giant cells (inclusion bodies) is a less sensitive method than antigen detection and it is not VZV specific.[1–4]

The detection of IgG serum in the acute and convalescent phase is a specific method with low sensitivity. The detection of IgM in the acute stage is a specific method but it is not the most reliable to either confirm or rule out infection.[3,4]

The serology to assess past infection or response to vaccination is difficult to interpret. Absence of antibodies does not imply susceptibility since cellular immunity controls viral replication. About 20% of individuals older than 55–65 years of age do not demonstrate measurable cellular immunity, despite having antibodies and a history of varicella.[3–5]

Treatment

The treatment of varicella with acyclovir or valacyclovir (administered orally) reduces the duration and severity of cutaneous and systemic manifestations. It is not recommended for healthy children. It is indicated during the first 24 hours of the disease (72 hours maximum) in: individuals older than 12 years of age, carriers of mucocutaneous and chronic pulmonary diseases, immunocompromised individuals under treatment with corticosteroids for extended periods (chronically or intermittently), and individuals under acetyl salicylic acid (ASA) treatment. Some experts recommend treatment of secondary intrafamilial cases. Intravenous therapy is indicated in immunosuppressed individuals. Valacyclovir is approved for the treatment of varicella in individuals between 2 and 17 years of age. Treatment of herpes zoster (orally) should be initiated promptly (before 72 hours) in immunocompetent individuals. Immunocompromised individuals or patients requiring hospitalization should be treated intravenously with acyclovir.[2–4]

Prevention

Primary prevention of VZV infection may be active through vaccination or passive through the administration of specific anti-VZV antibodies (immunogenicity).

Varicella Vaccine

The live attenuated vaccine is prepared with natural Oka strain, disseminated in cellular cultures and attenuated. In 1970, it was developed in Japan by Professor Michiaki Takahashi. It contains gelatin and residual amounts of neomycin. The monovalent vaccine is approved in immunocompetent individuals aged 12 months and older. The tetravalent vaccine (MMR) was approved more than 10 years ago in children between 12 months and 12 years of age. According to the Latin American Society for Pediatric Infectious Diseases (SLIPE), in some Latin American countries this vaccine is approved for administration as of 9 months.

As of 2016, vaccine availability in Latin America includes: Varivax (Merck & co.), Varilrix (GSK), Priorix Tetra (GSK), Varicella Vaccine (Biken) with Oka strain, and Suduvax (Green Cross) with Corea MAV/06 strain. The WHO does not specify the minimum number of plaque-forming units (PFU) required. The licensed varicella vaccines guarantee a PFU content range between 1,000 and 17,000 PFU. In several randomized studies, the efficacy of

one vaccine dose has been shown to range between 90% to 100% with 10,000 and 17,000 PFU. The vaccines are administered subcutaneously. Upon reconstitution of the freeze-dried solution, vaccine administration should occur within 30 minutes. The freeze-dried vaccine is stored refrigerated (2–8°C) and protected from light, to ensure stability for the two years of its shelf life.[1,23,24]

Immunogenicity

Between 76% and 85% of healthy children vaccinated with one dose develop a humoral immune response to VZV at levels considered protective: ≥5 units/ml in glycoprotein-based enzyme-linked immunosorbent assays (gpELISA) or fluorescent antibodies to membrane antigen (FAMA) ≥1:4. The individuals who were administered two doses reached significantly higher seroprotection levels (close to 100% for ≥5 units/ml gpELISA). The cell-mediated immune response is higher in individuals receiving two doses.[3,4]

The efficacy of the one-dose schedule ranges between 70% and 90% for infections of any type and it reaches 95% for severe disease. Effectiveness, upon certification, to prevent any type of infection is around 85% for the Oka strains vaccines, with few studies showing lower or higher values. One vaccine dose has 97% effectiveness or higher for the prevention of severe varicella. In a case-control study, the effectiveness of a single dose of universal varicella vaccine in South Korea, where the most common vaccine contains the Korea MAV/06 strain, was 54%.[25]

The two-dose schedule is 3.3 times less likely to result in varicella due to secondary vaccination failure (breakthrough varicella), as compared to the one-dose schedule during the first ten years after vaccination. This schedule demonstrated 98% effectiveness for all types of infection and disease severity.[1–3]

The vaccine may be administered simultaneously with other childhood vaccines. If not administered simultaneously, the interval between the MMR and varicella vaccines is 28 days. The vaccine virus is susceptible to acyclovir, valacyclovir, or farmacyclovir, therefore the administration of these products should be avoided between 1 and 21 days following vaccination.[3]

The vaccine is properly tolerated and safe. Adverse events are usually mild and occur in 5% to 35% of healthy children and in 20% to 30 % of adults. The most common side effects are local erythema, swelling, and pain, within 3 days post vaccination.

Between 1 and 3% of vaccinated individuals develop localized vesicles during the first week after vaccination, and from 3% to 5% develop varicelliform rash with few lesions between 7 and 28 days after vaccination. The vaccine virus is transmitted only if the vaccinee develops exanthema. It should be noted that a measles-like rash occurs in 2% to 3% of the vaccinees who were administered the MMRV or the monovalent vaccine + MMR vaccine. Fever occurs in 22% of children aged 12–23 months after one dose of the tetravalent MMRV vaccine and in 15% of the individuals receiving the varicella + MMR vaccines separately. A fever and a rash occur within 5 to 12 days post-vaccination. They are usually short-lived and leave no sequela. There is a slightly higher risk of febrile seizures and higher likelihood of experiencing fever after the first dose of the MMRV vaccine, as compared to MMR + monovalent varicella vaccine. After one dose of the MMRV vaccine, an additional febrile seizure is expected in every 2,300 to 2,600 vaccinated young children, as compared to the MMR + monovalent varicella vaccine. When administering the second dose to children older than 4–6 years of age, there was no difference in the incidence of fever, rash or febrile seizures amongst MMRV and MMR + varicella vaccinees.[1–3]

In immunocompromised individuals, the adverse reactions may be more severe; 20% to 40% may develop a varicelliform rash. Visceral dissemination of the attenuated virus is unusual.[1–3]

Post-certification surveillance shows that healthy vaccinated children have a lower risk than unvaccinated children to develop herpes zoster.[2–4]

Herpes Zoster Vaccine

In 2006, a vaccine against herpes zoster was licensed and prepared with the Oka strain with 19,400 PFU for administration in individuals aged ≥ 50 in a one dose schedule.[2,4]

Contraindications

The varicella vaccine should not be routinely administered to children who suffer from congenital or acquired T-cell immunodeficiency, including individuals with leukemia, lymphoma, and other malignancies affecting the bone marrow or the lymph system, as well as children under long-term immunosuppressive medication. Exceptions include certain children infected by HIV (children with no evidence of earlier disease and with 15% or higher CD4 T cell count). It is contraindicated in pregnant women and pregnancies should be avoided one month after vaccination.[3,4]

Monovalent vaccines do not contain egg proteins. The measles and mumps vaccines included in MMRV are produced in chicken embryo cultures. The amount of egg protein for cross reactions are negligible. Children with an egg allergy may receive the MMRV vaccine without prior skin testing.[3,4]

Other Strategies to Control Outbreaks and Avoid Disease in Exposed Individuals

Avoiding Infection in Susceptible Individuals Exposed to VZV

- Only vaccination ensures long-term protection; vaccinate ≥12 months over the first 3 days and no later than 5 days post-exposure. A second dose is recommended (minimum 3 month interval).
- Administration of anti-VZV antibodies (purified immunoglobulin from human plasma, with a high content of VZV specific antibodies), during the first 96 hours post-exposure. Standard immunoglobulin is an alternative option to consider.
- Administration of acyclovir orally, within the first 7 days post-exposure, may be useful to prevent or attenuate the disease in healthy individuals.

Educational or Residential Institutions Where Children, Adolescents, and Adults Coexist

- Individuals affected by varicella should stop attending school or individual isolation shall be established at the institution where they reside. They will be assisted by individuals who are not susceptible. They will be reintegrated once the exanthema is in the crusting phase.
- The same recommendation applies for herpes zoster.

Protecting Patients, Health Workers and Visitors from Hospital Exposure to Varicella

- Vaccinate individuals who did not acquire varicella or did not receive two doses of the vaccine. If one dose was administered (common situation in Latin America), administer the second dose (if the previous dose was administered at least three months ago). Prophylaxis should not be delayed to perform serological studies intended to confirm vaccine-based immunity or natural infection (to be performed if easily accessible).
- Discharge any susceptible individuals who were exposed as soon as possible upon performing active or passive prevention, or with antiviral drugs, as the case may be.
- Susceptible cases that cannot be discharged must be isolated from the eighth day of exposure (after the incubation period) until 21 days post-exposure.[3,4]

Conclusions

VZV infection is prevalent in children and it is potentially severe in some groups with special clinical situations. There are treatment and prevention strategies for varicella that have changed the epidemiology and clinical aspect of this disease, and they must be taken into consideration to take health actions at the population and individual level.

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Yellow Fever

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Introduction

Yellow fever is a rapidly-evolving acute hemorrhagic disease caused by a single-stranded RNA arbovirus of the family *Flaviviridae*. The virus is spread through the bite of an infected mosquito.

According to historians, the first confirmed epidemic of yellow fever in the Americas was in 1647 in Barbados.[1] However, records of yellow fever outbreaks in the Americas date back at least two centuries before the classic Mayan period. The *Popol-Vuh,* sacred book of the Quiché Mayas, refers to the epidemic of a disease called "*xekik*" (black vomit or bloody vomit) prior to the arrival of the Spaniards, from 1480 to 1485, affecting monkeys and human-beings later on, who developed a yellowish skin color. The book clearly narrates the disease transmission path from monkeys to human-beings*: "by a mosquito created by the Gods".*[2]

In 1881, at the end of the 19th century, a Cuban clinician and researcher, Carlos Juan Finlay y Barrés, discovered and described the importance of a biological mosquito vector — *Aedes aegypti (*then known as *Stegomyia fasciata*)– in the transmission of yellow fever. His theory on the transmission of yellow fever through an intermediary agent was not well accepted by the health community. However, he was able to publish it in the New Orleans Medical and Surgical Journal.[3]

Also in 1881, Finlay verified his hypothesis through clinical research conducted on volunteers and discovered that an individual bitten once by an infected mosquito remained protected against future yellow fever outbreaks. He presented his findings to the Havana Academy of Medical Sciences.[4] Shortly afterwards, the Yellow Fever Commission, led by Army physician Walter Reed, documented yellow fever as a viral disease. William Gorgas applied the same principles on vector control as indicated by Finlay and was able to turn around the situation in the Panama Isthmus, future site of the Panama Canal.

It should be noted that the first conference held by the Pan-American Sanitary Bureau (PASB), the oldest international health agency in the world (predecessor to the Pan American Health Organization [PAHO]), was held in Washington, D.C. in November 1902. An important agreement point during the event was the recognition of yellow fever transmission through the bite of an infected mosquito.[5]

The Agent

Yellow fever is caused by a single-stranded RNA arbovirus of the family Togoviridae, of the genus Flavivirus, with only one serotype and five genotypes. This virus is related to the West Nile, the San Louis encephalitis, and the Japanese encephalitis viruses. It replicates in the cytoplasm of affected cells. The virions are 40 nm in diameter and the viral envelope comprises a host-membrane derived lipid bilayer. The E protein on the surface is responsible for the initial phases of the infection in the host cells and it is also the main target for the host immune response.[6]

Epidemiology and Transmission

Yellow fever is endemic in 10 Latin American countries and more than 30 Sub-Saharan African countries. Based on recent World Health Organization (WHO) reports, globally there are an estimated 130,000 to 200,000 cases of yellow fever yearly, causing 44,000 deaths in endemic African countries, which account for 90% of the cases.[6,7]

There are three transmission cycles: 1) the jungle cycle involves non-human primates as the reservoir and *Haemagogus* as the mosquito vector species; 2) the urban cycle involves human to human transmission and *Aedes aegypti* is the mosquito vector; and 3) in Africa, the intermediate (savannah) cycle involves transmission of the virus from monkeys to humans and from humans to humans though *Aedes simpsoni* and *Aedes bromeliae* mosquitos resulting in small outbreaks in villages.[6,7]

Despite its lesser magnitude as compared to the African continent, yellow fever continues to be a public health problem in the Americas, where the risk of yellow fever transmission still prevails. Based on the definition by WHO, these are countries or areas where "yellow fever has been reported currently or in the past, plus vectors and animal reservoirs currently exist." From 2000 to 2013, more than 1,100 laboratory-confirmed cases were reported. Ninety-five percent of the cases were concentrated in four countries: Peru (54%), Bolivia (18%), Brazil (16%), and Colombia (7%). These countries are not holoendemic. Only some areas of the country are at risk for transmission of yellow fever.

Risk Factors

The main risk factor is to enter any enzootic region without previous immunization against the yellow fever virus. Individuals from the tree felling sector face a higher risk since after tree cutting mosquitoes descend to the ground level. The disease is often more frequent at the end of the rainy season when vector density is high and individuals cut trees to prepare the land for crops or livestock. This explains why young adults aged 15 to 40 years are the most affected and the impact on men is fourfold higher than in women.[6,7]

Factors currently conditioning the urbanization of yellow fever are associated with land-use changes, climate change, and the high degree of infestation by *Aedes aegypti* in urban areas. A viremic individual who exits the jungle may be bitten by the urban vector and initiate the transmission chain. Migration of populations induced by social, political, and economic conflicts affecting any endemic country determine the emergence of temporary settlements of unvaccinated populations in the jungle.

Climate change and increased rainfall are impacting and will continue to impact, both directly and indirectly, the spread of vector-borne diseases.[8] Since *A. aegypti* is the main urban vector for the transmission of dengue, chikungunya, Zika, and yellow fever, there is widespread interest in the potential impact of climate change on the bionomics and transmission of pathogens by this mosquito. Low temperatures limit vector distribution by killing larvae and mosquito eggs; however, *Aedes (Stegomyia) aegypti* has a broad distribution in tropical and subtropical areas of the Americas. It has been adequately established that warmer water temperatures shortens larva maturation and increases their capacity to produce more offspring during the transmission periods of several vector-borne disease.[8-10]

The extrinsic incubation period of dengue and yellow fever viruses is also dependent on temperature: the warmer the ambient temperature, the shorter the incubation period from the time the mosquito imbibes the infective blood until the mosquito is able to transmit by bite. A warmer temperature would not only imply wider distribution of *Ae. aegypti* and faster mosquito metamorphosis but also dengue and yellow fever viruses as well as other viruses would have a shorter extrinsic incubation period, cycle faster in the mosquito and thus increase the rate of epidemic transmission.[9]

Recent Yellow Fever Outbreaks in the Americas

Starting in the 1970's the area of emergence of jungle yellow fever cases has been restricted to the Northern region of the South-American Hemisphere. From 1985 to December 2007, a total of 3,837 human cases of jungle yellow fever and 2,229 deaths were reported. In 2007 and early 2008, there were intense and widespread jungle yellow fever epizootics in an area comprising six Brazilian states (Goias, Distrito Federal, Mato Grosso do Sul, Minas Gerais, Tocantins, and São Paulo). The epizootics were laboratory confirmed and/or used clinical-epidemiological criteria for confirmation through the state Health Departments. In January and February 2008, human cases were reported in three states (Goias, Mato Grosso do Sul, and Distrito Federal): 26 were confirmed cases with 13 deaths. The affected areas have high vaccination coverage. However, as part of the control measures the health authorities intensified their vaccination activities for previously-unvaccinated individuals aged six months and older, residing or travelling to the affected areas.[11,12]

Re-Emergence of Urban Yellow Fever in Paraguay, 2008

In 2008, jungle yellow fever cases were documented in the departments of San Isidro and San Pedro in Paraguay. A few weeks later, 24 cases of yellow fever with 8 deaths were confirmed (several more individuals were assumed to have been infected) in the districts of San Pedro, Caaguazú, Laurelty district, and the metropolitan capital area of Asunción. This marked the first urban outbreak of yellow fever in Paraguay since 1942.[13]

The urban-rural transmission cycle may have been affected by environmental and demographic changes. The presence and transmission of the virus in urban-rural districts were confirmed; entomological studies did not detect *Haemagogus*; human transmission was assumed. The lethality of the outbreak was 33%. As a result of the support provided by PAHO/WHO, 850,000 vaccines were sourced from Brazil, 144,000 from Peru and 2 million doses were shipped by WHO Global Fund. With the support of the Spanish Cooperation Agency for International Development (AECID) and the Office of U.S. Foreign Disaster Assistance at the United States Agency for International Development (OFDA/USAID), PAHO/WHO was able to implement emergency projects to escalate epidemiological surveillance, vector control, laboratory diagnosis, communication of risk, and complete vaccination in areas at risk.[14] Upon implementation of vector-control measures and a mass immunization campaign, no more cases were reported.

The Disease: Clinical Presentation

After the virus is acquired and the 3-to-6-day incubation has elapsed, the infection may be in one of two phases: the acute phase or the toxic phase. In the acute phase of the infection, the disease ranges from a non-specific mild febrile state with myalgia, myalgia with intense back pain, migraines, shivers, loss of appetite to nausea or vomiting. It may be misinterpreted as severe malaria, hemorrhagic dengue fever, leptospirosis, or vital hepatitis (in particular the lethal manifestations of hepatitis B and D). Later on, most of the patients improve and symptoms disappear after 3 to 4 days. In the second phase, known as the classic manifestation, 15% of patients enter a more toxic phase within 24 hours of the initial remission. The patient quickly turns jaundiced and complains of abdominal pain with vomits. There may be oral, nasal, ocular, or gastric bleeding with bloody vomit or bloody stools and kidney function is impaired. Half of the patients who enter the toxic phase die within 10 to 14 days, while the rest recover without significant organ damage.[15]

Pathogenesis and Immunity

Knowledge of the pathogenesis of yellow fever is derived from experimental studies of the disease induced in non-human primates that usually express the viscerotropic infection, including virus replication in lymph nodes, the liver, the spleen, the heart, and the kidneys. Pathological changes in the liver and kidneys with apoptotic changes in Councilman bodies are also present. An increase of TNFα, IL-1 and IL6 has been confirmed in vaccine studies.[15]

A fast immune response follows infection with the yellow fever virus. During the first week of the disease, IgM antibodies are produced reaching their peak during the second week and decreasing over 1 to 2 months. By the end of the first week, specific neutralizing antibodies are developed as the main mediators of protection and lasting several years. These antibodies bind to proteins in the viral envelope and interfere with the binding and penetration of the yellow fever virus to the host-cell membrane. Some structural proteins (NS1 and NS2) of the virus are associated with the infected host-cell membrane and targeted for elimination through the immune system.[6,15]

Diagnosis

Diagnosis of yellow fever in tropical areas is challenging and may be misinterpreted as other hemorrhagic fevers (the Bolivian, Argentinean, and Venezuelan hemorrhagic fevers, and other Flaviviruses such as West Nile and Zika viruses), and other diseases. Diagnosis of yellow fever is usually based on clinical data.

Detection of neutralizing antibodies is the only useful test to determine immunity to yellow fever. Blood tests detect specific antibodies against the virus and diagnostic confirmation entails demonstration of a fourfold increase in the neutralizing antibody titers in patients without recent history of yellow fever vaccination, and exclusion of cross-reactions to other Flaviviruses. Otherwise, demonstration of the presence of the yellow fever virus, its antigens, or genome in tissue, blood, or biological fluids is difficult, particularly in the early stages. Other techniques are also used to identify the virus in blood samples or liver tissue obtained from an autopsy. These tests require highly-trained laboratory personnel, specialized material, and equipment.[16]

Treatment and Prognosis

There is no specific treatment or cure for people infected with the yellow fever virus, which underscores the importance of vaccination. In severe cases, treatment is symptomatic, aimed at reducing symptoms in particular through rehydration and control of potential hypotension. Global mortality is 5% amongst indigenous populations residing in endemic regions despite the fact that, for severe cases, epidemics or other non-indigenous populations, up to 50% of the patients may die. Some cases result in acute kidney failure and dialysis becomes a significant treatment. Severe cases need management at intensive care units. Notwithstanding the severity, once the disease is acquired infected persons gain immunity for life.[6,7,15]

Prevention

Yellow fever is a vaccine-preventable disease and vaccination is the most efficient measure against transmission. The vaccine was developed by Max Theiler and colleagues in 1936,[17] and his contribution afforded him the Nobel Prize in 1950. The vaccine is considered effective and safe and it has been used for more than 70 years for the active immunization of children and adults against the infection caused by the yellow fever virus. From its creation, more than 600 million doses have been administered globally. The live attenuated 17D or 17DD vaccines from chick embryo tissue are safe and confer effective immunity with neutralizing antibodies for 90% of the vaccinated individuals within a 10-day period and for 99% at thirty days with only one dose. One dose provides immunity during ten years as of the tenth day of administration.[15]

Other preventive measures entail reducing human exposure to mosquito bites and controlling mosquito reproduction. Some measures include: physical control associated with the protection of water reservoirs, elimination of mosquito breeding sites through environmental rearrangement and waste collection, and chemical control (i.e. the application of insecticides and larvicides to control pockets and biological control to focus on larva elimination).

Vaccine Prevention

The vaccine prevention strategy in regions at risk for yellow fever transmission is comprised of two components. The first one is the inclusion of the yellow fever vaccine in the national vaccination schedules at twelve months of age. The vaccine should be administered subcutaneously in one 0.5 mL dose, on the upper arm. Administration may be concomitant with any other vaccine, even with other live injectable vaccines, such as measles, MMR (measles, mumps and rubella), MR (measles, rubella) and chicken pox, provided they are administered with a separate syringe on different injection sites. The only exception is the cholera vaccine, which should not be administered concomitantly with the yellow fever vaccine; or any other attenuated vaccine such as MMR, chicken pox or herpes zoster. These vaccines should be administered with a minimum three-week interval to generate an adequate immune response. If the yellow fever vaccine is NOT administered concomitantly with other injectable live vaccines (measles, MMR, MR, and chicken pox), a four-week interval at least shall be observed in between applications. The vaccine is not recommended for pregnant women, individuals with egg allergies, immunocompromised individuals, or children aged <9 months.[6,17,18]

The second component of vaccine prevention is the implementation of mass vaccination campaigns to protect vulnerable groups of older adults in at risk areas. Assessment of the risk level may help establish priority areas for mass vaccination campaigns. The vaccine is not recommended for children <9 months and adults >60 years, individuals with egg allergies, pregnant women, breastfeeding women, individuals with primary immunodeficiencies and HIV due to potential adverse events.[6,18]

Likewise, the introduction of the yellow fever vaccine into the vaccination schedule as part of the National Immunization Program is recommended for countries with enzootic areas. As of 2016, 13 countries in Latin America with enzootic areas have introduced the yellow fever vaccine in their vaccination schedules as part of the Expanded Program on Immunization (EPI) (Figure 1). In Argentina, Brazil, and Suriname, the vaccine is administered exclusively in areas of potential risk. Vaccination coverage for children aged 1 year in yellow fever endemic countries has been close to 70% in the 2007–2011 period, yet it has been significantly impacted by vaccine shortage.

Figure 1. Countries at Risk for Yellow Fever Transmission and the Vaccination Strategies Used in the Region of the Americas, 2013

Source: World Health Organization Yellow Fever Working Group. Available at: http://www.who.int/ith/yellow-fever-risk-mapping/risk_mapping/en/.

Severe Adverse Events Associated with the Yellow Fever Vaccine

The yellow fever vaccine is considered one of the safest attenuated vaccines, with few associated adverse events. Adverse events, such as pain on the administration site, muscle pain or headaches, and potentially a febrile state have been reported. Vasconcelos et al.[19] reported two deaths caused by the administration of the 17D-derived vaccine and recommended a safety review of this vaccine.[6,16,18] Such events are extremely rare and need to be further studied as the authors noted "host factors, probably idiosyncratic reactions, might have had a substantial contributed to the unexpected outcome."[19]

To establish the incidence of adverse events associated with the 17D or 17DD-derived yellow fever vaccine, Thomas et al. conducted a systematic review of six studies on vaccination campaigns with open populations that included 94,500,528 individuals, with data mainly from Brazil (99%) resulting in an estimate of 0.51 Events Supposedly Attributable to Vaccination or Immunization (ESAVIs) /million doses administered.[20]

In five retrospective reviews of the clinical histories of 60,698 individuals, no severe ESAVIs were confirmed. Most of the data (96%) was from the Hospital for Tropical Diseases in London: two studies with 35,723 children; four studies with 138 pregnant women; six studies with 191 HIV-positive individuals and a review of HIV+ patients, without severe ESAVIs reported.[20]

Every country has their own database with different definitions, protocols, and surveillance mechanisms to identify and report cases and adverse events, as well as strategies for the clinical follow-up of cases. Drug monitoring from databases offers three estimates: low estimate for data from Brazil and Argentina; intermediate estimate for the United States Vaccine Adverse Event Reporting System (VAER) data and a high estimate for data from the United Kingdom and Switzerland. Active surveillance estimates are lower (authors suggest they were influenced by data from Brazil) while passive surveillance estimates are lower (and strongly influenced by the data from the London Hospital for Tropical Diseases dating back to 1950).[20]

Neurotropic or Viscerotropic Disease

Severe adverse events include yellow fever vaccine-associated viscerotropic disease and yellow fever vaccine-associated neurotropic disease, known as YF-AVD and YF-AND, respectively. The neurotropic event has been reported in 26 cases (typically with full recovery) and the viscerotropic disease has been reported in 10 cases since 1990 (seven since 1996), with eight deaths (six of them had been vaccinated as a travel requirement to an endemic area and four affected inhabitants of endemic areas). Signs of an immune response elicited by the 17D-vaccine were found in tissue of the deceased individuals. The onset is abrupt at 3 to 5 days post vaccination, with multiple organ failure, and typical pathological findings. No risk factor has been identified.[20,21] These cases have underscored the importance of guiding vaccination campaigns exclusively for populations exposed to the risk of acquiring the disease and the need to continuously promote the development of new vaccines against yellow fever.[22]

Yellow Fever and International Health Regulations

WHO recommends the administration of the vaccine for travelers beyond urban areas in countries located in areas of Central and South America and parts of South-Saharan Africa. Yellow fever has unique status in the International Health Regulations (IHR, 2005), which outline requirements for proof of vaccination for people who travel to specific countries or enter select countries from an area where yellow fever is endemic.

The International Health Regulations indicate that travelers may be required to produce evidence of yellow fever vaccination as a condition to enter a country that so requires. Travelers without vaccination evidence could have the vaccine administered at the point of entry to the country or could be detained for up to six days to guarantee they are free from the yellow fever infection. The yellow fever vaccine is only administered at designated vaccination clinics where a sealed and signed "international certificate of vaccination or prophylaxis" (yellow card) is provided upon vaccination. This certificate is valid for 10 years after vaccination.[18]

Previously, a booster dose was required every 10 years. In 2014, the WHO World Health Assembly adopted the recommendation to suspend the requirement for application of a booster at ten years of vaccination to persons at risk of exposure to transmission as established in the International Health Regulations as of June 2016.[18]

PAHO/WHO Response Strategies to Outbreaks of Yellow Fever in the Region of the Americas

PAHO/WHO has developed a detailed map of yellow fever risk areas in the Americas (Figure 1) and enables countries to carry out mass preventive vaccination campaigns during inter-epidemic periods. Evidence-based plans have been developed to provide support and technical guidance to all countries facing outbreaks with the purpose of requesting support, including vaccine mobilization through the PAHO Revolving Fund.

Conclusion

Yellow fever is a significant cause of hemorrhagic fever in several African countries with more than 30,000 deaths yearly and, sporadically, in some South-American countries. Given the emergence of other diseases borne by the vector *Aedes aegypti*, such as dengue and, more recently, the Zika virus, there is great interest in the impact global warming may have and also in the risk of re-urbanization for yellow fever not only in tropical areas but also in more temperate areas.

The yellow fever vaccine is the most effective measure to avoid transmission. The WHO recommends the administration of the vaccine for any travel beyond urban areas in enzootic countries located in regions of Central and South America and areas of South-Saharan Africa. Likewise, the introduction of the yellow fever vaccine into the vaccination schedule as part of the Expanded Program on Immunization is recommended in enzootic countries.

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Zika Virus

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Introduction

The scientific data collected, and the lessons learned following Zika virus (ZIKV) introduction into the Western Hemisphere, first in Brazil and then spreading very rapidly in the Americas, provided a huge amount of unexpected information and have been crucial in informing a better understanding on several aspects related to the transmission of the virus, its clinical manifestations, neurological complications and particularly the risk of microcephaly and other neurological malformations in fetuses born to mothers infected with ZIKV during pregnancy.[1] In this context the development of potential therapeutic interventions and preventive strategies, including vaccines, are of paramount importance.

This chapter summarizes the current knowledge on the ZIKV infection in humans and provides a perspective on the issues and challenges related to the development of a safe and efficacious vaccine against ZIKV.

Etiology

ZIKV is an emerging arthropod-borne, single-stranded RNA virus, member of the *Spondweni* serocomplex (genus *Flavivirus*, family *Flaviviridae*) and related to other mosquito-borne viruses that cause yellow fever, dengue, West Nile disease, St. Louis encephalitis, and Japanese encephalitis. Two major lineages, African and Asian, have been identified through phylogenetic analyses.[2]

Epidemiology

After initial identification in 1947 from a sentinel rhesus monkey (i.e., monkeys held captive with the purpose of identifying yellow fever activity) in a forest in Uganda,[3] ZIKV was associated only with few sporadic cases in humans in Africa and Asia over the next 60 years.[4] However, since 2007, when the first outbreak of ZIKV outside Africa and Asia was reported in the Federated States of Micronesia (Yap), it has been identified in subsequent outbreaks in French Polynesia and other Pacific islands.[4] In May 2015, the Ministry of Health in Brazil confirmed autochthonous transmission of ZIKV associated with an outbreak of "dengue-like syndrome" cases in Northeastern Brazil. The ZIKV outbreak continued to evolve, spreading geographically very rapidly.[5]

Since then, in the Americas, 49 countries and territories reported local transmission, 24 countries and territories have reported microcephaly and/or central nervous system (CNS) malformation cases potentially associated with ZIKV infection and 15 countries and territories have reported Guillain-Barré syndrome (GBS) potentially associated with ZIKV infection. As of 14 April 2018, Uruguay is the only country in the Americas with evidence of established competent vector, but no known documented past or current transmission of ZIKV (Figure 1).[5]

Figure 1. Areas With Risk of Zika in Latin America and the Caribbean

Brazil was the most affected country in the Americas, reporting 216,207 probable cases in 2016, 17,594 cases in 2017 and only 1,174 cases by week 10 in 2018.[6] Since 2015, the Ministry of Health in Brazil confirmed 3,071 cases of microcephaly and/or CNS malformation associated with ZIKV infection, with the majority (60%) occurring in the Northeast, followed by the Southeast (24%) and Central-West (7%) region.[7]

In 2018, a substantial decline in cases of Zika virus infection has been reported in most affected countries in the Americas, probably because of "herd immunity" of the population that became immune after being infected in previous years, reducing the number of susceptible, naive subjects, and thus, limiting the transmission of the virus in the community.

Transmission and Incubation Period

ZIKV is transmitted to humans primarily by *Aedes aegypti* mosquitoes (and less commonly by other *Aedes* species, like *Aedes polynesiensis, Aedes hensilli, Aedes africanus*, and *Aedes albopictus),* the same vector that can transmit dengue, chikungunya, and yellow fever viruses.[8] ZIKV has already been isolated from other non-*Aedes* mosquitoes. However, it is important to emphasize that the isolation of ZIKV from a mosquito is not evidence that transmission is feasible by this mosquito. Human and nonhuman primates are the main reservoirs of the virus, with humans acting as the primary host.

Additionally, non-vector modes of transmission have been identified, including: perinatal, *in utero*, sexual, blood transfusion, and laboratory exposure.[8] Although ZIKV RNA has been detected in breast milk, transmission through breastfeeding has not yet been demonstrated, reinforcing the current recommendations that mothers with ZIKV infection should continue to breastfeed their infants.[8]

Intrauterine transmission of ZIKV was confirmed in Brazil by the detection of virus genome, by reverse transcriptase-polymerase chain reaction (RT-PCR), in amniotic fluid samples of women with symptoms of ZIKV infection during the first trimester of pregnancy whose fetuses have been diagnosed with microcephaly, in placental tissues from early miscarriages, and also in the blood and brain tissue of infants with congenital neurologic anomalies, including microcephaly.[8,9]

Reports of cases with ZIKV possibly transmitted by blood transfusion are being investigated in Brazil. Interestingly, during the French Polynesian outbreak, 2.8% of blood donors tested positive for ZIKV by RT-PCR, with all of them asymptomatic at the moment of blood donation.[10]

The incubation period in humans prior to onset of symptoms is thought to be between 3 to 14 days after the bite of an infected mosquito. Infected people, both symptomatic and asymptomatic, can transmit ZIKV to mosquitoes throughout the viremic period that usually ranges from a few days to one week.[8]

Diagnosis

Clinical diagnosis is limited by the non-specific signs and symptoms of ZIKV infection which are similar to other arboviral infections (e.g., chikungunya and dengue) common in endemic areas. Abnormal laboratory findings, including mild thrombocytopenia, leukopenia, and elevations in acute-phase markers of inflammation, serum lactate dehydrogenase, or liver transaminases have been observed in symptomatic patients.[8]

ZIKV specific diagnosis in nonpregnant symptomatic individuals is primarily based on the detection of ZIKV RNA by RT-PCR performed on serum and/or urine specimens collected <14 days after onset of symptoms.[8] ZIKV-specific immunoglobulin M (IgM) and neutralizing antibodies can be detected by enzyme-linked immunosorbent (ELISA) assays in serum specimens collected by the end of the first week of illness and up to 12 weeks post onset of symptoms. As the immune response develops, IgM titres rise in peripheral blood and the level of viral RNA generally declines. Serum IgM antibody testing should be performed if the RT-PCR result is negative or when ≥14 days have passed since illness onset. IgG antibodies develop within days after IgM and can be detected for months to years. However, false-positive results due to cross-reaction with related flaviviruses (e.g., dengue and yellow fever viruses) are commonly observed. During the outbreak of ZIKV infection in the Yap state of Micronesia, the presence of low levels of cross-reactive IgM was demonstrated in all patients with secondary *flavivirus* infection.[11]

Positive results in primary *flavivirus* infections should be confirmed with a four-fold increase in the titer of neutralizing antibodies to ZIKV with plaque reduction neutralization test (PRNT). In endemic areas, where a great proportion of the population may have been previously infected with other flaviviruses or vaccinated against a related *flavivirus* (i.e., secondary *flavivirus* infection), neutralizing antibodies might still yield cross-reactive results in these individuals.[8]

Clinical Manifestations

It is estimated that approximately 80% of persons infected with ZIKV are asymptomatic. When symptomatic, the infection is considered to be associated with a mild, self-limited disease, lasting few days and characterized by low fever, pruritic rash, edema of extremities, conjunctivitis, headache, and myalgia. Less common manifestations include gastrointestinal symptoms, retro-orbital pain and lymphadenopathy.[1,8,12] Clinical manifestations in infants and children with acquired infection are similar to the findings observed in adults with ZIKV infection. The presence of arthralgia in infants and young children is difficult to detect and can manifest as irritability, limited moving or refusing to move an extremity. During outbreaks of ZIKV, cases were reported in all age groups with higher incidence rates in adults compared to children.[1,8,12] The World Health Organization (WHO) developed interim case definitions with the purpose of providing global standardization for classification and reporting of Zika virus cases: patient with rash and/or fever with at least one of the following signs and symptoms: arthralgia; arthritis or conjunctivitis (non-purulent conjunctival hyperemia). A confirmed case is a suspected case with laboratory confirmation of recent Zika virus infection: presence of Zika virus RNA or antigen in serum or other samples, e.g., saliva, tissues, urine, whole blood; or IgM antibody against ZIKV positive and $PRNT_{90}$ for ZIKV with titer ≥20 and ZIKV $PRNT_{90}$ titer ratio ≥4 compared to other flaviviruses; and exclusion of other flaviviruses.[13]

Neurological and Autoimmune Complications

Neurological complications, such as Guillain-Barré syndrome (GBS), meningitis, acute disseminated encephalomyelitis and myelitis have been reported following ZIKV infection, mainly in adults. French Polynesia, Brazil, Colombia, Venezuela and several other countries from Central America and the Caribbean reported an increase in the rates of GBS during the recent ZIKV outbreak.[14] The reported incidence of GBS was higher among males and consistently increased with age, with males over 60 years having the highest rates, findings that are in line with previous reports on the epidemiology of GBS. This epidemiological situation reinforces the hypothesis of a link between ZIKV infection and the occurrence of GBS, highlighting that ZIKV should now be included in the list of potential infectious pathogens that can trigger the development of GBS.[14-16]

Congenital Syndrome

The most striking finding during the ZIKV outbreak in Brazil, however, was the strong cumulative evidence that provided the basis to establish a relationship between ZIKV infection during pregnancy and congenital abnormalities. A wide range of congenital malformations was described, characterized predominantly by CNS alterations and associated symptoms: microcephaly (with significant cranium-facial disproportion), spasticity, convulsions, marked irritability, and brainstem dysfunction including feeding difficulties. The results of neuroimaging studies suggest that intrauterine ZIKV infection is associated with severe brain anomalies, such as cerebral calcifications, hydrocephalus, lissencephaly with agenesis of the corpus callosum, pachygyria, cerebellar dysplasia, and white-matter abnormalities.[1,8,16-18] The severity of the neurological alterations appears to be related to the period of gestation when the women are infected, i.e., the earlier the infection during pregnancy, the more severe the neurologic outcomes to the fetus. Arthrogryposis, microphthalmia, funduscopic alterations in the macular region, as well as optic nerve abnormalities were also described in infants with suspected congenital ZIKV syndrome.[1,8,16-18]

The true burden of the congenital disease associated with ZIKV is probably underestimated assuming that it is likely that a significant proportion of the affected newborns have subclinical manifestations at birth, without microcephaly, preventing these infants from being diagnosed by the current ascertainment methods, at least until later stages of childhood/adolescence when cognitive, developmental, and/or visual limitations can be detected.

The unique characteristics of the ZIKV outbreak in Brazil, where the population was completely susceptible (naïve) to the virus, affecting highly populated urban areas with high density of *Aedes aegypti*, and the established surveillance reporting system, are possible reasons to explain why the role of ZIKV as a potential cause of congenital disease has only been recognized after circulation in Brazil. Furthermore, if ZIKV infection is associated with life-long immunity, it is expected that in endemic places in Africa and Asia, where the virus is circulating for years, a proportion of the women in childbearing age is likely to be previously infected, limiting the number of susceptible women.

It is also possible that the more severe outcomes of ZIKV infection observed in Brazil and other countries may be related to mutation in virulence characteristics of the ZIKV circulating strain or even immune interaction between consecutive *Flavivirus* infections. Interestingly, after the reports from Brazil[17-19] raised a causal relationship between ZIKV infection in pregnancy and microcephaly and other congenital malformations, a retrospective study performed in French Polynesia found an association between ZIKV and microcephaly.[20]

Treatment

We currently do not have any available specific antiviral treatment for patients with ZIKV disease. Only supportive care is indicated, including rest, fluids and symptomatic treatment (acetaminophen to relieve fever and antihistamines to treat pruritus). Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) should be avoided to reduce the risk of hemorrhagic complications. One recent study showed that chloroquine exhibited antiviral activity against ZIKV in VERO cells, human brain microvascular endothelial and neural stem cells. In this study, the authors were able to demonstrate that chloroquine reduced, *in vitro,* the number of ZIKV-infected cells, virus production and cell death promoted by ZIKV infection without cytotoxic effects.[21]

Vaccines

Preliminary studies identified a single ZIKV serotype and suggested that immune response after ZIKV infection induces broadly neutralizing antibodies against multiple strains (South American, Asian, and early African ZIKV strains proved to be similarly sensitive to neutralization by ZIKV convalescent human serum), paving the way for the development of an effective vaccine.[22] Similarly to other flaviviruses, neutralizing antibodies appears to play a critical role in protection against infection.

Several ZIKV vaccine candidates using different technologies, based on plasmid DNA, modified mRNA, purified inactivated virus, recombinant live attenuated vaccines and viral vectored vaccines, showed promising results in mouse and non-human primate studies and are now advancing to clinical trials in humans.[23,24] Taking in account the need to protect women at childbearing age, vaccination strategies should be prioritized to target individuals of both sexes of reproductive age (to prevent sexual transmission), nine years of age or older. The recent results with the live-attenuated chimeric dengue vaccine, showing increased risk of severe dengue among dengue-naïve subjects vaccinated compared to the unvaccinated control group,[25] highlights the importance of long-term safety surveillance, to evaluate the duration of the protective immune responses of the current candidate ZIKV vaccines.[26] It will be also critical, when planning future vaccine trials, to have a better knowledge on the immune responses after subsequent infections with these flaviviruses. It is still unknown whether a previous infection with other flaviviruses, like dengue, or the presence of antibodies against yellow fever in populations where vaccination is routinely recommended, will increase the risk of severe disease, neurological complications like Guillain-Barré syndrome, or congenital disease in pregnant women.

Inovio Pharmaceuticals is developing a synthetic DNA plasmid vaccine against the ZIKV virus, which encodes for the premembrane-membrane and envelope regions of the virus, currently in Phase I trials, assessing the safety, tolerability, and immunogenicity of the candidate vaccine in adults 18–65 years of age.[27]

The National Institute of Allergy and Infectious Diseases (NIAID) has a candidate ZIKV DNA vaccine that is currently in Phase II trials. Previous studies demonstrated that the vaccine was safe and elicited a neutralizing antibody response against Zika virus.[28] The current Phase II/IIb trial, started in 2016, is divided in two parts: a safety and immunogenicity study and an efficacy study, enrolling at least 2,490 healthy participants in areas of confirmed or potential active mosquito-transmitted Zika infection, including: the United States and Puerto Rico, Brazil, Costa Rica, Mexico, Panama, and Peru. The U.S. National Institutes of Health (NIH) has also a partnership with the Butantan Institute in Brazil to develop a live attenuated vaccine against Zika, currently in early phase trials.

Although DNA vaccines, as well as subunit vaccines are safe and potentially easily manufactured, they have limited immunogenicity compared to other vaccine types such as live-attenuated vaccines.[29]

Prevention and control currently relies on personal strategies to avoid mosquito bites and community-level programs to reduce vector densities in endemic areas. Personal measures include using insect repellent containing DEET, picaridin, oil of lemon eucalyptus, or IR3535. Permethrin-treated clothing and gear can repel mosquitoes.[8]

Future Research and Challenges

Despite the advances that were recently achieved on the understanding of several aspects related to ZIKV infection, it is important to acknowledge that we still have many research gaps and unanswered questions about ZIKV. Crucial areas of future research include the need for a better understanding of the full spectrum of fetal outcomes resulting from fetal ZIKV infection; evaluation of potential risk factors for vertical transmission (viral load, co-infections, timing, virulence of the circulating strain); development of more specific diagnostic tests; the role, if any, of non-*Aedes* mosquitoes in the transmission, as well as other potential modes of non-vector transmission; the pathogenesis of neurological and auto-immune complications following ZIKV infections.

Finally, novel methods of vector control, and the development of specific antivirals drugs and vaccines will be of paramount importance to control the disease and decrease the burden of ZIKV infection.

Conflict of Interest Statement

The authors have no funding or conflicts of interests to disclose.

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Module 2:
Decision-Making on New Vaccines

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Introduction of New Vaccines in Latin America and the Caribbean: Decision-Making

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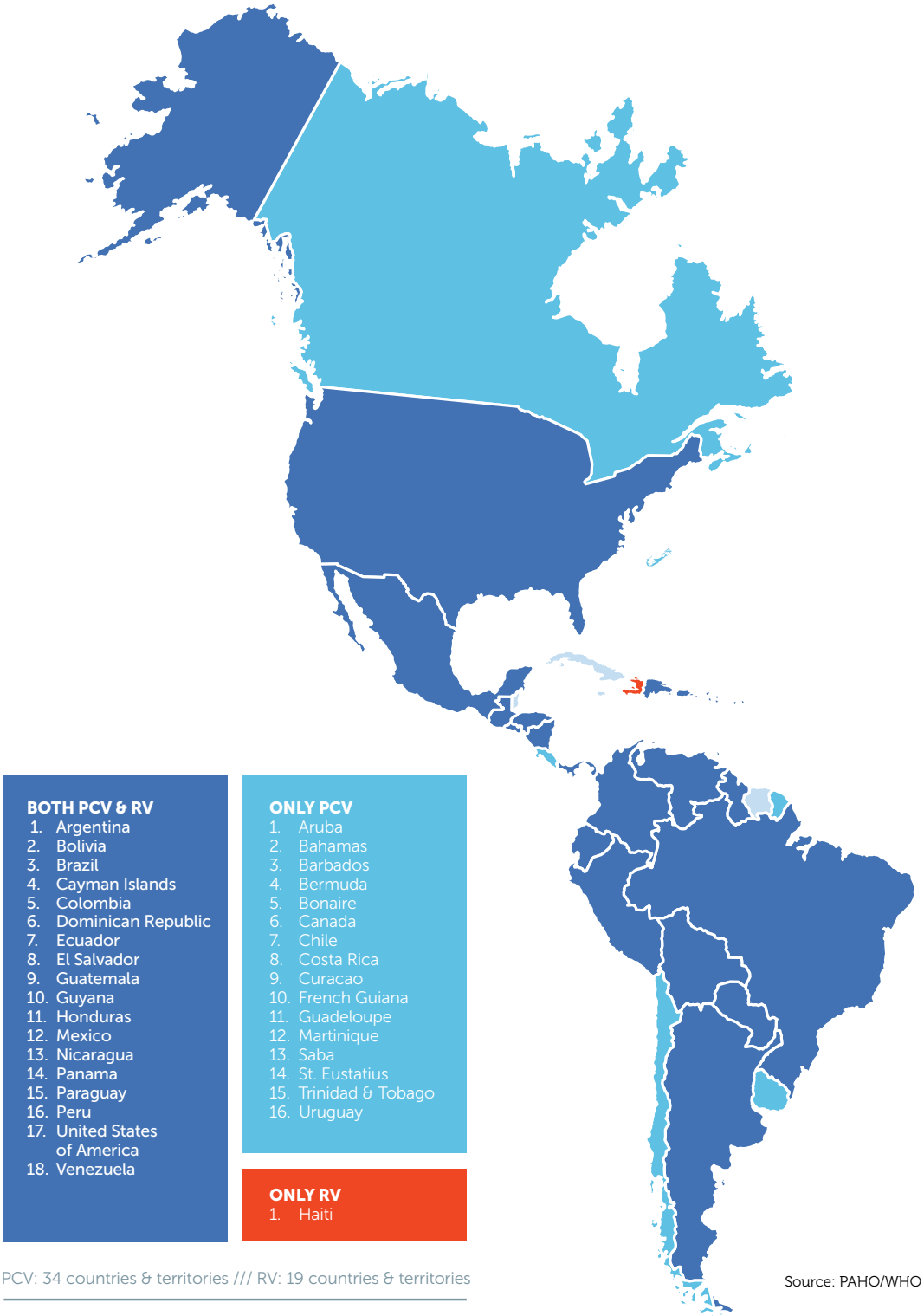
Introduction

Biotechnological advances in recent decades have resulted in the proliferation of new vaccines at an unprecedented pace. The Region of the Americas has been at the forefront of the introduction of new vaccines in the national immunization schedules, particularly in connection with the introduction of the rotavirus, pneumococcus, and human papillomavirus (HPV) vaccines.[1,2]

In general, new vaccines have demanded more years of research for their development and have frequently required the use of new and more complex technologies, which make them more expensive.[3] To minimize the inequalities resulting from the lack of access to vaccines in developing countries, it is necessary to support the decision-making process with a broader and more solid evidence base to make a case for such investment.[4]

Consequently, the Ministries of Health in Latin America and the Caribbean (LAC) adopted a Pan-American Health Organization (PAHO) resolution to call for broader evidence to be used for informed decision-making on the introduction of vaccines with the technical support of PAHO. They urged Member States to mobilize additional resources to support analyses for the introduction of new vaccines.[5]

Figure 1. Universal Introduction of the RV and PCV Vaccines in the EPI, Region of the Americas, 2016



BOTH PCV & RV
1. Argentina
2. Bolivia
3. Brazil
4. Cayman Islands
5. Colombia
6. Dominican Republic
7. Ecuador
8. El Salvador
9. Guatemala
10. Guyana
11. Honduras
12. Mexico
13. Nicaragua
14. Panama
15. Paraguay
16. Peru
17. United States of America
18. Venezuela

ONLY PCV
1. Aruba
2. Bahamas
3. Barbados
4. Bermuda
5. Bonaire
6. Canada
7. Chile
8. Costa Rica
9. Curacao
10. French Guiana
11. Guadeloupe
12. Martinique
13. Saba
14. St. Eustatius
15. Trinidad & Tobago
16. Uruguay

ONLY RV
1. Haiti

PCV: 34 countries & territories /// RV: 19 countries & territories

Source: PAHO/WHO country reports

Criteria for Decision-Making

The decision-making process for the introduction of a new vaccine is complex and should be undertaken with absolute caution and responsibility to guarantee a successful and sustainable introduction in the long term. Ultimately, upon introduction of a vaccine in the national immunization schedule, withdrawal is not a good practice given its ethical, political and social implications.

To ensure that vaccine introductions achieve the greatest sustainable impact, experts have identified three essential factors.[6]

1. **Decisions should be nationally based**, since countries have different burdens of disease, infrastructure, and budget.
2. **Scientific evidence used to support decisions must be broad based**, including cost-effectiveness and financial sustainability measures.
3. **Infrastructure must be in place to support nationally based decision-making**, including a National Immunization Technical Advisory Group (NITAG) or another independent advisory body.

Additionally, based on vaccine introduction guidelines issued by the Word Health Organization (WHO), PAHO developed the Field Guide for the Introduction and Implementation of New Vaccines, describing the various criteria to be taken into account when considering adding a new vaccine to the national schedule.[3,7] These can be broken down into political and technical considerations, and program and feasibility considerations.

Political and Technical Considerations

Political and technical considerations lay the foundational support and scientific evidence upon which a recommendation to introduce or to not introduced a health strategy will be based. Beyond the political considerations which fall under the responsibility of high level authorities, all technical aspects are usually reviewed and discussed by the National Immunization Technical Advisory Group (NITAG) or similar national committee. This important independent advisory body makes technical recommendations to the Ministry of Health on the best control measures against the disease to be prevented.

Public Health Priority. The Ministries of Health first assess if the vaccine-preventable disease is a public health priority in their country. Therefore, the opinion of various key stakeholders needs to be established and documented through qualitative evaluations. The more awareness there is about the problem, the broader the acceptance of the vaccine introduction will be, and strategies for awareness raising are often times the first step in the policymaking process. Importantly, awareness raising requires supporting data and evidence about the magnitude of the problem, and this is where burden of disease comes into play.

Burden of Disease. To make an informed decision, it is essential to be aware of the magnitude of the disease burden intended to reduce. This requires national studies on incidence, prevalence, disability, hospital admissions, and mortality. Ideally, this information can be obtained through surveillance data and/or special studies. When country-level research and surveillance data are available, disease burden is easily recognized, facilitating policy development. On the other hand, when there is a lack of sufficient national-level data, disease burden is difficult to estimate, and policymakers are left with neighboring country or even broad regional or international estimations to inform their decisions.

Vaccine Efficacy and Safety. Vaccine efficacy and safety are established in clinical trials under ideal conditions prior to the licensing of the vaccine and during the post-marketing phase. This critical piece of information is considered by Ministries of Health to determine the potential added value of the intervention as an effective and safe public health strategy.

Comparison with Other Interventions (Including Other Vaccines). For some diseases, there are a number of vaccines to select from and other non-vaccination interventions available. Comparison of the various control interventions requires the proper analysis level for each of them. The key aspects to consider in a comparative analysis are burden of disease, effectiveness, costs of each intervention, and cost-effectiveness results.[8,9] Comparisons between interventions require the use of national-level data to the extent possible, since there is great variability in disease burden, immunization program and healthcare costs, and even vaccine effectiveness data, where it exists.

Economic and Financial Criteria. Assessment of the economic and financial implications of the new vaccines provides valuable information for the decision-making process of governments and their partners. Different types of economic analyses assess health interventions, including: cost analysis, cost-minimization analysis, cost-benefit analysis, cost-effectiveness analysis and return on investment analysis. These types of analyses are described further in the chapter, *Stronger Immunization Policy in Latin America and the Caribbean: Vaccine impact, costs and cost-effectiveness evidence*.

Program and Feasibility Considerations

Program and feasibility considerations are related to the characteristics of the product to be introduced. They need to be assessed by technical experts since they imply an analysis of the functioning, logistics, supply, performance, and delivery of the vaccine by the immunization program. These considerations often have the final say in the decision-making process, since sometimes technically sound recommendations cannot be put in practice in imperfect real-world conditions.

Vaccine Characteristics and Supply. Countries should determine if the vaccine characteristics, administration route, and formulation available in the market is adequate for the national program or if problems with the program logistics or functioning can be anticipated, including additional cold chain capacity requirements, or the need to change the delivery strategy, among others. Likewise, vaccine supply needs to be considered to ensure an uninterrupted offer of the product to the country's population.

Performance of the Immunization Program. The introduction of a new vaccine may increase community demand and/or weaken the immunization program. Therefore, prior to the introduction of a new vaccine, it is important to assess the current performance of the program and work on the aspects that need to be strengthened or prepared in advance for a successful introduction.

Decision-Making Process

The criteria above constitute the necessary elements to be considered by the national immunization program manager to justify a preliminary technical decision. This decision should also consider discussions among the interdisciplinary groups of the Ministries of Health that are relevant to the health problem being addressed. This includes the teams performing cost-effectiveness studies with PAHO's ProVac tools, as presented in greater detail in the chapter, *Stronger Immunization Policy in Latin America and the Caribbean: Vaccine impact, costs and cost-effectiveness evidence*. This analysis usually concludes with a two-way decision: recommend the introduction of the new vaccine or defer its introduction. Often times, however, decisions to scale up or change a vaccination strategy are also made, with the aim to improve vaccination coverage and the performance of the program.

Regional Evaluation of New Vaccine Introduction in Latin America

A regional study performed in 2009 analyzed the existence and functioning of the national immunization technical advisory groups in the Americas as components of the decision-making process on the introduction of new vaccines.[10] Of the 29 countries that returned the questionnaire, 17 reported that they had a NITAG. The participating countries underscored the need to strengthen the immunization policy decision-making process by:

- Creating or strengthening the existing NITAG;
- Improving coordination among stakeholders of the decision-making process;
- Increasing political commitment for immunization;
- Strengthening national data collection systems;
- Securing vaccine financing; and
- Generating economic evaluations.

Since then, countries in the Americas have been making steady though variable progress on these dimensions. These conditions allow national governments to make better technical decisions about immunization programs, take responsibility for helping to pay for and distribute vaccines, and reduce the gap between developed countries and developing countries in the prevention of cases and deaths due to vaccine-preventable diseases.[11]

To improve knowledge and promote understanding of the new vaccine introduction process in the Region of Latin America, in 2012 a systematic qualitative assessment was performed with specific emphasis on the rotavirus vaccine (RV) and the pneumococcal conjugate vaccine (PCV.)[1] The decision-making process, the existing program structure, and the key factors that influenced the introduction of new vaccines were assessed, including national data on morbidity and mortality available and considered before the introduction of vaccines, sources of financing and mechanisms for vaccine introduction, implementation challenges and evaluation of vaccine impact.

The countries included were Bolivia, Brazil, Nicaragua, Peru, and Venezuela. Their decision-making process was assessed through interviews with key participants in each country and a systematic review of published data, gray literature, official technical documents and country-specific health indicators.

The study results showed the potential of new vaccines to reduce mortality, as established in the Millennium Development Goal Number 4, was an important consideration that led to the introduction of vaccines in all of the countries assessed. Additionally, other key components of the decision-making process in these countries included availability of funds, existence of adequate evidence for vaccine introduction, and the feasibility of sustained financing.

Finally, the study concluded that the decision-making process in the countries assessed does not follow a systematic approach. However, available evidence on efficacy, potential impact, and cost-effectiveness, even without local data, are significant elements in the decision-making process for the introduction of vaccines in Latin America.

Conclusions

For the introduction of new vaccines to be successful several criteria should be considered and agreed amongst various stakeholders. Latin America and the Caribbean countries have introduced new vaccines at a very high pace, and in doing so, some countries have followed the criteria described in this chapter more rigorously and comprehensively than other countries. As new and more expensive vaccines continue to emerge and as the national immunization programs require an increasingly higher budget, it will become important to count on the scientific evidence base to achieve successful and sustained introductions of new vaccines.

The creation and strengthening of NITAGs allow for a broad and independent evaluation of all the vaccine-related technical evidence under consideration. Lastly, quality national-level data on vaccine-preventable diseases is a critical component to be able to support the decision-making process within the national context.

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Introduction of New Vaccines in Latin America and the Caribbean: Measuring Impact and Effectiveness

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Rationale Behind a Surveillance System of New Vaccines

All of the vaccine-preventable diseases require an epidemiological surveillance system.[1] To be able to estimate more reliably the impact of a new vaccine, the national surveillance system should include the disease to be prevented with the vaccine before its introduction in the national schedule. If this has not been done previously, it would be key to include the disease in the surveillance system as part of the plan of action before the introduction of the new vaccine.[2]

Surveillance is a fundamental tool to leverage the process for the introduction of a new vaccine. The surveillance system provides significant support before the introduction of the new vaccine by collecting information in a systematic and standardized fashion to estimate the burden of disease (population-based surveillance) and the scope of the problem. Likewise, it helps to better understand the epidemiology of the disease and generate useful data for studies that inform the decision to introduce the vaccine, such as cost-effectiveness studies. In the stage prior to the introduction of a vaccine, the surveillance system allows for the monitoring of the epidemiological shifts in the disease, monitors changes in serotype circulation and generates data to analyze temporal trends or other studies to measure the vaccine impact.[2]

Measuring the Impact of a New Vaccine

It is important to differentiate between the impact and the effectiveness of a vaccine. The (direct) effectiveness of a vaccine may be estimated by comparing vaccinated and non-vaccinated individuals within the same immunization program. The impact of a vaccination program, on the other hand, is measured by comparing populations with and without the vaccination program. In general, the same population is used before and after the implementation of a vaccine in the program.[3]

There are different methods to measure the impact of a vaccine, depending on the characteristics of the disease to be prevented and the existing surveillance system in the country. Out of the methods to assess the post-introduction impact of a vaccine, the following should be considered:

- Assess the concordance between vaccination coverage and disease incidence.
- Estimate the effectiveness of the vaccine through the evaluation of vaccination records for all cases of disease, to determine the proportion vaccinated. Care should be taken when interpreting the data because there could be compounding factors (for example, if the vaccinated individuals have more access to the health services in general, then it is possible to diagnose more cases of disease than amongst non-vaccinated individuals).[4]
- Perform special studies, for example, in the case of the hepatitis B vaccine, whose impact will not be seen until decades after its introduction. In this case, a serological survey may be performed to estimate the prevalence of chronic disease.

The Case of Rotavirus Vaccine in Latin America

As an illustration, below is a description of the various methods used by Latin American countries to estimate the impact and effectiveness of the rotavirus vaccine.

Estimating Vaccine Impact in Bolivia. Bolivia estimated the percentage of positive samples of rotavirus amongst children younger than 5 years of age hospitalized due to diarrhea at six sentinel hospitals in the country.[5] Bolivia introduced rotavirus vaccine in August 2008, and to assess the impact of the program, the percentage of positive rotavirus samples from the previous two years was compared to samples two years after vaccine introduction. Table 1 shows an increasing trend of positive samples before the introduction of the rotavirus vaccine, followed by a declining trend of positive samples in the two years after the introduction of the vaccine. Despite not being able to establish causality with this type of estimate, these data suggest that the rotavirus vaccine in Bolivia may have had an impact on the decline in the number of rotavirus-related hospital admissions in children younger than 5 years of age.

Table 1. Number of Diarrhea-Related Hospitalizations Among Children < 5 Years of Age and Percentage of Positive Rotavirus Samples in Six Sentinel Surveillance Hospitals, Bolivia, 2006–2010

Estimate	2006	2007	2008*	2009	2010
All-cause admissions	11,119	11,377	11,080	10,827	12,408
Diarrhea-related admissions	2,296	2,139	2,015	1,848	1,885
Eligible for rotavirus surveillance	1,856	1,782	1,645	1,579	1,685
Cases investigated	1,272	1,585	1,379	1,393	1,509
Positive rotavirus cases	492	637	678	498	422
% of positive rotavirus cases	39%	40%	49%	36%	27%

*Year of introduction of rotavirus vaccine, in the month of August.

Source: Data from the Ministry of Health of Bolivia.

Estimating Vaccine Effectiveness in El Salvador. El Salvador performed a case-control study to assess the effectiveness of the monovalent rotavirus vaccine.[6] The country introduced the vaccine in October 2006. To perform the study, the Ministry of Health selected seven sentinel hospitals, representative of about 48% of the diarrhea-related hospital admissions in children younger than 5 years of age. From January 2007 to June 2009, 323 children younger than 2 years of age were hospitalized with laboratory-confirmed rotavirus diarrhea and 969 healthy controls matched for age and neighborhood with similar demographics were recruited. The study assessed the vaccine status of the cases and controls to estimate the effectiveness of the vaccine for the prevention of rotavirus-related diarrhea and severe disease. Based on this study, the effectiveness of the two-dose monovalent vaccine to prevent diarrheas requiring hospitalization in El Salvador was 76%.

A subsequent study assessed the impact of the program in El Salvador for all-cause diarrhea.[7] The study compared all-cause diarrhea and confirmed rotavirus-related diarrhea hospitalization rates during pre-vaccine year 2006, against the rates for the post-vaccine period of 2008–2009. Data were provided by seven sentinel surveillance hospitals. Results showed a decline of 81% for rotavirus hospitalizations in these sentinel hospitals for children younger than 5 years in the post-introduction period. Additionally, diarrhea cases declined 48% (95% CI: 47%–48%) during the rotavirus season in 2008 and 35% (95% CI: 34%–35%) in 2009 compared to the mean rate for 2005 and 2006.

Estimating Impact Using Interrupted Time Series. PAHO conducted an interrupted time series study to assess the impact of the rotavirus vaccine on hospitalizations and all-cause diarrhea deaths in Bolivia, El Salvador, Honduras and Venezuela.[2] The study compared the hospitalization trend and all-cause diarrhea-related deaths in these countries, all which had introduced the rotavirus vaccine, to the trend observed in Argentina, which had not introduced the vaccine at the time of the study and was considered a control country. The analysis period was from 2002 to 2010, and included 2 to 4 years post-vaccine introduction depending on the country. The results of the study showed a declining trend for hospitalizations and a trend which had an even more pronounced decline on the number of deaths in the countries that had introduced the vaccine, as compared to the trend observed in Argentina as the control country. Figures 1 to 4 show the rate of rotavirus-related diarrhea cases against the total number of diarrhea cases (Y axis), for the year included in the study (X axis).

Figure 1. Annual Rate of Diarrhea-Related Hospitalizations and Deaths Due to Rotavirus Over the Total Number of Diarrhea Cases, Bolivia, 2006–2010[2]

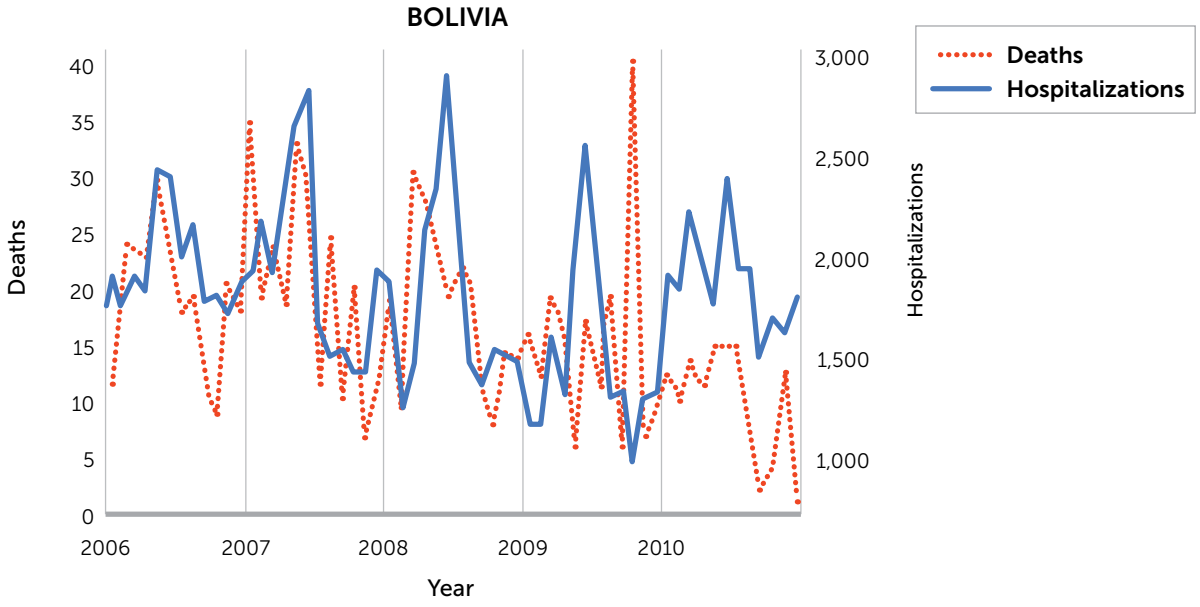


Figure 2. Annual Rate of Diarrhea-Related Hospitalizations and Deaths Due to Rotavirus Over the Total Number of Diarrhea Cases, Honduras, 2004–2010[2]

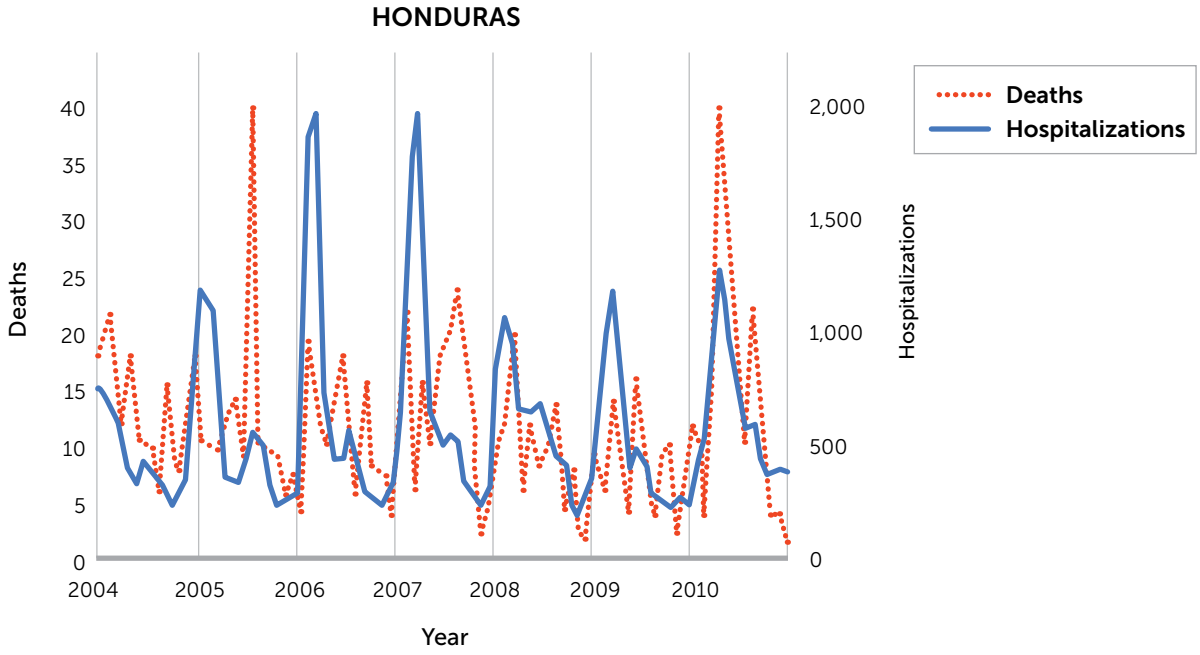


Figure 3. Annual rate of diarrhea-related hospitalizations and deaths due to rotavirus over the total number of diarrhea cases, El Salvador, 2002–2010[2]

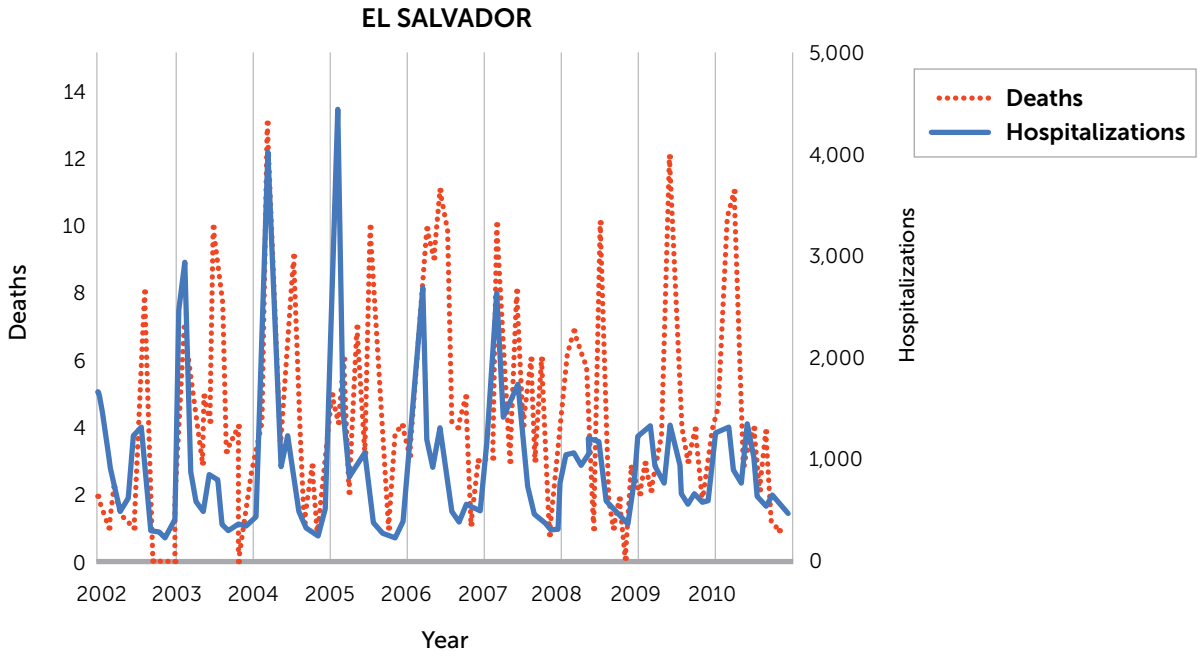
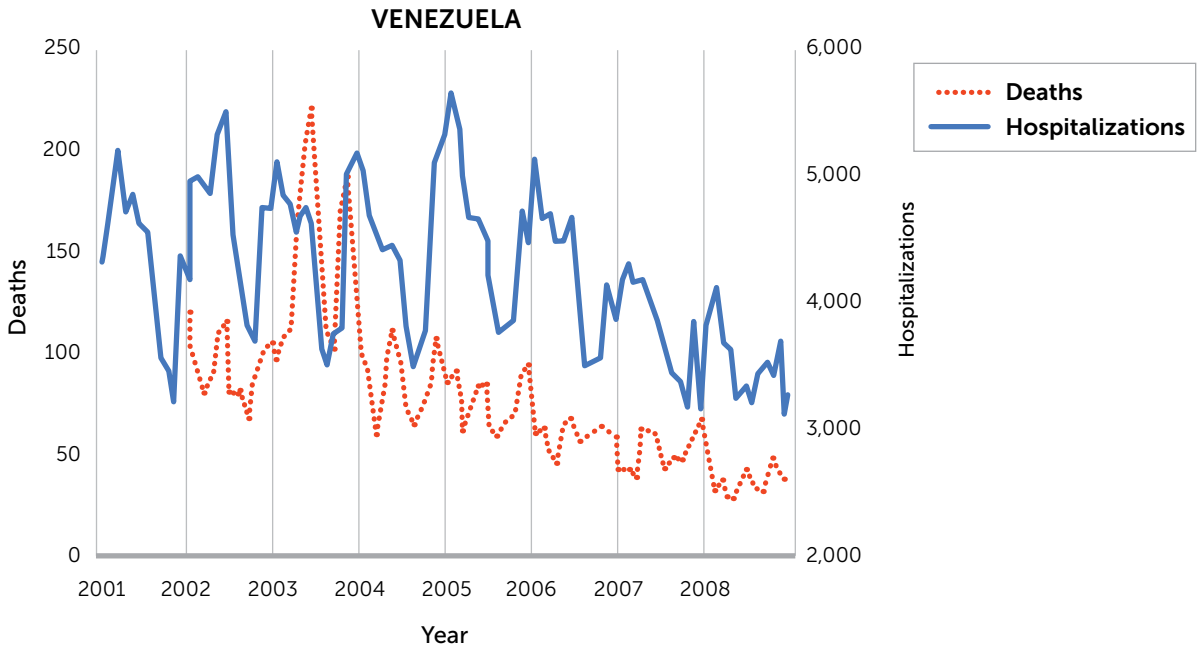


Figure 4. Annual rate of diarrhea-related hospitalizations and deaths due to rotavirus over the total number of diarrhea cases, Venezuela, 2001–2008[2]



The Case of Pneumococcal Conjugate Vaccine in Latin America

Again as an illustration, below is a description of the various methods used by Latin American countries to estimate the impact of the pneumococcal conjugate vaccine.

Estimating Vaccine Effectiveness in Brazil. In March 2010, Brazil introduced the 10-valent pneumococcal conjugate vaccine (PCV10) in the national immunization schedule. A case-control study was performed comparing the vaccination status of 316 children who had laboratory-confirmed invasive pulmonary disease and the vaccination status of 1,219 controls of the same age and neighborhood.[8] The effectiveness of the vaccine was 83.8% against the vaccine serotypes and 77.9% against the vaccine-related serotypes. No protection was observed against the non-vaccine serotypes. Therefore, the conclusion was that PCV10 is effective to prevent invasive pneumococcal disease caused by vaccine serotypes and may provide cross protection against some vaccine-related serotypes.

Estimating Vaccine Impact in Brazil. Subsequently, Brazil performed a study comparing the trend in pneumonia-related hospitalizations in children under 5 years of age, during the previous ten years and two years after the introduction of the pneumococcal conjugate vaccine in 2010.[9] The incidence of pneumonia-related hospitalizations was compared to the incidence of hospitalizations due to non-respiratory causes. The results showed that pneumonia-related admission rates decreased steadily by 12.6% in the 2010–2012 period compared to the 2002–2009 period, while admissions due to non-respiratory causes remained stable.

Estimating Vaccine Impact in Argentina. Argentina introduced the 13-valent pneumococcal conjugate vaccine (PCV13) in 2012. Subsequently, to assess its impact, the pre-vaccine and post-vaccine periods were compared against the rates of all-cause pneumonias, confirmed pneumococcal pneumonia and pneumococcal meningitis, as well as the pneumonia-related admission rates in children under 5 years of age.[10] Data were obtained from the national reporting system for pneumonias and meningitis, sentinel surveillance hospitals, and laboratory surveillance from the regional surveillance system network for bacterial agents responsible for pneumonias and meningitis (SIREVA). Results showed a reduction of pneumonia rates by 28% in children under one year of age and by 30% in children under 5 years of age, a reduction of confirmed pneumococcal pneumonia cases by 47%, a reduction of confirmed pneumococcal meningitis cases by 39%, and a reduction of pneumonia-related admissions by 41% in this age group.

Conclusions

Measuring the impact and effectiveness of a new vaccine is essential to justifying the investment and for making changes to the strategy and/or vaccination schedule as needed. Countries should perform epidemiological surveillance, time series studies, effectiveness studies and other types of studies to assess the impact of the vaccine. National governments are increasingly underscoring the importance of generating and having relevant, objective, and quality evidence for informed decision making in the health sector. This is the perfect opportunity to strengthen technical capabilities at the country level in connection with the generation and collection of national evidence. Once the technical teams, politicians and society get used to solid evidence-based decision making, there is no return. This is precisely the way to promote a lasting shift in the decision-making culture of the public health sector.

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Case Study: The Policy for the Introduction of New Vaccines in Brazil

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Introduction

The National Immunization Program (NIP) of the Ministry of Health (MoH) of Brazil was created in 1973, and the first national immunization schedule was published in 1977 with four mandatory vaccines in the first year of life (tuberculosis, poliomyelitis, measles, and DTPw [diphtheria, tetanus and pertussis]).[1]

During this time, vaccine production in the country was going slowly. The private sector considered that the national vaccine market was limited in contrast to other areas within the pharmaceutical sector, given its low profitability as compared to other profitable lines of business within the sector. This was a discouragement for the entry of private vaccine manufacturers to the national vaccine market.[2]

Despite the institutional effort to maintain the flow of supplies offered by the PNI, a significant crisis erupted in connection with the shortage of immunobiologicals as a result of the closure of Sintex of Brazil that was a privately-owned foreign-capital company that addressed the demand for products such as sera and the DTP vaccine. So, in 1985, the need for such products demanded the creation of the National Program for Self-Sufficiency in Immunobiologics (PASNI).

With PASNI, the MoH sought to establish coordinated actions by stimulating investments and improving the quality of the production between the national vaccine manufacturers: Instituto Butantan (São Paulo), Instituto de Tecnologia em Imunobiológicos Bio-Manguinhos/Fiocruz and Instituto Vital Brazil (Rio de Janeiro), Instituto de Tecnologia do Paraná (TECPAR) (Paraná), and Fundação Ezequiel Dias (Minas Gerais).

PASNI led to the expanding production of sera and vaccines in the Brazilian market aiming to meet the NIP's[1] demands and allowing for an increased supply of vaccines for other population segments other than just infants under one year of age.[3]

Current Situation

Presently, Brazil is one of the countries that offers the highest number of vaccines distributed free of charge as part of a defined schedule covering all age groups. The immunization schedule for children includes 14 vaccines, for adolescents and adults it includes five vaccines, and for older adults it includes four vaccines (Table 1).

Table 1. National Immunization Schedule, 2018

CHILDREN

1. BCG
2. Hepatitis B vaccine
3. Pentavalent vaccine (DTP/Hib/Hep B)
4. IPV (Inactivated poliovirus vaccine)
5. OPV (Oral poliovirus vaccine)
6. RV (Human-attenuated oral rotavirus vaccine)
7. PCV-10 (10-valent pneumocccal vaccine)
8. Yellow Fever vaccine
9. MMR (Measles, Mumps & Rubella vaccine)
10. DTP (Diphtheria, Tetanus & Pertussis vaccine)
11. MenC (Meningococcal C conjugate vaccine)
12. Influenza vaccine
13. MMRV (Measles, Mumps, Rubella & Varicella vaccine)
14. Hepatitis A vaccine

ADOLESCENTS AND ADULTS

1. Hepatitis B vaccine
2. Td (Tetanus, Diphtheria)
3. Yellow Fever vaccine
4. MMR (Measles, Mumps & Rubella)
5. Tdap (pregnant women)
6. Influenza vaccine
7. HPV (Human papillomavirus vaccine)
8. MenC (Meningococcal C conjugate vaccine)

OLDER ADULTS

1. Influenza vaccine
2. PCV-23 (23-valent pnemococcal polysaccharide vaccine)
3. Td (Tetanus, Diphtheria)
4. Yellow Fever vaccine
5. Hepatitis B vaccine

Personalized immunization schedules are available for the indigenous populations and for groups under special conditions such as immunodeficiencies at the Reference Centers for Special Immunobiologicals (CRIE). In total, NIP purchases 45 types of immunobiologicals (including vaccines, sera and immunoglobulins) and every year an estimated 300 million doses are distributed.

As the immunization schedule expansion request is increasing every day, the MoH has adopted new criteria for the introduction of new vaccines. This policy implementation has guaranteed an efficient and quick expansion still in observance of the regulations for immunization actions throughout the country.

So, the introduction of new vaccines relies on an epidemiological criterion which considers the population needs to reduce morbidity and mortality rates for a specific disease. In addition, other aspects are considered as the vaccine itself (immunobiological factors) as well as the operational, socioeconomic, technological, financing and legal factors.[4]

Sustainability of National Production

The MoH sustainability policy is based on the strengthening of the national health industrial park, where the main strategic supplies must be produced by the public laboratories. This action guarantees the self-sufficiency of national production, avoids product shortages and any restrictions due to market forces besides the maintenance of high vaccination coverages in all Brazilian municipalities. Two main mechanisms were adopted to foster national production: the incentivization of development of national products and the identification of partnership (private manufacturers) with the purpose of technology transfer to the Brazilian's public manufacturers. These actions have enabled the national production of all the main strategic supplies.

In this context, the introduction of new vaccines favors and implements the policy of supporting financial investments in the public vaccine manufacturers, strengthens the national market, decreases import costs and benefits the national trade balance in Brazil. This complex process involves several social actors from various other sectors besides the MoH. This policy has guaranteed the provision of vital strategic inputs and so the NIP has efficiency contributed to the control, elimination and eradication of vaccine-preventable diseases.

In the event that acquiring immunobiologicals from the national producers is not possible, the acquisition of these inputs is sought by the Revolving Fund for Strategic Public Health Supplies that was created in 2000 by the Pan American Health Organization (PAHO) as a request of the Member States. This initiative was intended to facilitate the purchasing and access of medicines and strategic supplies and by facilitating low cost procurement on behalf of the Member States. The chance of acquiring immunobiologicals through the Revolving Fund has made it possible to guarantee the NIP's supply of the needed inputs, especially for those inputs in which there are no established technology transfer partnerships or in situations where national production does not meet the country demand.[5]

New vaccines introduction demands additional resources which requires a budget proposal and the approval by the National Congress. The States and municipalities also need to allocate resources to guarantee the payment of human resources, the logistics of storage and distribution of the immunobiologicals and the acquisition of needles and syringes supplies. Once this additional budget has been approved, there is an annually guaranteed allocation of funds (Law 12.919 of 12/24/2013) as a mandatory action, which does not allow contingency of this action.

Furthermore, the inclusion of a new vaccine in the National Immunization Schedule requires consideration of the cold chain networks capacity at all the three government levels (national, state and municipal). For this, a structured cold chain network is essential from the manufacturer to the vaccination room, with responsibilities defined by the receipt, storage and distribution of the immunobiologicals. The MoH maintains the National Center for Storage and Distribution of Inputs — CENADI, responsible to receive all the national and international products purchased by NIP and to distribute them to the States and the Federal District where they are stored in central cold chains for redistribution to regional and/or municipal centers, and from there to the vaccination rooms.

The MoH has been putting in its investment plan the restructuring of this network as a priority action. However, it is an extremely expensive and complex process that demands effort and financial resources.

Between 2006 and 2015, an additional eight new vaccines were introduced in the National Immunization Schedule and even some conjugated vaccines already included in the schedule. Such vaccine additions have reduced the total number of vaccines in the national schedule without impacting the number of diseases

targeted for prevention, such as the pentavalent vaccine (diphtheria, tetanus, pertussis, *Haemophilus influenzae type* b and hepatitis B vaccine).

During the same period the following vaccines were introduced into the National Immunization Schedule for children: oral rotavirus vaccine (2006)[6]; 10-valent pneumococcal vaccine (2010); meningococcal serogroup C conjugate vaccine (2010)[7]; DTP/Hib/HB vaccine (2012); inactivated polio vaccine (2012) as part of the sequential schedule with the oral polio vaccine (OPV)[8]; tetravalent measles-mumps-rubella-varicella (MMRV) vaccine (2013); and the hepatitis A vaccine (2014). In 2014, the quadrivalent human papillomavirus vaccine (HPV4) was added for adolescent girls aged 11–13 years and the diphtheria-tetanus-acellular pertussis (dTpa) vaccine for pregnant women.[9]

Despite the progress in the implementation of those new vaccines, NIP faces the challenge of achieving and maintaining high vaccination coverages for all the vaccines included in the vaccination schedule. Vaccination coverage plays an important role in changing the morbidity and mortality profile of the country, allowing for the control and above all, the elimination of the transmission of diseases, such as the elimination of the transmission of autochthonous measles virus.

Conclusions

Vaccination strategies either in routine schedules or campaigns have increased the offer of vaccines and have reached the target populations established in the National Immunization Schedules.

In Brazil, the proven impact of the vaccination program, such as the eradication of poliomyelitis, the elimination of rubella and congenital rubella syndrome, and the contribution to the drastic reduction of vaccine-preventable diseases – has prioritized vaccination goals amongst public health policies. Consequently, ongoing epidemiological studies become necessary to measure the impact of new vaccines on the disease burden. Proper documentation for each disease and new vaccine introduced in the schedule is critically important.

The guarantee of the maintenance of the national health industrial park has been essential for the success achieved, as an important health benefit to the population, as well as to the country's economic sector, since the continuity of the national production guarantees the sustainability of the supply of the 36,000 vaccine rooms in the country, reducing the acquisition costs of acquiring immunobiologicals and strengthening the national productive sector.

However, given the complexities surrounding the introduction of new vaccines into the national immunization schedule, not only vaccine impact on disease morbidity and mortality should be taken into consideration, but also the cost-effectiveness of the vaccine (i.e., whether it produces benefits for health and reduces the disease-related costs of treatment, hospitalization, and work/school days lost by the patient and/or their relatives, plus their survival) as well as all the operational aspects that ensure sustainability and quality of the product to be offered in the country's service network.

Policies for introducing vaccines need to be standardized to guarantee efficiency, allow new vaccines to be incorporated into the National Immunization Schedule and to become available to other population groups in the light of scientific evidence.

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Phase III Clinical Studies for Vaccine Evaluation

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Introduction

The following chapter addresses how a researcher or a practitioner in charge of quality in a clinical trial may respond to the efficacy and safety of a candidate vaccine. Design par excellence is a randomized, double-blind, controlled trial where one group of the study receives the candidate vaccine while the other group receives the placebo as the control group. Several methodological considerations are discussed: how to structure a null hypothesis, how to select the study population and the importance and methods of randomization to avoid biases.

Since it is very difficult for an experiment to include the entire target population of the study, the sample size is explained and the important role of the researcher in defining the magnitude of the effect expected through the candidate vaccine, its significance and potency. The measurement, analysis and interpretation of study results, through intention to treat (ITT) or per-protocol analysis (PPA) methodologies are presented. Since one of the cornerstones of clinical study design is the ethical aspect of research conducted in human beings, a special section is included to explain the general principles behind research and the importance of informed consent. Finally, guidelines on how to design clinical studies and assess their quality are presented.

Overview

Based on the assignment to exposure, epidemiological studies fall into two types: observational and experimental studies. The former includes descriptive and analytical studies; the latter are conducted to verify a hypothesis and allow for the establishment of comparisons amongst various groups.[1]

The study factor in experimental studies is controlled by the research team and the studies are intended to evaluate the safety, efficacy, and optimal dose for one or more drugs, medical or technical devices for diagnostic, therapeutic, or prophylactic purposes, based on eligibility criteria, whose effects may demonstrate favorable or unfavorable effects for individuals. This design implies that the ethical requirements of research in human beings fulfill a key role in their execution.

Within experimental studies, the most important design is the randomized clinical trial (RCT).

The clinical study phases range between I and IV. Phase I is the first stage in an experimental study in human beings where the dose, the immunogenicity, and the administration path are assessed. Phase I is conducted in healthy individuals and requires about 100 individuals. Phase II is also conducted in volunteers, about 300 or 500 individuals, to study the immune response and product safety.

Phase III clinical studies share some common characteristics:

1. They are prospective;
2. They are closed: they use blinding techniques;
3. The researcher uses a research hypothesis with a clearly defined goal,
4. The outcome of a trial to demonstrate the protective efficacy of a vaccine depends on the case definition and the specificity for case detection and confirmation methods;[2]
5. They are designed to assess the efficacy and the safety of the intervention;
6. They are controlled and randomized;
7. They are the last phase of clinical research before the product is registered by the Regulatory Authority and authorized for entry into the market; therefore, the conditions of the study should attempt to replicate the conditions for regular use of the candidate medicine or product;
8. They use an estimated sample size to determine if there are statistically significant differences between the therapy and the placebo.[3]

Phase IV studies are conducted upon approval for distribution or marketing by national Pharmaceutical Regulatory Authorities (PRAs). Once a vaccine has been registered and is in use, pharmacovigilance is key to permanently maintaining information about its safety in the population. These studies are also conducted when the intention is to establish a new clinical indication.

Methodological Considerations

A clinical trial is initiated to answer a question on the efficacy and safety of a vaccine and it requires careful planning, ongoing monitoring of its execution, and follow-up of individuals to ensure no biases are present and the results are valid.

The research question is the most important step for research design and development. It should be:

- **Feasible:** adequate number of individuals, technical expertise, affordable in terms of time and financing.
- **Interesting:** it conveys the effect and safety of the vaccine to be used afterwards to solve a public health problem.
- **Novel (Original):** it confirms or refutes previous findings; it provides new results.
- **Ethical:** the benefits outweigh the damages and the main principles of research in human beings are respected.
- **Relevant:** to scientific knowledge, future research paths, or clinical and health policies.

Next, the meaning of each of the above-mentioned characteristics of Phase III studies shall be explained.

1. **Study hypothesis and purpose:** to detect through research whether the candidate vaccine is more effective than placebo. The null hypothesis will indicate that the effect of the candidate vaccine is similar to the placebo.
2. **Study population:** is selected from the population used to define the inclusion and exclusion criteria. The inclusion criteria define the population to be included in the study. Upon the understanding and signature of informed consent by the study participant, or their legal representative if the participant is under age, studies on preventive vaccines should be conducted in a healthy population of the same age group, sex and place of origin for which the vaccine will be recommended. Exclusion criteria are not the negation of the inclusion criteria: in general, experimental studies exclude individuals with an underlying pathology, pregnant women, vulnerable populations, and populations that would be unable to attend periodic checkups, or have contraindications to vaccine administration such as a history of allergy to some of the vaccine components.
3. **Controlled and randomized studies:** refer to the conditions which need to be controlled in every aspect, such as selection of individuals, storage, and medicine administration, registration of all variables and parameters that could impact the study, and measurement of results. They must have at least two groups or research arms: the study group (candidate vaccine) and a control group (placebo, the best available therapy shall be administered; it may be a vaccine with demonstrated efficacy).[4] Randomization in both groups is intended to distribute every participant at random in one or the other arm to obtain a balanced distribution of the demographic characteristics of the study population. Randomization is intended to attain as much homogeneity as possible in both groups to avoid selection biases since the only difference should be the intervention being studied.

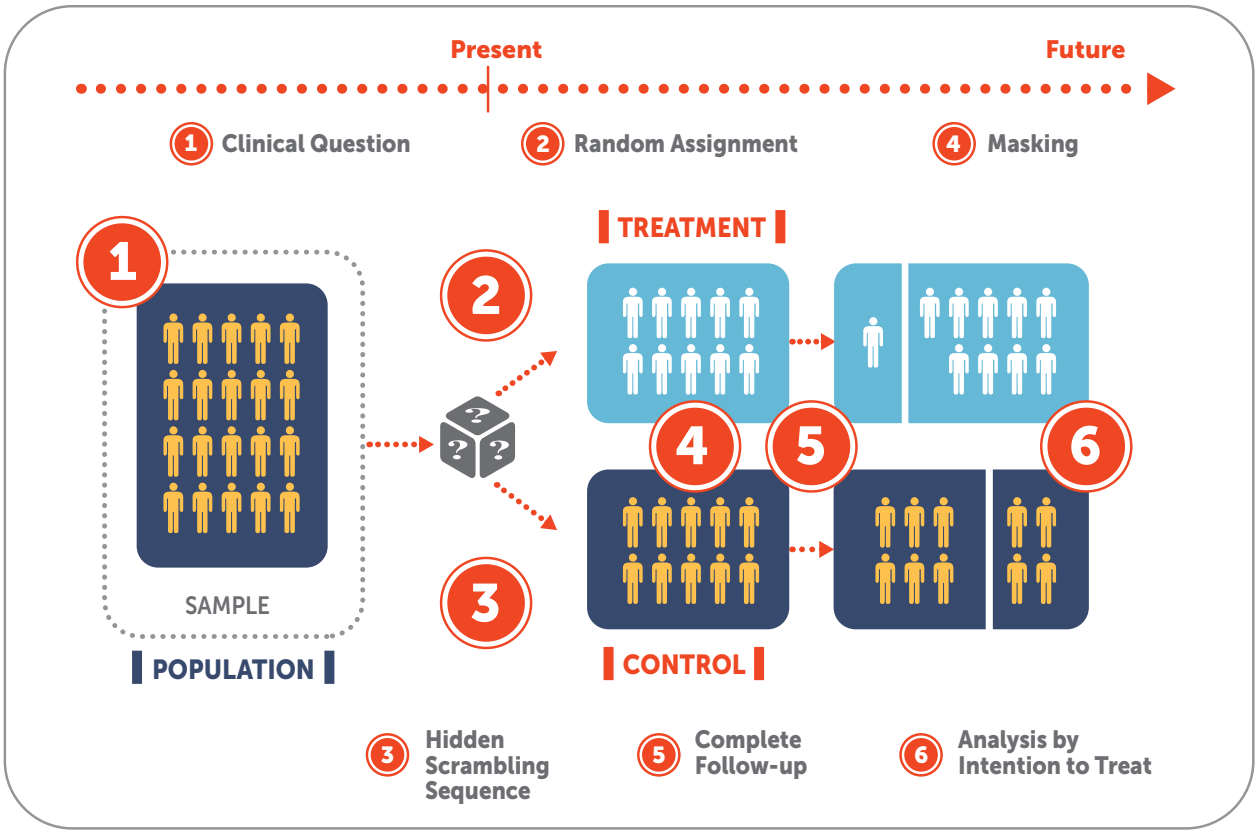
   Statistical randomization techniques include:

   - *Simple randomization:* it is the most straightforward way to randomize the intervention; uses as a basic tool the table or series of random numbers to avoid any type of bias; whenever possible, it should be computer generated and the person in charge should not be a member of the recruitment and follow-up team. With small samples, the use of this method may lead to imbalances in the number of individuals assigned to each group. Another drawback that must be taken into account is that sometimes repeated sequences of the same intervention may occur.[5]
   - *Block randomization:* this method counters the drawbacks under simple randomization. It comprises a series of blocks of cells which include an equal number of intervention options; the number of blocks to use depends on the number of patients to be assigned to an intervention, thus: number of blocks = number of patients/number of cells per block. The use number for each block is determined by the random number table and the assignment is done one patient at a time following the order obtained. The drawback with this method is that it does not balance the potentially modifiable variables of the effect or confounders.
   - *Cluster randomization:* is a simple or block randomization method in which the allocation unit is the group, rather than the individual. For this method it is important to estimate the measure of intracluster correlation (ρ) to determine the degree of response similarity amongst the group members; a positive ρ indicates that the variation in the observations amongst the different groups exceeds the variation within them.

4. **Blinding:** is a procedure used to prevent study participants from knowing the treatment they are receiving, to avoid the bias of the observer, and therefore, prevent them from impacting the answer. Types of blinding include:

    - *Single blind:* the participants are unaware of the intervention each individual receives.
    - *Double blind:* both the participants and researchers are unaware of the intervention.
    - *Triple blind:* in addition to the participants and researchers, other individuals are unaware of the treatment received by each individual, such as the statistician or those who assess the outcomes.

Figure 1. Phase III Clinical Study Design



Source: Molina Arias M, Ochoa Sangrador C. Ensayo clínico (III). Aleatorización. Enmascaramiento. *Evid Pediatr.* 2015;11:15.

5. **Sample size calculation:** is intended to determine the desired effect (answer to the research question) with a level of statistical significance and adequate power.[6,7] Based on the hypothesis, the research should issue findings on:

    - The scope of the candidate vaccine impact, i.e., how much the incidence of disease will diminish as compared to the control group or how much the number of deaths will decrease (depending on the outcome of the study). It is necessary to have baseline information, derived from epidemiological surveillance or through official morbidity or mortality registries, to estimate incidence in the population prior to the study.
    - The level of significance refers to the type I (α) error (Table 1); it is the error made when stating that the difference between the results obtained in the experimental group and the control group is significant at random. In general, this error is specified as 0.05, or there is a 5% probability of rejecting the null hypothesis when it is true, i.e., there is no difference between both treatment groups.
    - Another way to understand the size of the type I error is by interpreting its complement (1−α) as the level of evidence reached to reject the null hypothesis. In other words, this complement is the level of certainty with which the null hypothesis is rejected.
    - Power is derived from the difference of 1−β; rejecting the null hypothesis when it is false. Its complement is the type II β error, i.e., the probability of affirming there are no differences between the study groups when that is not the case. It is the capacity to detect a minimum difference of clinical significance, rejecting the null hypothesis when it is false.

Table 1. Type I and Type II Errors

	H_0 is True	**H_0 is False**
DO NOT REJECT H_0	Correct decision Confidence level Probability p=1−α	Type II error Probability p=β *H_0 is not rejected even though it is false (–)*
REJECT H_0	Type I error Level of significance Probability p= α *H_0 is rejected even though it is true*	Correct decision Test capacity Probability p=1−β

Source: Adapted from Biostatistics 2013.[8]

Table 2 suggests that as long as the researcher intends to obtain high power, error II is the lowest. In studies to determine vaccine efficacy, the working power is 90%.

Table 2. Statistical Power and Errors

Power	**Type II Error**	**Interpretation**
1.0	0.0	If there are differences between the group that received the candidate vaccine and the control group, it will be detected 100% of the time.
0.8	0.2	If the vaccine has an impact, it will be detected 80% of the time.
0.5	0.5	If the vaccine has an impact, it will be detected 50% of the time.

Sample size is important since it should be representative of the target population for the vaccination, and therefore, the results derived from the research may be extrapolated (external validity). There are various ways to estimate sample size depending on the outcome variable to be measured. One of them is estimation based on proportion difference:

Formula 1. Estimating Sample Size

$$N = \frac{(p1*q1) + (p2*q2) * f(\alpha, \beta)}{(p2 - p1)^2}$$

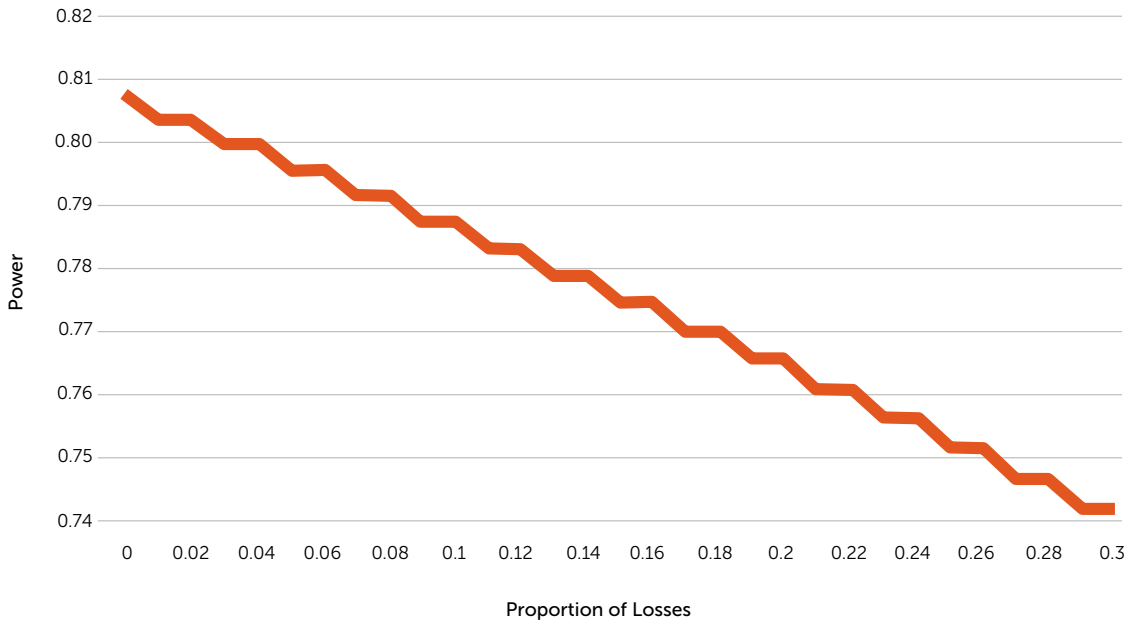
Note that p1 is the expected incidence in the experiment group and p2 is the incidence in the control group.

Follow-up begins once the study is initiated and individuals are randomized to an arm. The protocol should include strategies for adherence to the study since dropouts may result in a significant study bias and the loss of sample potency, more so if the loss is not random or homogenous in both groups.

Follow-up should take as long as necessary considering the natural history of the disease and the available background information on vaccine safety. The protocol should present follow-up strategies to avoid losses.

The loss should not be greater than 10% in population-based studies. To compensate for a 10% loss, the sample should be increased by 23%. If the loss is close to 20%, the sample should be increased by 56% as a number that will be added to the sample size.

Graph 1. Power Versus Losses



By accepting to lose no more than 5% of the initial power (80%), the acceptable loss ranges between 10% and 20% (Simulation by G. Cavada and M. Teresa Valenzuela). Therefore, a loss of up to 20% is considered acceptable with the assumption that losses need to be random with respect to the treatment arms.

Result Analysis

The first analysis should be a description that characterizes both populations, the candidate vaccine recipients and the placebo recipients, to guarantee similarity between the groups. Next, the results are analyzed to determine whether the null hypothesis should be rejected or not.

To that end, two types of analysis are conducted:

1. **Intention to treat:** the randomized individuals are analyzed based on the originally allocated treatment. If individuals are excluded upon randomization, biases may be introduced. Additionally, the individuals who left the study and their rationale for leaving shall be recorded; their exclusion from the analysis restricts generalization of the results.
2. **Per-protocol analysis:** the individuals are analyzed based on treatment completion, regardless of the original allocation.

How to measure the efficacy of the candidate vaccine versus the control group vaccine:

Using the incidence rate or incidence density of the disease in both groups, the subject years are estimated by adding the years each individual was free of the disease since enrollment until sickness within the follow-up period, and also the follow-up period for those who became sick. Based on this information, the incidence rate in both groups is established and the rate reduction percentage is determined.

The relative risk (RR) expresses the strength of association between vaccination and the decrease of cases of disease. As the RR value decreases below 1, the higher the vaccine efficacy.[9]

Formula 2. Calculating Vaccine Efficacy

$$\text{Vaccine efficacy} = \frac{\text{Incidence rate in the control group} - \text{Incidence rate in the vaccinated group}}{\text{Incidence rate in the control group}} \times 100$$

Formula 3. Calculating Relative Risk

$$\text{Relative risk} = \frac{\text{Risk for individuals exposed to the candidate vaccine}}{\text{Risk for individuals not exposed (control)}}$$

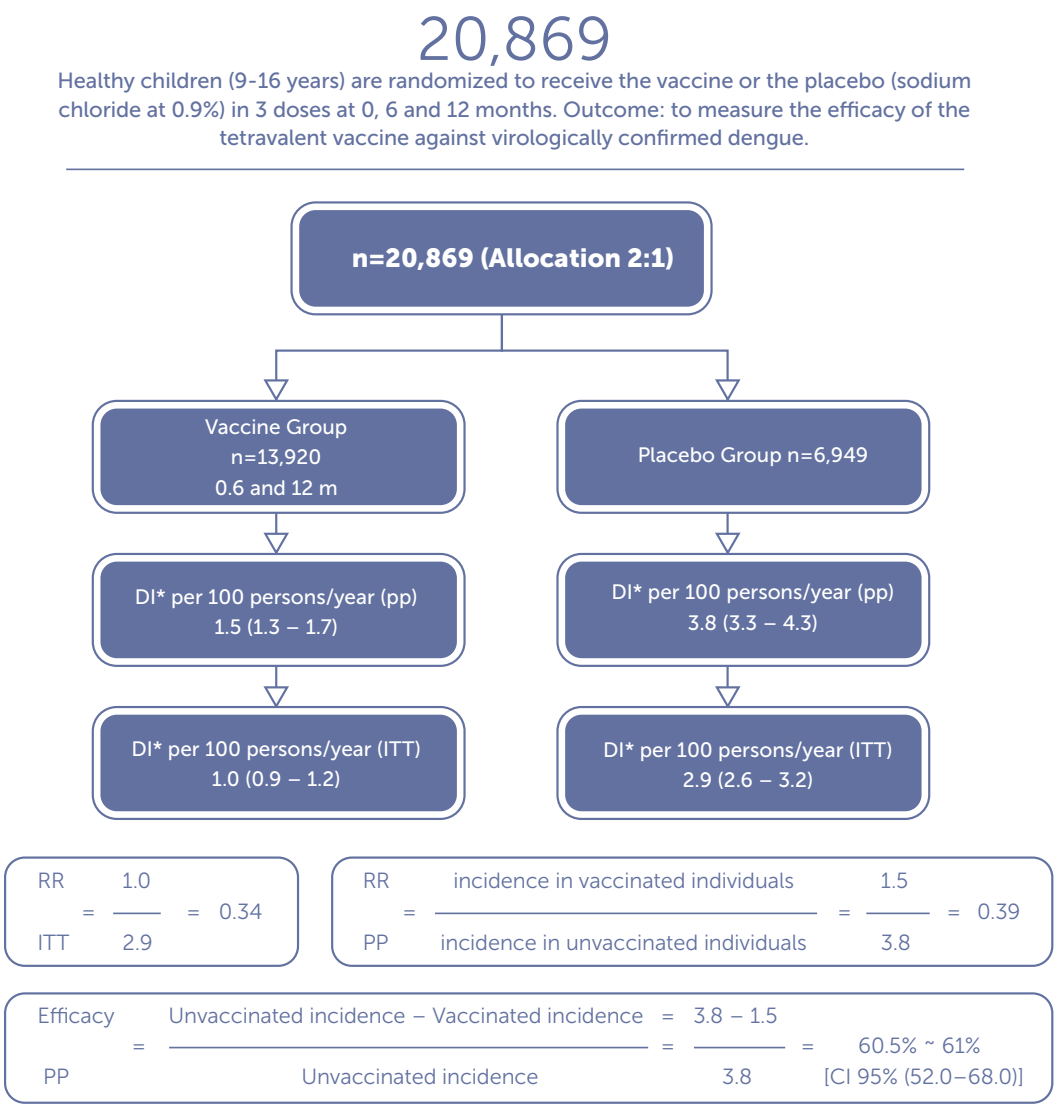
If the vaccine affords protection, the relative risk value obtained will be below 1.

Vaccine efficacy = 1–RR
The values obtained are specific and the 95% confidence intervals (95% CI) are estimated to have an accurate measure of the estimate. Values between the lower and upper bounds of the 95% CI include the specific value 95% of the time.

If the CI included one (1), the results would agree with the null hypothesis, i.e., there is no difference between the groups under study, and this means that the difference between the groups does not have statistical significance at a 0.05 α value.[10] . As an example, the candidate vaccine efficacy results expressed as RR are 0.62 (95% CI: 0.4–1.3); despite the RR value below 1, i.e., protective, the upper bound for 95% CI exceeds 1, therefore there is insufficient evidence to conclude that the findings are *statistically significant*.

Below follows an example of a Phase III trial:

Example 1. Efficacy of the Tetravalent Dengue Vaccine in Latin America[11]



Source: Adapted from NEJM 2015; 372:113

Notes: DI: Density of incidence or incidence rate; PP: Per-protocol analysis, and ITT: Intention-to-treat analysis.

Ethical Considerations

When planning a clinical study, key components to consider are the essential ethical principles to be complied with, including:

1. Respect for people
2. Principle of beneficence
3. Principle of justice

Respect for people implies acknowledging human autonomy for deciding their voluntary participation in a study and the protection of individuals with diminished autonomy.

The principle of beneficence refers to avoiding harm and maximizing the potential benefits while minimizing the potential damages.[12]

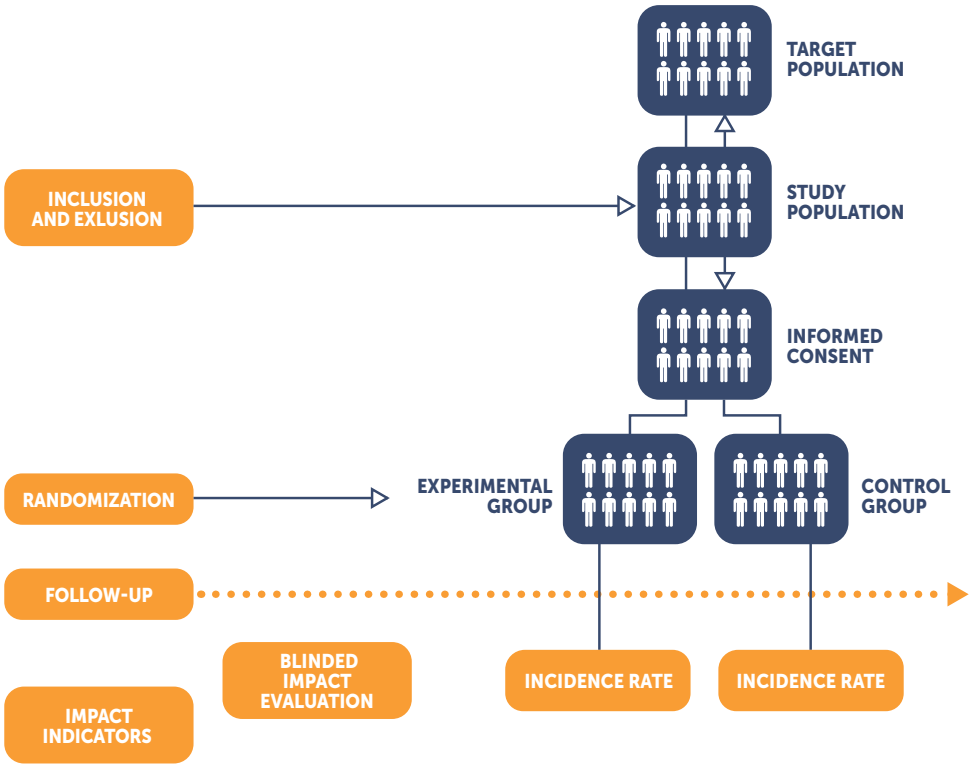
The principle of justice implies that to conduct a study, more vulnerable individuals cannot be systematically chosen, for example, individuals who are deprived of freedom and the elderly, nor can they be manipulated into entering a research investigation.

The application of the general principles to conduct a research study requires every participant to freely submit a written informed consent. This document shall contain clear, straightforward and easy to understand information on the study objectives, the potential risks and benefits expected, the vaccines to be received, the number of visits to be held, and the type and number of samples to be taken.

The informed consent shall be put in writing and explained to every study participant or their legal representative in a language that is adapted to their level of understanding, in a calm manner, with enough time to make sure it was understood and that the person had a chance to ask all the questions deemed necessary.

The individual or their legal representative shall accept participation voluntarily, without pressure or undue influence.[13]

Figure 2. Summary of Phase III Clinical Study Processes



Assessment of Clinical Trials

To develop a Phase III controlled clinical trial,[6] the following guidelines (CONSORT) should be followed:

1. **Title and summary:** must contain the randomized patient selection method.
2. **Scientific background and study justification.**
3. **Methods:** participants, interventions, objectives, results, sample size, randomization, blinding and statistical methods.
4. **Results:** flow diagram of participants, recruitment, baseline data, numbers analyzed, results and estimation, supplementary analyses and adverse events.
5. **Discussion:** interpretation, generalization and global evidence.

Furthermore, it is highly advisable to critically review the validity of the results of a clinical trial. To assess a clinical trial, three main questions need to be considered:

1. Are the results of the study valid?
2. What are the results?
3. Are the results useful?

For more information, the Critical Appraisals Skills Programme (CASP) offers a checklist to appraise a Randomized Controlled Trial: http://www.casp-uk.net/casp-tools-checklists.

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National Immunization Technical Advisory Groups

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Introduction

At the global level, the World Health Organization (WHO) Strategic Advisory Group of Experts (SAGE) on immunization makes recommendations about the use of vaccines, taking into account characteristics of the disease, safety and efficacy of the vaccine, cost-effectiveness, and other factors. Regional and National Immunization Technical Advisory Groups (NITAGs) play an increasingly important role in developing national immunization policies and assisting Ministries of Health in monitoring progress against vaccine-preventable diseases.

NITAGs are needed in order to adapt global (SAGE) recommendations to the local or national situation. With the increasing number of new vaccines being developed and increasing complexity of immunization schedules, they are critical to assist national policy makers in establishing feasible approaches to provide optimal protection of the population. In addition, with the increasing costs of new vaccines and the increasing scrutiny of government expenditures and decisions, they provide an evidence-based assessment of the wisdom of adding additional vaccines to the schedule. Donor partners are increasingly demanding evidence both of the national need for a given vaccine and of the process for developing recommendations for its use.

The 2011 Global Vaccine Action Plan (GVAP) of WHO and its key partners calls on countries to "...create, or strengthen existing, independent bodies that formulate national immunization policies (e.g., NITAGs or RITAGs)..."[1] In addition, the PAHO Directing Council calls for "...promoting the formal establishment of and strengthening existing NITAGs or regional policy bodies that serve the same purpose, as is the case of the Caribbean Advisory Committee..." and "...grounding immunization policy-making in a broad national evidence base..."[2]

NITAG Functions

NITAGs are technical resource and deliberative bodies designed to empower national authorities and policy makers to make evidence-based decisions.[3] They are not implementing, coordinating, or regulatory bodies. They also are not the same as Interagency Coordinating Committees (ICCs), which are composed of representatives of partner agencies charged with ensuring coordination of all the players in immunization.

NITAGs typically conduct policy analyses and recommend optimal immunization policies for existing programs and for use of new vaccines. They guide the formulation of strategies and provide advice on monitoring the immunization program to measure impact. They also advise on the collection of information about diseases and vaccines and identify the need for further data for policy making.

NITAG Establishment and Operations

NITAGs are usually established by ministerial decree or legislative action and report to a high level official in the Ministry of Health, often the Director of the Immunization Program or higher level authority. Formal terms of reference are developed and Secretariat support is provided by the Immunization Program. Funding to support the NITAG is budgeted by the Ministry of Health.

NITAGs typically have 10–15 core members who make decisions by vote or by consensus. They are appointed by a senior level government official on the basis of their individual expertise. Core members should represent a range of disciplines and be independent of government, manufacturers, and have no conflict of interest. In addition to the core members, there are typically non-voting *ex officio* members representing government agencies and *liaison* members representing professional societies, WHO, UNICEF, or other non-governmental organizations. Typically a formal orientation is provided for new members.

Operationally, NITAGs develop standard operating procedures addressing items such as whether meetings will be open or closed (or a mix) and the role of industry and observers. In addition, they define the process to review and share evidence and the process for decision-making (e.g., formal vote, consensus). They also address the establishment, composition, and functioning of working groups to address specific issues. Working groups often include non-NITAG members but are chaired by NITAG members and do not decide NITAG policy — rather, they recommend policy to the NITAG, which may then accept, reject, or amend the recommendation. The procedures also address more mundane issues such as meeting frequency, methods of communication and reports.

NITAG Recommendations

In developing immunization policy recommendations, the following elements should be considered:[4]

- **Vaccine and immunization characteristics** such as safety, efficacy and effectiveness, and indirect effects of the vaccine.
- **Vaccine delivery characteristics** such as number and timing of doses and method of administration.
- **Disease characteristics —** burden of disease, clinical characteristics, use and costs of health care occasioned by the disease, social impact, existence of alternative preventive and control measures, as well as regional and international considerations.
- **Economic and operational considerations —** vaccine related costs and use of resources (human and financial), vaccine availability, vaccine affordability, economic impact of the vaccine on the overall immunization program and on the health sector.
- **Health policy and programmatic issues —** interaction with other prevention and control strategies, feasibility (e.g. cold chain requirements), vaccine registration and regulations, affordability and sustainability, ability to evaluate, acceptability, equity, and social considerations.

The process for developing evidence-based recommendations should be transparent and should include a thorough search for data, using a systematic, standardized, and reproducible approach. The quality (design and execution) of studies should be assessed along with possible biases. Consistency and generalizability of results from different studies as well as effect size should also be considered. Finally, cost-effectiveness and feasibility of implementation should be considered. These activities are often carried out by work groups and reported back to the NITAG for consideration/decision-making.

NITAG Functionality

Six global process indicators for NITAG functionality have been developed:

- Legislative or administrative basis for the advisory group,
- Formal written terms of reference,
- At least five different areas of expertise represented among core members,
- At least one meeting per year,
- Circulation of the agenda and background documents at least one week prior to meetings, and
- Mandatory disclosure of any conflict of interest.

Blau *et al.* developed a more detailed list of 31 proposed indicators of NITAG functionality, including process, output, and outcome indicators.[5] Following testing in 14 countries, the list was narrowed to 17 (Table 1). These provide a more complete picture of NITAG functionality, and can be used in self-assessment.

Table 1. NITAG Indicators for Country Self-Assessment

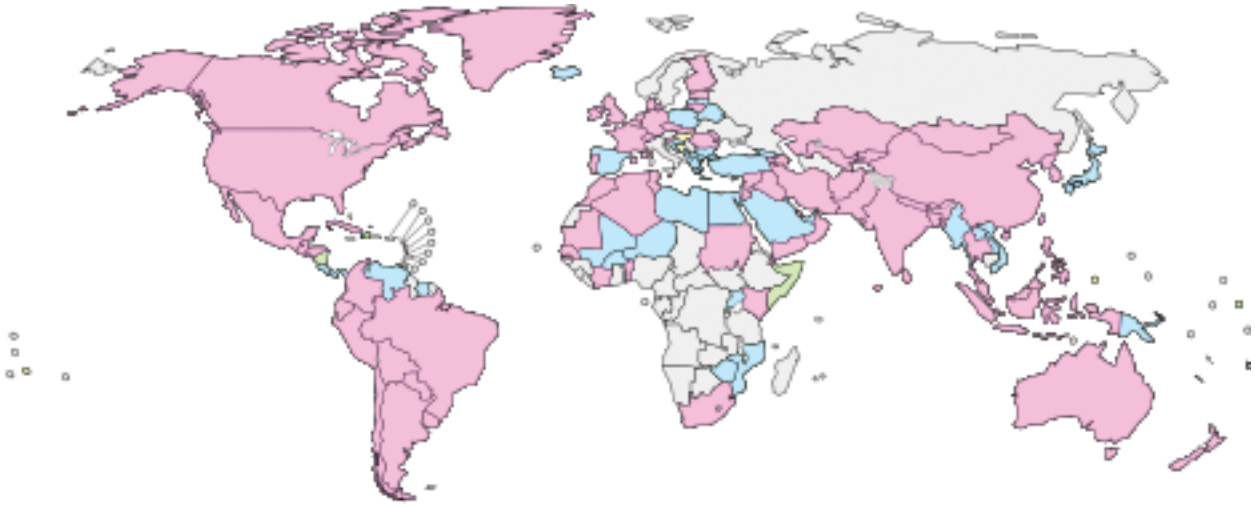
Process Indicators
▪ Legislative/administrative basis ▪ Advisory role only ▪ Terms of reference ▪ Membership ▪ NITAG functioning Standard Operating Procedures ▪ Independent chairperson ▪ Number of meetings ▪ Agenda and background documents distribution ▪ Declaration of interests ▪ Official requests for recommendations received and addressed
Output Indicators
▪ Evidence-based methodology for recommendations ▪ Country-specific criteria for recommendation ▪ Vaccine availability and delivery capacity criteria for recommendations
Outcome Indicators
▪ MoH decisions made in consultation with NITAG ▪ Recommendations accepted by MoH ▪ Recommendations which were not adopted by scientific or professional organizations ▪ Recommendations implemented in the country

NITAGs Globally

Figure 1 is a map displaying the global status of NITAGs. With respect to the proportion of countries having NITAGs, the Americas (63%) are doing about the same as the world overall (64%). However, 98% of the population in the Americas is covered by NITAGs compared to 87% globally and 92% are covered by fully functional NITAGs, compared to 75% globally. Some of the challenges facing NITAGs include:

- Identifying a qualified pool of members with the time and interest in participating,
- Identifying and managing potential conflicts of interest,
- Availability of national data to support decision-making,
- Assuring continuous support in the face of political change, and
- Identifying and supporting a dedicated secretariat.

Figure 1. Global Status of NITAGs 2014



- 81 countries meeting the 6 NITAG criteria
- 115 countries having a NITAG with administrative or legislative basis
- 116 countries reporting the existence of a NITAG with terms of reference
- 123 countries reporting the existence of a NITAG
- No NITAG/not available
- Not applicable

Map production: Immunization Vaccines and Biologicals, (IVB) WHO. Date of slide: 16 July 2015.

Source: WHO/UNICEF coverage estimates 2015 revision.

NITAGs in the Americas

The report of the 23d meeting of the PAHO TAG (held in Varadero Cuba, July 1–3, 2015) states that:[6]

> "PAHO has provided technical assistance in the form of trainings and facilitation of technical exchanges between committees since the 1990s. In the past five years, 12 countries have worked with PAHO to revise their terms of reference (TOR) and standard operating procedures (SOP). Argentina published their revised TORs in Vaccine as a brief report last year in an effort to share with other countries. As of 2014, 22 of the 23 countries that report an active NITAG have formal terms of reference. Though, the systematic declaration of conflicts of interests by core members is still absent in some countries. Four of the 23 countries with NITAGs do not meet all six indicators for a well-functioning NITAG because these committees have not introduced these procedures. Still, the number of national-level decisions backed by NITAG recommendations in the Region indicates that governments generally recognize the value of NITAGs in ensuring a credible, transparent and evidence-based process for decision-making. This process is only possible with the presence of a strong executive/NITAG secretariat within the national immunization programs. The executive/NITAG secretariat is responsible for the preparation of the technical content and evidence inputs required for the committees' deliberations. In this sense, since 2004, PAHO ProVac Initiative has assisted countries in the development of evidence inputs for vaccine policymaking, primarily vaccine cost-effectiveness and impact data. These studies have been an important input into decision-making for new vaccine introduction. Fourteen countries have completed analyses and presented results from them to their national authorities and in May of this year much of this data was published in a special issue of the journal Vaccine. Important advances in strengthening the process for evidence-based immunization policy at the country-level in the Region have been made. To sustain this progress and achieve the goals set forth for this decade, countries will need to continue their commitment to strengthening their committees and establishing them where they do not yet exist. The English-speaking Caribbean is a special case where countries in this sub-region have generally worked as a sub-regional block towards harmonized policies for immunization. This model is unique in the world and the governments in this sub-region may consider strengthening the formality of this model.
>
> Recommendations
>
> - TAG reiterates the independent advisory role of NITAGs and encourages all countries in the Americas to formally establish these committees, considering the guidance developed by PAHO.
> - Where NITAGs already exist, they need to be guided by independent experts using the scientific evidence available to make recommendations with a transparent and structured process.
> - In the English-speaking Caribbean, there are existing sub-regional collaborations on immunization policy development. PAHO should support countries in a coordinated effort to formalize this technical advisory structure."

Conclusions

NITAGs are increasingly an essential part of national immunization activities. Although establishing and sustaining NITAGs is complex, it is feasible, particularly given the technical assistance available in the Americas from PAHO, WHO and the NITAG Resource Center.

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Stronger Immunization Policy in Latin America and the Caribbean: Vaccine Impact, Costs and Cost-Effectiveness Evidence

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Introduction

Public health priorities are set on the basis of multiple criteria which may vary from country to country. Most governments prioritize immunization for their population, considering vaccines "a best buy" in public health. New vaccines often challenge this assumption because they are more expensive and complex. As a result, decision makers increasingly request evidence on the distributional impact, costs and cost-effectiveness of new vaccines before their introduction. This chapter reviews economic evaluation methods to estimate the impact, costs and cost-effectiveness of new vaccine introduction and their importance for a robust decision making process. The chapter concludes with real world experiences from PAHO's ProVac Initiative to support evidence-based decision making on new vaccine introduction in Latin America and the Caribbean.

From Disease Elimination to New Challenges

In 2015, the Pan American Health Organization (PAHO) declared the Americas free of rubella and rubella congenital syndrome.[1] This declaration came on the heels of the last case of congenital rubella syndrome being reported in 2009.[2] Thanks to high and sustained levels of vaccination coverage, other vaccine preventable diseases such as neonatal tetanus, diphtheria, whooping cough, polio and measles are no longer the substantial public health problem they used to be in Latin America and the Caribbean.[3]

Today additional benefits are being reaped from even newer vaccines that target big childhood killers such as pneumonias and diarrheas, further dramatically reducing needless death and suffering from these diseases. In countries of the Latin America and Caribbean region, vaccines to prevent invasive pneumococcal disease and rotavirus diarrhea, responsible for approximately 28,000 and 15,000 deaths every year in children <5 respectively[4,5], were the first series of new vaccines marketed for sale in the early 2000s. Human papilloma virus (HPV) vaccines that prevent 70% of all cervical cancers in women were introduced to the market in the mid-2000s.[6,7] With the risk of pandemic and seasonal influenza, vaccines to protect against flu have also become

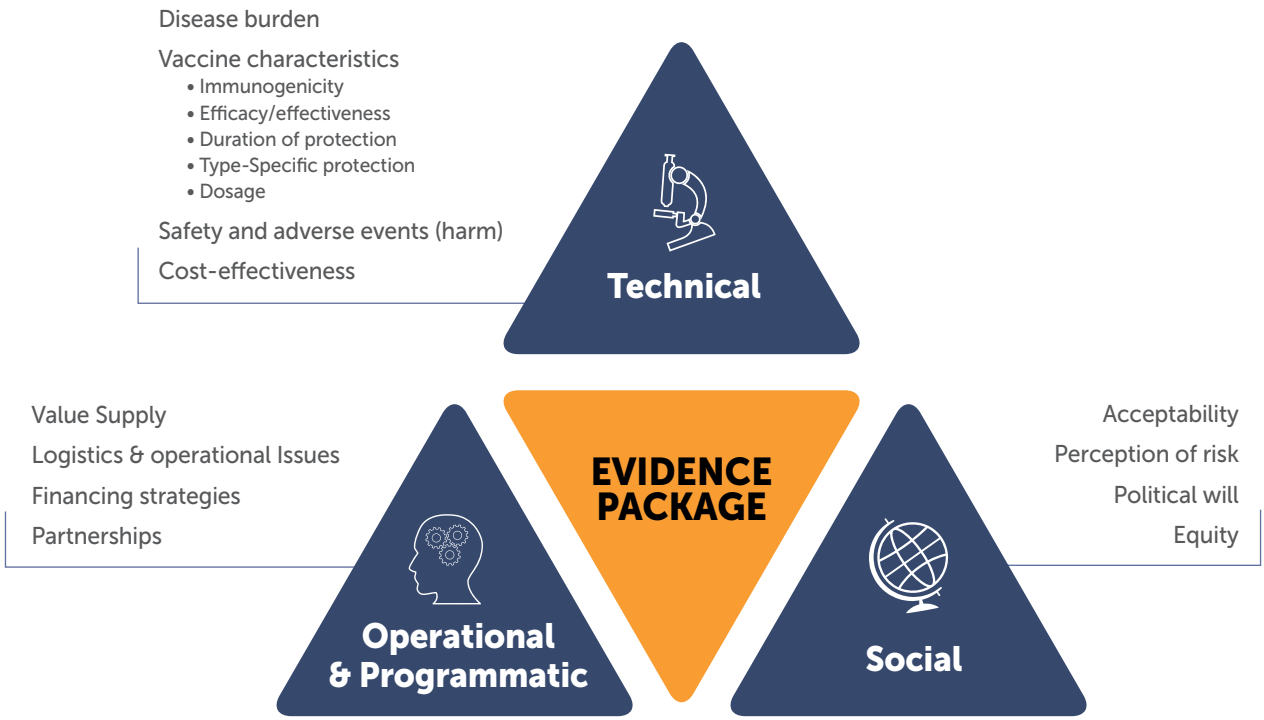
more accessible and widely used in national immunization programs. Recently vaccines that prevent dengue and cholera as well as second generation or better, safer product vaccines have arrived to the market.[8]

All these new vaccines have three characteristics in common:

1. Higher initial prices when introduced to the market compared to traditional vaccines already offered in the basic routine immunization schedule;
2. Generally efficacious products but they target smaller pockets of disease burden than the predecessor vaccines such as polio, measles, rubella, pertussis, diphtheria and tetanus;
3. Multiple products available (or under research) for the respective antigen; they often target different strains and types with differential effectiveness by strain/type, adding to the complexity of evidence.

The shared characteristics of each of these new vaccines have several implications for policymaking, both at the global and local levels.[9] Disease burden and epidemiology vary by country and region. Also, available resources and feasibility of expanding fiscal space for the adoption of these newer vaccines may drastically vary from country to country. Technical, programmatic and operational and social considerations for immunization policy formulation are summarized in Figure 1.

Figure 1. Considerations for the Introduction of a New Vaccines into a Routine, Universal Immunization Program



Source: Andrus, JK., Toscano, CM., Lewis, M., Oliveira, L., et al. 2007, "A model for enhancing evidence-based capacity to make informed policy decisions on the introduction of new vaccines in the Americas: PAHO's ProVac Initiative", *Public Health Reports*, 122(6): 811-816.

Evolution of Global-, Regional- and National-Level Policymaking Structures

Governments have traditionally relied on global and regional level advisory bodies for recommendations on the inclusion of vaccines in their basic routine immunization schedule. In 1989, the PAHO Technical Advisory Group (TAG) on VPDs was officially established to serve this purpose, initially with the aim of providing guidance on evidence-based strategies to eliminate wild poliovirus in the Americas.[10] The World Health Organization (WHO) established a similar advisory group called the Global Advisory Group (GAG). Later in 1999, the Strategic Advisory Group of Experts (SAGE) was created to support the policy deliberations at the global level and advise on official WHO vaccine-specific policy positions.[11] Despite the immense resource these two policy and advisory bodies have been to governments in the Americas, local processes and infrastructure for evidence-based decision making around new vaccine introduction and more generally immunization policy were critically needed. To that end, ministries of health across the Region have moved to establish National Immunization Technical Advisory Groups (NITAGs) to serve this purpose.[12]

NITAGs consider data that evidences the public health problem (i.e. disease burden and economic burden), the potential for shift in this problem (i.e. changing epidemiology of the disease), and the existing public health infrastructure to address the problem (i.e. available non-vaccine interventions and cost to maintain these interventions). These bodies are charged to review data, in particular local data, in combination with the data on available vaccines that target the public health problem. This approach to emphasizing the use of local data in helping determine the potential impact of new vaccines in a country contributes to making more sound recommendations.[11] More recently, policy makers, both in advisory and decision maker roles, are finding evidence on the cost to health benefit tradeoff an important, and often compelling, data point to any decision on new vaccine introduction or policy changes to existing recommendations.[13]

Assessments of Costs and Cost-Effectiveness as Important Considerations for Immunization Programs

Data on the tradeoff between costs and health benefit helps decision makers determine the return (health gains and costs saved) on their investment in public health. High level authorities in the public sector should want to use scarce financial resources to achieve the greatest possible health impact for their beneficiary populations. Cost-effectiveness analysis is one method that guides decision makers when setting priorities about where and how to invest public monies. These analyses compare the net costs to the net benefits.[14] Table 1 summarizes the types of evaluations undertaken to assess costs and cost to benefit tradeoffs.

Table 1. Types of Cost and Economic Evaluations Used for Decision-Making

Type of Analysis	Description	Includes Costs?	Includes Health Impact?
Cost Analysis	Identifies quantities of resources consumed and values them to estimate total cost of delivery of services and/or interventions.	**X**	
Cost-Minimization Analysis	Compares the cost of two or more interventions that have **identical outcomes** to assess which provides the least costly option to deliver the same outcome.	**X**	
Cost-Benefit Analysis	Compares the monetary value of health (converting the value of life gained or lost to monetary units) achieved from two or more interventions that may provide different outcomes. Because health consequences are valued in monetary terms, this form of analysis enables comparisons between the health sector and other sectors (i.e. transportation, agriculture, education, etc.).	**X**	**X**
Cost-Effectiveness Analysis or Cost-Utility Analysis	Compares the net increase in cost to the net health gain (expressed in natural units) of two or more interventions to determine the cost to health benefit tradeoff, or cost-effectiveness, of one intervention compared to another. Cost-utility analysis is a special type of cost-effectiveness analysis that uses health-adjusted life years to measure health gain, which includes both duration and quality of life extended due to an intervention.	**X**	**X**
Return on Investment	Compares the monetary value of health achieved from two or more interventions to the costs invested to deploy the two interventions. The results are expressed as percentage or ratio.	**X**	**X**

In the case of a vaccine, cost-effectiveness analysis compares the total incremental costs of the new vaccine to the health system (i.e. immunization program) minus the potential costs averted by the health system due to vaccination with the total incremental health benefit provided by vaccination (Figure 2). Both costs and health benefits are discounted in this type of analysis to represent the time preference of decision makers.[15] Health benefits are often measured in a unit called Disability-Adjusted Life Years (DALY). DALYs are a composite measure of mortality and morbidity. This allows for the comparison of interventions that may target high mortality disease with interventions that target only morbidity, for example.[14]

Figure 2. Equation for Estimating the Cost-Effectiveness of a Health Promotion Intervention

$$\text{Incremental cost-effectiveness ratio (ICER)} = \frac{\text{(Intervention associated costs-averted costs to treat disease)}}{\text{Health benefits gained}}$$

The primary outcome of a cost-effectiveness analysis is the incremental cost per health benefit gained, generally represented as the cost per DALY.[15] This outcome is often compared to the gross domestic product (GDP) per capita to determine willingness to pay or cost-effectiveness. When the cost per DALY is between one and three times GDP per capita, the intervention may be considered cost-effective. If the cost per DALY falls below one GDP per capita, the intervention may be considered highly cost-effective. Of course, above three times GDP per capita, the cost per DALY would no longer be considered cost-effective.[15] These rules of thumb

thresholds for cost-effectiveness are currently supported by the World Health Organization, but many academic and policy groups are working to adapt these thresholds to reflect a more realistic and applicable representation of willingness to pay at country level.[16] Because budget envelopes for health and specifically for vaccines will vary from country to country, willingness to pay must consider the direct budget impact of an intervention to the health sector. Therefore, affordability is a key part to the equation and currently is not directly accounted for in any cost-effectiveness calculations.

Experiences from Latin America and the Caribbean

Latin American and Caribbean (LAC) governments continue to routinely consider recommendations from PAHO and WHO on immunization. Nonetheless, as mentioned above, countries in the Americas identified a need to incorporate local economic evidence into their decision making process on new vaccine introduction and other immunization policy considerations in the mid-2000s.[17] PAHO responded with support to strengthen the capacity at country-level to develop and use economic evidence, among other types, in the decision making process with a focused, capacity building initiative called ProVac. Since 2004, PAHO's ProVac Initiative has provided training and technical assistance to countries to conduct and to use cost-effectiveness analysis.[17]

The ProVac Initiative empowers national teams at ministries of health to conduct their own economic analyses, leading to more informed decisions about the introduction of new and underutilized vaccines into national immunization programs. The country-led process increases trust and interest in the results by national authorities. ProVac helps to build local technical capacity. This process in turn provides a number of important indirect benefits such as increased collaboration between national institutions, more effective planning for vaccine introduction, improved infrastructure for decision making, and a solid platform for the wider promotion of evidence-based decision making.[18] A key element of this process involves establishing a national working group to collect, summarize, and assess the evidence inputs to a cost-effectiveness analysis. National teams then populate ProVac tools and models with the best available evidence estimates to generate and interpret cost-effectiveness outcomes.

One of the most important lessons in the implementation of this ten-year initiative has been that the process matters much more than the point estimate results of a cost-effectiveness analysis.[19] Inputs to cost-effectiveness analysis include: disease burden, vaccine effectiveness (strain-specific efficacy over time adjusted for real world programmatic error, such as drop-out, suboptimal coverage), vaccination program costs and cost of treating existing disease burden.[15] This work helps ministries of health put together the package of evidence required for new vaccine policy. Also, in the process of applying a quality assessment framework, decision makers can transparently assess the quality of available evidence so as to identify gaps, weaknesses and uncertainty in the evidence base underlying a new vaccine decision.

Importantly, the quality of data for key parameters like vaccine impact—efficacy and coverage—hinges on the risk of bias, precision, consistency and directness. For example, many data considerations that inform decisions on new vaccines are drawn from randomized-control trials (RCT) or case-control studies designed to assess the safety, immunogenicity, efficacy and sometimes effectiveness of a vaccine in the intervention populations versus the control population. If the populations included in the studies are not the same as a given country's vaccination target population, this may represent a risk of 'indirectness' because the results of the studies may

not apply directly to a given country's population. However, these quality risks or uncertainties can be dealt with in sensitivity analysis of economic evaluations and therefore provide a systematic approach to communication uncertainty in an analysis to decision makers.

Communicating results from economic evaluations to decision makers is no easy task. Most ministry of health authorities may be familiar with the concept of DALYs or other morbi-mortality measures. Still, DALYs averted does not translate easily for public health practitioners. Another important lesson from the work of the ProVac Initiative in the Americas is the critical role of program managers and their technical teams in translating cost-effectiveness data into appropriate evidence-based policy messages. Country teams often make use of other results than the cost per DALY averted. For example, public health decision makers are keen to understand the mortality and morbidity trends projected pre and post introduction of a vaccine. Economic evaluations, including those supported with ProVac tools, can report the number of disease cases and deaths should epidemiological trends persist under existing control conditions as well as the number of disease cases and deaths averted due to the introduction of a new vaccine.

Other results such as the total resource requirement for introducing a new vaccine and the projected cost savings to the health system due to avoided healthcare treatment are useful inputs into discussions with ministries of finance or departments of planning and budget within a ministry of health. These types of results should be reported in simple policy briefs that address a specific audiences questions in the decision making process. An effective program manager plays a critical role in crafting the appropriate messages for the relevant decision making audiences.

ProVac's experience has demonstrated that ministry of health technical teams can adhere to quality standards with the help of user-friendly, standardized economic evaluation tools and methods. The ProVac tools and approach provide a clearly defined process for selecting and assessing the quality of evidence for each parameter. A frequent challenge has involved the need to weigh the quality of local versus regional or international data. As described above, countries may ultimately choose to assess more than one data sources if uncertain or concerned about quality risks. To date, ProVac has supported over 30 countries in the Americas and other regions to conduct economic evaluations and other modeling exercises and to use the results to inform their national decision making processes. Several of these countries have formalized ProVac multidisciplinary country teams through ministerial decrees or linking their work to NITAGs. Additionally, more than 400 public health professionals both from immunization programs and their advisory bodies have been trained in the development and use of cost-effectiveness data in the Americas for vaccine decision making.

Conclusion

Countries of Latin America and the Caribbean (LAC) rapidly introduced and deployed traditional vaccines against childhood diseases, essential for increasing life expectancy in the Region. Currently, new vaccines against invasive bacterial diseases, diarrhea, and other causes of premature mortality among children and their mothers will be essential for achieving the targets in the Global Vaccine Action Plan (GVAP) (For more information, please refer to the "Global Vaccine Action Plan" chapter). Such vaccines include those against rotavirus, pneumococcal, seasonal influenza, and human papillomavirus (HPV) diseases. Several factors remain as obstacles to the introduction of these new vaccines, including vaccine price. New, more expensive vaccines require a broader base of evidence and knowledge in order to make informed policy decisions. However, many

countries lack the capacity to expedite evidence-based policy decisions. This chapter shared insight gained by PAHO's experience implementing the ProVac Initiative, whose mission is to build national capacity in making evidenced-based policy decisions for the introduction of new vaccines in developing countries. PAHO's ProVac Initiative has been working more than a decade towards addressing gaps in national technical capacity around evidence-based decision making for new vaccines in Latin America and the Caribbean (LAC) and more recently at a limited scale in the WHO Regions of the Eastern Mediterranean (EMR), Europe (EUR) and Africa (AFR). This work moves beyond establishing technical advisory resource groups such as National Immunization Technical Advisory Groups (NITAGs) to additionally focus on strengthening skills at the national level to develop economic and health impact evidence to inform decision making, which is critical to improving country ownership and sustainability.

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Module 3:
Implementation and Expansion of Immunization Programs

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Information Systems for the EPI

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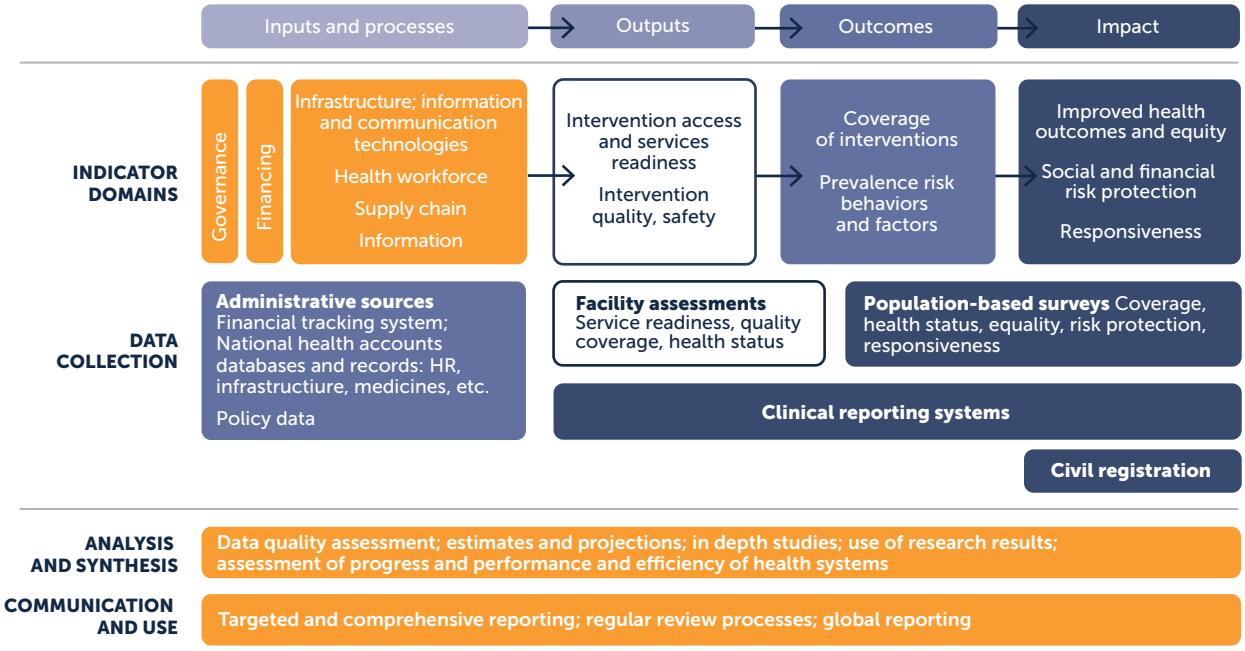
Introduction

Information systems are key to producing the information that will guide the strategic, managerial, and operative decision-making process within the Expanded Program on Immunization (EPI). Moreover, essential data for monitoring and accountability, from the administrative to the managerial level to the general target population, will be created. The ultimate goal of having proper information is for EPI to make adequate decisions that contribute to reducing the morbidity and mortality of vaccine-preventable diseases (VPD) and improve Program performance.[1-3]

The data-centered strategic and policy decisions within the EPI include focusing vaccination strategies and methods on reaching vulnerable and undervaccinated populations, communications, community education and outreach, as well as adjustments to vaccination schedules. Managerial decisions relate to the vaccine and supply inventory at all levels, secure cold chains and trained vaccinators to provide safe and quality vaccination services covering all of the population. Finally, operative or routine decisions include the determination of an approximate number of vaccinees every week/month, tracking of individual schedules and the strategies necessary to attain them as well as the required vaccines and supplies for vaccination at the medical facilities and in the community.

Progress and accountability are monitored through the analysis of wide-ranging performance data and indicators. The International Health Partnership, or IHP+, has proposed a theoretical framework for monitoring and managing inputs, processes, outputs, outcomes, and impact of health programs,[4] as shown in Figure 1.

Figure 1. Theoretical Framework for Monitoring and Managing Impact in Health Programs



Source: International Health Partnership or IHP+

In the area of vaccination, the four indicator categories include the following:[3]

1. **Inputs and processes:** Resources such as vaccines, supplies, staff, and financial resources, and processes that make them available where needed.
2. **Outputs:** Availability for the provision of safe and quality vaccination services for the population and the informed population requesting the service.
3. **Outcomes:** The main EPI indicator of this type is vaccination coverage, to be measured through facility reports or coverage surveys. This indicator results directly from the availability of vaccination service supply and the demand of the population under item 2 (outputs).
4. **Impact:** Improvements to health, for instance through the reduction of the morbidity and mortality of vaccine-preventable diseases, to be detected through the epidemiological surveillance of vaccine-preventable diseases (VPD).

The information systems for EPI should monitor the above-mentioned indicators. Often times an information system is mistakenly equated with software. However, information systems include a range of elements focused on data management and administration to produce information. These elements include individuals, data, activities or work techniques, and material resources (typically, though not necessarily, information and communication resources).

In general, EPI requires at least four types of information systems or subsystems for decision-making: 1) vaccines administered (mainly used to estimate vaccination coverages), 2) the supply chain, 3) VPD epidemiological surveillance, and 4) surveillance of events supposedly attributable to vaccination or immunization (ESAVI). This list includes only information obtained regularly rather than from specialized studies or surveys, neither does it include information on finances or human resources as this type of information is usually within the health system in general. This chapter focuses on the first two types of information, i.e., vaccination coverage and the supply chain as well as the use of information and communication technologies (ICT) for EPI.

Vaccination Coverage

As previously mentioned, vaccination coverage is the most widely used outcome and performance indicator to monitor an immunization program. This indicator is measured and monitored systematically and periodically at the various management levels since it allows to detect problems and to implement corrective actions wherever and whenever required.

Coverage should be considered as an indicator that is estimated rather than measured directly. This is done by dividing the number of vaccine doses administered (persons vaccinated) for each vaccine type and dose (first, second, third) at a specific place and period, by the target population at that place and period, expressed in percentage form, as presented in the formula below.

$$\textbf{Administrative coverage (\%)} = \frac{\text{(Number of vaccine doses administered)}}{\text{(Target population)}} \times 100$$

Despite the fact that some countries derive coverage estimates from surveys only, most of the countries use the administrative method whereby EPI uses aggregated data on vaccine doses administered. Under the administrative method, the determination of the number of vaccine doses administered, i.e. the **numerator** to estimate coverage, typically starts with the recording of the number of doses for each biological and the doses (for instance, first, second, third) administered on one day at a health clinic or community-based vaccination activity. Then the data are consolidated based on the tier (district, regional, or similar), up to the national tier with aggregated data for the vaccinated total for a specific vaccine and dose in a specific time period.

The **denominator** to estimate coverage will be the target population for each vaccine and dose. This data is usually derived from population estimates based on census projections or recorded births, even though some countries have comprehensive immunization registries used as population denominators.

The information system to estimate coverages is the vaccination record that, in general, includes several tools for data collection, including vaccination cards, individual vaccination records and home-based records of vaccine doses administered.[2,3]

Vaccination cards, either only including data on vaccines administered or where vaccines are included in health cards or records of other data such as growth, are provided to the user. The cards record the vaccination and doses administered and the date and, in many cases, provide information on the upcoming visits.[5] To see a global repository of vaccine cards, go to: http://www.immunizationcards.org/.

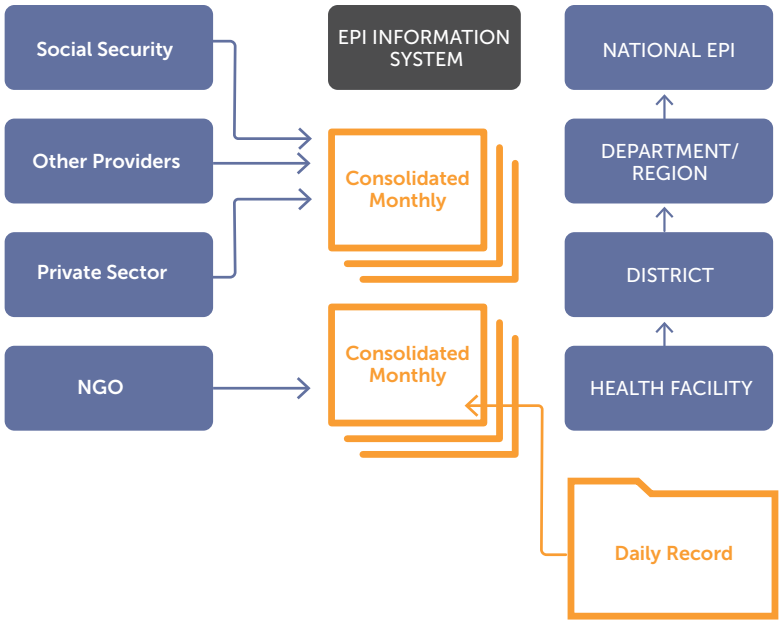
Individual vaccination records include information on the vaccinee and data on each vaccine administered. They may be books or copies of the vaccination cards organized by date of birth, by date of first contact with vaccination or by ID number. The most important characteristic of these records is their sorting to readily identify the user and to monitor the individual vaccination schedule, allowing for the identification of the vaccines received and others still pending based on their age or risk group.

Tickler files (picture below) are very practical systems to organize vaccination cards showing at the beginning of each month the cards of the individuals requiring vaccination and, by the end of the month, the individuals requiring vaccination who failed to attend the health clinic to be vaccinated are readily identified.

Daily records or tally sheets of doses administered are sheets or books to capture each vaccine dose administered, in general organized by age group and, in some cases, by hospital or community-based strategy and/or place of residence of the user. The main goal of this record is to facilitate counting and consolidation of doses administered every month (or every week, in some countries). This data is later reported on a **consolidated monthly form** (Figure 2).

In some countries, a daily paper vaccination registry has been developed whereby each line includes data on the vaccinee, such as name and date of birth. However, contrary to the individual vaccination records in tickler files described above, the records are ordered by vaccination date, limiting their use for monitoring the vaccination of each individual. Their role, as any non-individualized daily record, is to allow counting for consolidation of doses administered monthly. This type of record is not advisable since a simpler daily record or tally sheet fulfills the same function. Even worse, if this type of record or book replaces the individual vaccination record or tickler file mentioned above, the health clinics are left without a simple mechanism to identify and follow-up defaulters, i.e., individuals with outdated schedules.

Figure 2. A typical Data Flow for Vaccines Administered



There are flow variations, with more or fewer levels of data aggregation and computerization from various levels. Data entry into EPI information systems, usually into Excel sheets, or into health information systems for data on vaccines administered as well as other health interventions may take place at various levels, but the trend is to computerize at the level of the health clinic.

Data entered into the information system on coverages should at least include all of the vaccines and doses (first, second, third, and boosters) disaggregated by age group (or by indication, for example, influenza for pregnant women, patients with chronic diseases, etc.); the reporting period (weekly, monthly); and information on the facilities and geographical location data. They should also include the denominator used for each vaccine and dose.

Electronic Immunization Registries

Electronic Immunization Registries (EIRs) are confidential, population-based computerized information systems or databases, which include and consolidate vaccination data (doses administered) with information for each person. Electronic records facilitate the timely follow-up of individual vaccination schedules in addition to monitoring coverage according to vaccine, dose, geographical area, age, and provider (health clinic).[6-8]

Data shows that the EIRs help improve coverages through the following functionalities: patient reminders (upcoming vaccine and dose, overdue vaccines and doses); performance monitoring according to facility and feedback; and support for individual decision-making.[9, 10] In addition to facilitating data for decision-making, the EIRs may also introduce useful data for research, such as vaccine effectiveness, equality, vaccine safety, Program efficiency, and vaccine hesitancy data.

Currently, many countries, in particular in the Americas and Europe, are developing and implementing EIRs.[11] The rationale for this trend includes increasingly complicated vaccination schedules given the rapid introduction of new vaccines; mass use of new information and communications technologies (ICTs), and rapid increase in availability of computers, connectivity, and other devices.

An EIR should ideally have the following features:

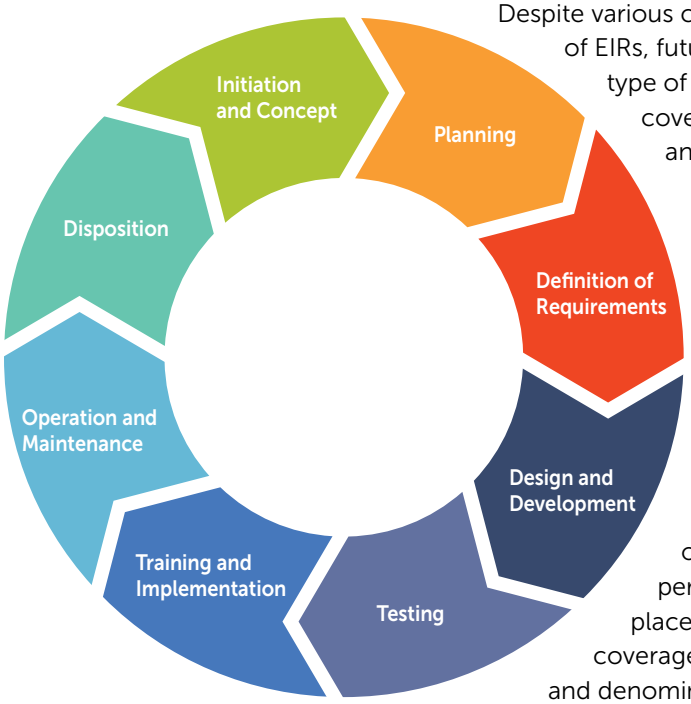
- Inclusion of all persons at birth, or as early as possible.
- Unique identifier, i.e. national identifier number or birth registration ID; a unique combination of variables (names, mother's name or her ID, date and place of birth); or biometric data (fingerprints, iris)
- Information about the vaccine given as close to the vaccination date as possible (in time and place)
- Data security and protection of patient confidentiality
- Flexibility to allow adjustment to changes in the vaccination schedules
- Information about each person, including information on geographical area of residence.
- Information about the vaccines given, dates and provider.
- Record deactivation features (deaths, migration)
- Timely individualized follow-up of vaccination schedules
- Aggregation of data by various geographical levels, age groups, and other relevant variables.

Several lessons are being learned from the increased development and use of EIRs:[12, 13]

- As with every information system, the development has a life cycle (Figure 3). Ignoring or incorrectly implementing a step impacts on quality and/or costs and/or time.
- The implementation of an EIR is a time-consuming process requiring adequate resources not only for its development but also for its operation and maintenance.
- The EIR design should take into account the operational levels and be useful for vaccinators. Design should be based on a clear understanding of vaccination processes and data registries. Consideration should also be given to the possibility of optimizing processes with this technology, i.e. it goes beyond replacing the individual vaccination registry form on paper with an electronic registry since processes can be re-designed and re-engineered.

- Properly defined processes need to be established to identify and manage potentially repetitive registries. They include:
  - mechanisms intended to prevent duplicated registries (search/verify before creating a new registry and system checks), and
  - processes for deduplication (system logs to detect registries suspected of being duplicate, establish how the registry is determined to be duplicated, how data are consolidated from two or more registries into one, etc.)
- The EIR development and implementation shall be monitored and assessed systematically and thoroughly. The following monitoring areas should be considered at minimum:
  - Infrastructure and equipment;
  - Integration and interoperability with other relevant systems;
  - Software performance and quality certification;
  - Trained human resources;
  - Most frequent consultations and problems;
  - User satisfaction at the various levels and in the various roles;
  - Compliance with the implementation schedule;
  - Management of information generated by the EIR and data quality;
  - Thoroughness of the registry. This is key to use registry data as a denominator for coverage estimation.

Figure 3. Life Cycle of Information Systems Development



Source: Public Health Information Institute (PHI)

Despite various challenges for the development and implementation of EIRs, future immunization programs will clearly include this type of information to optimize monitoring of vaccination coverages for all vaccines and improve EPI performance and efficiency.

Data Usage

Finally, notwithstanding the information system used, **data use** is the most important activity.

Coverage levels for vaccination should be analyzed in terms of persons (by age group, ethnic group or other vulnerable population, special indication), periodicity (monthly, quarterly, or yearly and trend), and place (for example, by district, region, country). Since coverage estimates are impacted by inaccurate numerators and denominators, "data quality" and coverages should always be monitored to look for data inconsistencies, if any, in the numerators and the denominators, assess the comprehensiveness and timeliness of the report, and verify data consistency at all levels.

In addition to coverages, other indicators associated with the vaccinees should be monitored. The **dropout** rate is the most important supplementary indicator for individuals who start but do not complete their vaccination schedules. A significant advantage of this indicator is that it is not impacted by denominator inaccuracies, since it only analyzes numerator data. The most-widely used dropout rate is between the first and the third doses of the diphtheria-tetanus-pertussis (DTP) vaccine or the DTP-Hib-Hep B (pentavalent) vaccine, estimated as follows:

$$\textbf{DTP1-DTP3 (\%) Dropout} = \frac{(\text{DPT1 Dose \#} - \text{DPT3 dose \# in children <1 year})}{(\text{DPT1 dose \# in children <1 year})} \times 100$$

The dropout rate should be lower than 5% in a country or region with a good follow-up system.

Supply Chain

The supply chain for immunization is defined as the **processes and elements** to ensure the vaccine and the vaccination supplies are in proper condition, where they should be, whenever needed and in adequate quantities. The supply chain processes include vaccine and supply r**eception**, **transportation** and **distribution**, as well as proper **preservation.** As previously mentioned, the elements of the information system for supplies include human resources, financial resources, and equipment.[14]

Given the varied organization of the supply chain depending on the country, this section is intended only as an overview of the processes and some indicators, for processes and equipment, to consider when designing or restructuring the information system for the immunization supply chain.

Typically, data for the immunization supply chain are developed at vaccine storage sites, ranging from the national warehouses to the refrigerators at health clinics. Most countries have national (and sometimes regional) warehouses with capacity to maintain vaccines refrigerated or frozen for long periods of time, and to freeze cold packages. At the subnational levels, there is equipment to maintain vaccines refrigerated for shorter periods of time than at the national warehouses. Finally, at the operational levels there are refrigerators, or cold boxes, with capacity for fewer vaccines for limited periods. Similarly, the storage capacity for vaccination supplies depends on the level.

Currently, the type of data used for managing the supply chain is varied and dependent on the supply chain level. Some of the most frequently used data include vaccine and supply inventory balance sheets; forecasted demand (monthly, quarterly, yearly); cold chain equipment inventory (with information to plan maintenance and replacement); and data on temperature.[3,15] The tools used for data collection range from kardex or requisition books for vaccines and supplies, and untabulated temperature control sheets to computerized inventory records and electronic temperature monitoring.

To conceptualize the information systems for the supply chain, it is important to consider the data purpose and to define the key performance indicators to monitor for guiding the process for strategic, managerial, and operative decision-making. Information systems for the supply chain should allow for:

- **Adequate Planning**
  - Vaccine and supply needs (what and how much to request)
  - Financial needs
  - Vaccine and supply acquisition (when and how)
  - Vaccine distribution

- **Efficient and Effective Management of Resources**
  - » Anticipate shortage of vaccines and/or supplies
  - » Anticipate excessive burden on cold chain equipment (since this impacts adequate preservation and heightens the risk of products reaching their expiry date while in storage)
  - » Reduction and prevention of unnecessary waste
- **Vaccine Access Improvement**
  - » Ensure supply meets the needs.
- **Ensuring User Safety**
  - » Traceability of the products used
- **Performance Monitoring**
  - » Using standardized data and indicators, with complete and timely statistics.

Below follows a list of the elements to consider when defining key performance indicators for the immunization supply chain:[15]

- Stock levels (by dose/month)
  - » Back-up stock
  - » Minimum and maximum stock
- Supplies
  - » Distributed versus needed
  - » Used vs. received
- Vaccine waste
  - » Open vials
  - » Closed vials
  - » By product, presentation, and place.
- Storage capacity
  - » Required vs. available
- Storage and transportation temperatures
  - » Continuous vs. twice daily
  - » Freeze indicators
  - » Alarm indicators
- Cost indicators
  - » Requested vs. used and wasted

Recently, the *Gavi Data for Management* task force proposed some standard indicators,[16] as follows:

- **Full stock availability:** The time range between vaccine and supply arrival and availability of all vaccines and supplies (or trace vaccines/supplies) at a warehouse or a health facility, i.e. without shortage periods (stock=0). This indicator is contrary to shortages which could have a negative connotation.
- **Stocked according to plan:** Health center ratio with vaccines and supplies at levels between minimum and maximum stock defined.
- **Closed vial wastage:** Ratio of closed vials discarded in a warehouse or at the health center. Vials are discarded based on expiry date, interruption of the cold chain (warming up or freezing), breakage of vials, diluent loss or damage, or because they were taken to a community activity.
- **On-time and in-full delivery (OTIF):** Ratio of orders completely delivered as planned and on time, at the national level or from the national level to lower levels, etc.
- **Temperature alarm ratio:** This indicator can be estimated with a digital device to measure temperature and generate alarms provided alarm occurrence is recorded. These alarms occur when temperature drops below -0.5 degree Celsius for at least 60 minutes (low temperature alarm), or when the

temperature increases above 8 degrees Celsius for 10 or more continuous hours (high temperature alarm). Despite that the goal of the alarms is to immediately correct the problem, their frequent occurrence might signal equipment problems and the need to have them assessed and repaired.

- **Operability of the cold chain equipment:** Ratio of functional cold chain equipment (cold chambers, refrigerators, freezers, cold boxes, thermoses) over the cold chain equipment total in a specific area; it can be estimated by equipment type.

The above-mentioned indicators are baseline and should be adapted according to the organization of the supply chain and the needs of the immunization program in each country.

Use of Information and Communications Technologies Under EPI

Currently, information and communications technologies (ICTs) play a very important role in the monitoring of health programs in terms of data collection and transmission online or through mobile devices, as well as the analysis and generation of dashboards and visualizations. Examples of ICT use under EPI include electronic immunization registries (EIRs), vaccination recall/reminders delivered through a short message service (SMS); development of mobile applications for health education; remote monitoring of temperature and integrated systems for stock and supply chain management, including the use of bar codes to facilitate traceability of supplies.[15, 17] Some of the uses are described below:

Data collection: Recording doses administered, or vaccinees, vaccine or supply stock transactions directly on a mobile device or an information system, for example an EIR.

Data transmission: Online or through mobile devices to have data at a higher level of the system, in real time.

Analysis: Automatic production of graphs, tables, maps, and interactive views which were not possible in the manual systems. ICTs allow for integrating data from various systems, including with geographic information systems (GIS), and the creation of **dashboards**.

EPI management dashboards: Offer simultaneous views of various indicators, such as the supply chain; coverage and dropout rates at specific places, time and based on specific individuals; and the impact as measured through the epidemiological surveillance indicators for VPD, among others.

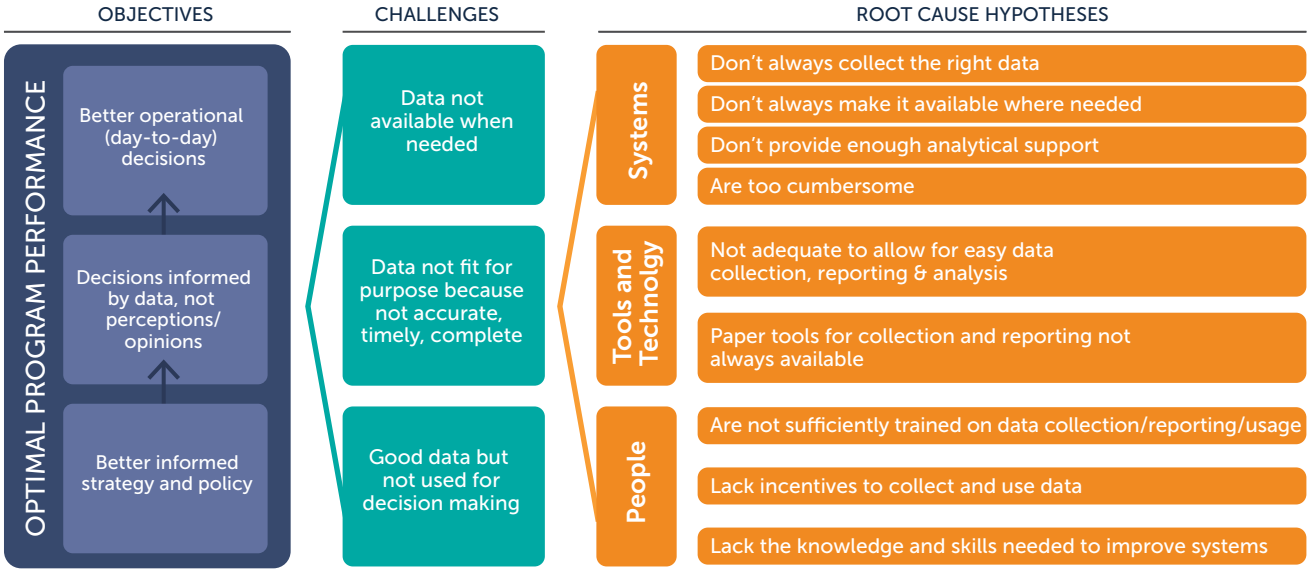
Geographic information systems (GIS) are part of a technology which is still underused but very promising for EPI management. GIS systems are designed to capture, store, manage, analyze, administer, and present all sorts of spatial or geographic data. They have been used successfully for risk analysis; microplanning of Program activities; campaign planning and follow-up (estimation of target population, progress follow-up); and to support the management and strategic planning of the immunization supply chain, among others.

Technology in itself cannot modify incentives or the behavior of users. However, the ICTs may motivate and empower skilled individuals to do a better job. The use of ICTs will only lead to improved information systems once technology and individuals work together to improve EPI performance.

Conclusion

The information system used by EPI, whichever it is, should be monitored and assessed systematically to understand the challenges and root causes impacting its performance and the quality of the data produced to allow for continuous improvement. Figure 4 below introduces a framework to assess challenges and root causes that may impact EPI information systems.

Figure 4. Challenges and Root Causes Affecting the Performance of Information Systems and Data Quality



Finally, coordination amongst countries and across the region to harmonize the immunization indicators used and to share data is key. This coordination allows for regional and global analyses to inform and guide strategies for the elimination and control of vaccine-preventable diseases.[18,19]

For more information associated with information systems and ICTs for EPI, the *TechNet Resource Library* comprises more than one thousand resources on this topic: https://www.technet-21.org/en/library/main. This library is available in English, Spanish, and French. To register, visit: http://www.technet-21.org/.

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Vaccine Coverage Monitoring

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Introduction

Vaccine coverage monitoring is critical and all levels must be monitored.[1,2] Despite important epidemiological and political information obtained through national-level monitoring, local monitoring may be the most important one since it provides us with the information needed to guide the program for all the areas in the community. The information received from the local level provides us with a snapshot of the program performance as to the area, the cluster or the centers that have good coverage or the lowest coverage. Managers can use this information to take action to improve or correct problems.

Use of Vaccination Data

Locally there are several key and necessary analyses to be performed by the centers, such as an analysis of the descriptive epidemiology by asking about the basics: *who, where,* and *when*.[3] This implies that centers need to have customer listings or records with information on identification and vaccination. At the same time, they need to assess program quality. BCG and/or DPT1 coverage may be analyzed as an indicator of program access. The dropout rate and simultaneity will provide us with information on the program. A high dropout rate, or a high number of children beginning to receive vaccines but failing to come back for a third dose, may indicate insufficient parental education or the absence of systems to remind families that their children need a vaccine or that the children have left the area. These indicators need to be monitored monthly and used to guide corrective actions.

Other vaccination program indicators include 1) the rate of districts with coverage > 95% and 2) the rate of children that live in districts with a coverage > 95%.[4] Both indicators should be monitored taking into account that each includes different types of program information. The former one provides us with information on the coverage of geographical areas but excludes population. The latter provides us with information on the infant population but not necessarily on geographical areas. Both indicators are key to the program; however, there are potential biases in their individual interpretation. For example, when the district rate is estimated based on coverage, every district receives the same value or weight for coverage estimation without taking their population into account. Therefore, it is likely for a country to have a high rate of districts with very high coverage and, at the same time, ordinary coverage at the national level. This is possible when most of the districts with small populations have high coverages and the few districts with low coverages have large populations. For the second indicator, i.e. the rate of children that live in districts categorized by coverage, there is no consideration of infant vaccination in a district when

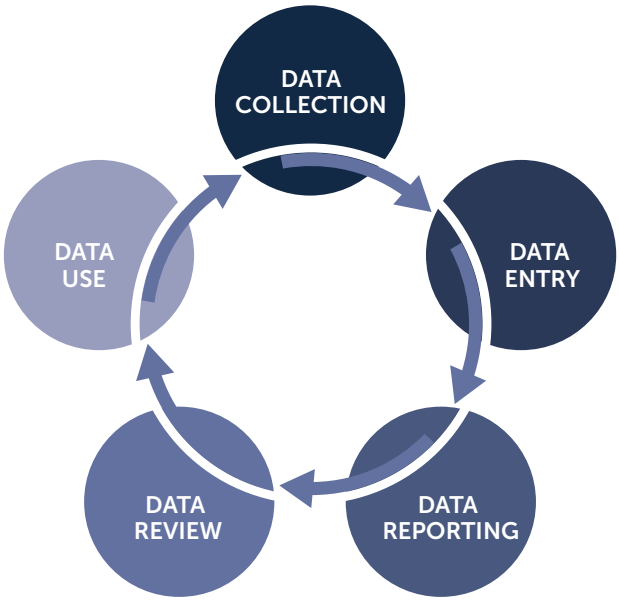
children were vaccinated in a neighboring district. Therefore, both have biases and should be used jointly for a more comprehensive understanding of vaccine coverage.

Finally, it is very important to pay a great deal of attention to the numerator and the denominator of the operation when monitoring coverages. The numerator may be inadequate if the record of the doses applied is wrong or the number of doses applied is inflated. Likewise, the denominator may be wrong if the population figures are incorrect or there is significant migration from rural to urban areas. In this case, population in the rural area would be overestimated and coverage would be underestimated. Likewise, population would be underestimated and coverage overestimated in the urban area. This does not imply that biases weigh more than their usefulness. The use of coverage as a key program indicator cannot be second guessed or underestimated. However, to interpret the coverages and their patterns biases need to be understood and analyzed together with the remaining program indicators.

Data Management Cycle

In an effort to understand and improve data from the immunization program, let us address the Data Management Cycle (Figure 1).[5] This cycle represents the immunization data management steps and applies to every country in the Americas. This cycle has at least five important steps. Firstly, there is data collection for which every program needs to have a system. Upon completing data collection, the programs enter data into a computer program or, at least, into an immunization record. The next activity is reporting the data to authorities for analysis. Finally, health workers need to use the data to guide and support the program. Every activity in this cycle needs a system that includes its own resources as well as trained individuals to perform the activities. However, several assessments have noted difficulties in every step of the cycle and they are not unique to a few countries, rather similar in all of the countries of the Americas.

Figure 1. Vaccination Data Management Cycle



In addition to the steps of the cycle, principles also apply to the management of vaccination data that should be recognized as a form of evaluation. In other words, there is a need for quality data that are thorough, accurate, reliable, and representative of the community implementing the vaccination program to avoid inconsistencies at the various reporting levels. The guaranteed application of these principles is a significant part of the cycle.

In an effort to plan a strong and successful system, it is important to recognize the challenges a manager may face during the data management cycle process. As to data collection, several local programs have limited capacity amongst workers who not only lack the necessary training but also the supervision required to complete seamlessly each step of the cycle. Ideally all workers should be trained at the same time to retain a consistent group of experts; however, as in every program, staff retires and new staff comes in without proper instructions. At the same time, there may be problems with the forms, including frequent changes and the lack of standardization at every monitoring level which may result in data errors. More importantly, there needs to be supervision to ensure the adequacy of monitoring for collection practices and collection accuracy.

The second challenge faced occurs during data reporting at every level of the monitoring process, which also has its own errors and results. In the data reporting process, the roles and responsibilities of every employee or entity need to be made clear, and the data flow also needs to be established, which may be confusing at times. Adequate monitoring is needed to guarantee the timeliness and completeness of data. However, often times there are challenges in this step of the cycle which later on result in problems with the transmission of the correct information. There could also be data transmission problems and the sites, especially locally, cannot be assumed to have Internet access since they may be relying on a manual immunization registry.

A significant challenge is at the point of data entry since several mistakes can be made. The first issue could be the lack of data standardization with inconsistent variables and different formats. In addition to this potential problem, data entry could be challenging since it is not always performed in a regular or timely fashion. The lack of data purge and validation threatens successful data monitoring. Likewise, the use of various tools for data entry may interrupt the process. Once again, data management and the tools used throughout the monitoring process need to be standardized since they could lead to significant variances amongst countries and their data entry elements.

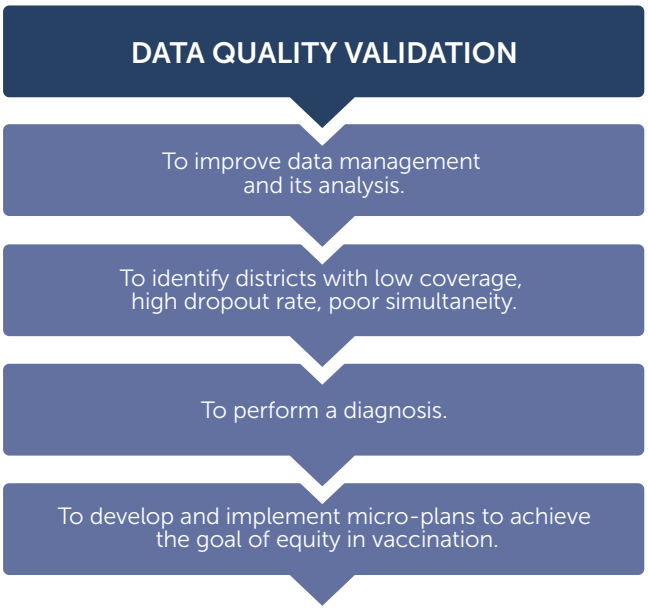
In many places, capacity and knowledge are insufficient to conduct a good analysis or it might not be done regularly or adequately. Data quality monitoring may be inadequate for several reasons, as we already mentioned, resulting in an incomplete cycle. Another important issue is the lack of feedback to the workers to ensure that they are aware of the situation, regardless of whether it is good or bad.

Finally, a very important challenge to pay attention to is the use of information which often times is not sufficient to guide the program or monitor progress for the achievement of the goals. In places with limited resources, there is a great need to complete systematic surveillance for the collection, management, and use of data during the data management cycle. This is especially important to be able to justify the need to obtain more resources and funds to guarantee that the programs achieve the same goals.

The introduction of new vaccines brings about unknown challenges. Moreover, their introduction results in schedule complications; new and standardized forms are needed; new data needs to be recorded and new analyses are required.

To control these added challenges, there are also potential solutions to analyze (Figure 2). Some necessary steps and tools may offer a solution to address the biases. First, the quality of data needs to be validated and this involves comparing the number of doses administered from various sources, official population data from various sources as well as coverages and dropout rates. Data management and its analysis need improvement to identify low coverage districts, high dropout rate, and poor simultaneity to develop a diagnosis and to implement plans for the achievement of equality in vaccination as a goal.

Figure 2. Potential Solutions to the Data Management Cycle Biases

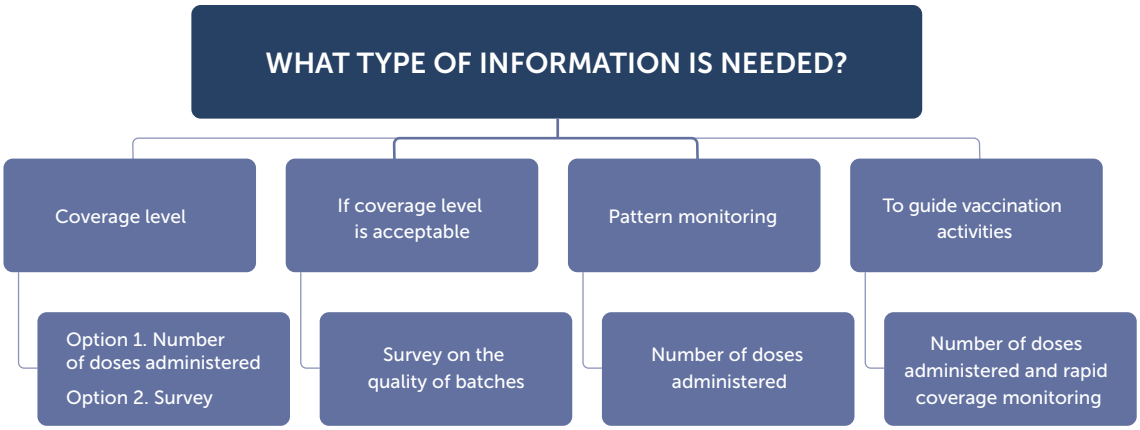


Indications Derived from a Coverage Survey

The recommended method for monitoring program progress is regular monitoring; however, frequently managers face the need of implementing a coverage survey. In fact, a survey provides more accurate information and, therefore, in general, it is the most useful, albeit with some disadvantages. Managers need to clearly understand not only what they want but also what they need (Figure 3).[3,6,7]

What is the accuracy needed to estimate vaccine coverage? What situations demand more accurate information? The use of applied doses is generally sufficient to monitor coverage patterns and vaccination activities. Comparing the use of doses applied, a survey may offer a more accurate coverage and managers may trust the survey figure

Figure 3. Guide for Selecting Evaluation Methods Based on Program Needs



significantly more. Moreover, during a survey, the programs may add questions to the questionnaire used. The questions may require information on a range of issues, family health issues, economic status, knowledge and concerns about vaccination. The analysis of the survey results may include an evaluation of the relationship of coverage and other factors. This allows for the identification of risk factors or factors linked to higher coverage levels. These evaluations provide us the necessary information to improve vaccination services. For example, this information may help identify populations and/or areas that require more program efforts in terms of activities, more education, more access, etc. Simultaneously, when the survey is linked to questions on knowledge and ideas on vaccination, the programs may develop messages and some educational material. Moreover, the information can be used to develop interventions or strategies for new activities focused on risk factors, such as isolated populations.

However, surveys have several disadvantages. Firstly, a survey is generally expensive and, therefore, most programs cannot perform too many surveys. Based on cost, before making an implementation decision, managers need to know clearly what they want to learn from the survey and how they plan to use the results. Vaccination program managers should communicate with other programs to identify the information they require on children. Often other questions may be added by requesting useful information on other programs.

Another important factor is that survey planning may be time consuming and require a significant amount of workers' time, thus interfering with their regular activities. Managers are well aware of the program but most have no experience with every aspect of the surveys. Therefore, technical skills are needed. It is absolutely important for managers to work with experts on statistics and sampling while performing sophisticated analyses.

Finally, results are useful but they are not usually ready in time so they may be of little use in real time. It does not mean that they are not useful but it usually takes 1 to 2 years upon completion of the survey for managers to receive the results. This delay prevents their use to monitor patterns when the goal is to guide the program on a daily basis. Another problem when using surveys to determine daily operations is that usually their results are national in nature and, sometimes, they are not very useful at the local level. However, truth be told, sometimes a survey is carried out for political reasons or to secure more financing.

Figure 3 presents a guide to help managers and health workers to better select the evaluation method based on the needs of the vaccination program. Firstly, authorities should clearly decide the information they need. Typically, the needs fall into one or more of four categories: to estimate coverage levels, to provide more accurate coverage levels, to monitor patterns and/or to guide vaccination activities. Frequently, only a coverage figure is needed and, in that case, a decision needs to be made whether the figure will be an estimate to guide the program or needs to be more accurate. In the former case, the use of doses administered is sufficient, with the abovementioned biases. But if accurate figures are needed or if the intent is to assess the relationship between coverages and some factors, a survey is the preferred methodology.

Sometimes it is only necessary to establish whether coverage reached an ideal or the expected level. In this situation, the exact figure is not required. The use of a quality control survey for the batches may be useful.[8] However, technicians are needed to help with planning and sampling as well as the analysis of the sample.

When managers only wish to monitor coverage patterns, using monitoring methodology for the doses applied is usually enough. The monitoring of the doses applied is the most common and inexpensive activity but it should be remembered that vaccines may be less expensive at the outset of the vaccination programs. With the rising cost of new vaccines, it would be more important to have more accurate results to monitor patterns ensuring or limiting expenditure. Therefore, some experts underscore surveys to have more accurate figures in an effort to minimize expenditures.

Finally, if the sole purpose is to guide program activities, the monitoring of the doses administered is sufficient. In addition, several programs use Rapid Coverage Monitoring (RCM).[9] RCM is a rapid methodology to estimate if the vaccination level in an area is acceptable. However, there is no information on the coverage figure.

It is very important to understand that the RCM is not a coverage survey since it samples the activity, which is supervision and it is not random. Therefore the results from RCM may not be generalized to other areas. In fact, the activity only indicates to managers whether revaccination in an area is necessary or not. With RCM, managers choose some residential blocks in low-coverage areas, with families at high risk for missing vaccination or without access to health services. The manager may also choose an area out of concern. There are several ways to perform the survey but PAHO has recommended selecting four separate blocks in the research area.[10] In each of the four blocks, the workers have to visit all of the homes, advancing in the same way in each block, for example clockwise. The work continues until at least five homes have been identified with children eligible for vaccination and whose vaccination data is available to be reviewed and recorded. Upon identifying 20 children with vaccination data, the RCM is deemed completed for the area.

Monitoring interpretation depends on the number of unvaccinated children identified every 20 children. If none or no more than one of the 20 children surveyed is unvaccinated, it is safe to assume that the area probably has been vaccinated in an acceptable fashion. However, if two out of the 20 children found are unvaccinated, the area has to be revaccinated or the RCM needs to be repeated. The identification of three or more unvaccinated children should result in the revaccination of the area and the implementation of another RCM upon revaccination. Meanwhile, the managers need to investigate the reasons why the area was not properly vaccinated. Frequent reasons include poor planning, lack of required resources, parental rejection or vaccination taking place when parents were not present such as during a market day or during working hours.

This monitoring activity can be implemented with any supervision activity, a vaccination campaign, or ordinary program activities. Moreover, it would be more useful to conduct the RCM without alerting the local authorities. Then, the current situation in the community cannot be changed due to any last minute activities. As mentioned above, RCM does not produce a coverage figure and results should not be considered coverages. RCM only provides managers with an idea on the additional activities to be considered for an area.

Conclusion

To conclude, routine monitoring at every level of vaccination data is key to guiding the program, identifying risk areas and activities to implement in order to achieve program goals. Vaccination coverage monitoring is one of the most important activities under the immunization program. The information derived from monitoring guides the program at the various geographic levels and may also be suggestive of the quality of program performance. Monitoring at the national level is a performance indicator for the program but it also offers information to politicians on the program and what needs to be done or invested in the program. Monitoring at the local level is the most important although monitoring is needed at all levels.

Monitoring should include data collection, reporting, entry, and review but mainly the use of data. Program managers should guarantee their information comprises quality data that are accurate, reliable and representative of the community. Managers should follow data collection activities, data entry into a computer program or data recording, and reporting to the authorities in charge and to data reviewers. There are several

indicators to monitor program performance within the vaccination program. Monitoring of the number of doses administered is indicative of coverage and an indicator to assess coverage distribution throughout time. Other indicators include the number of districts classified based on specific coverage and the proportion of children residing in the municipalities classified based on coverage. However, every indicator entails biases that need to be understood by managers.

Surveys are another option to monitor coverages. A survey may provide more accurate coverage and questions may be added to analyze together with the coverage for an identification of the risk factors. However, surveys are more expensive and time consuming and require technical expertise. Often, results are not published in time, therefore, they are of little use in real time and since they are national in coverage, they are not very useful at the local level. Surveys provide a great deal of useful information, but prior to making a decision or planning, managers need to identify the goal of the survey, its advantages and disadvantages. Finally, supervision and training are critical to guarantee that the available data are accurate and truthful for them to guide us to reach national objectives.

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Integration of Immunization Activities with Other Health Activities

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Introduction

There has been great interest in integrating several health services with immunization programs. In 2005, the World Health Organization (WHO) and UNICEF published "Global Immunization Vision and Strategies".[1] The document defines the recommendations issued by both organizations to help countries achieve their vaccination goals. The primary strategy (goal number 3) was the recommendation for countries to integrate vaccination activities with other health services. Eight years later, in 2013, WHO published the "Global Vaccine Action Plan 2011–2020" and emphasized the importance of strong systems within vaccination programs to help deliver other health services.[2] For several reasons both organizations recommended the integration of health services.[3, 4]

Firstly, vaccination programs in the Latin American countries are typically the strongest and most developed programs within the countries. At the same time, vaccination coverage, i.e. the rate of the population covered with the vaccination program, is higher than the coverage afforded by the other programs. Considering that the age target for several health services geared to children is similar and also that children have multiple contacts with the immunization programs during the first 2 years (when other interventions are more effective), several experts recommended integration as a strategy to improve additional health services. Numerous program managers indicated also that integration may prevent duplication of the activities performed by health workers, resulting in savings for the health system. Community representatives and family advocates also suggest a reduction in the number of family visits to health units and family savings on transportation costs.

Here we discuss the evidence for and against integration as a useful strategy, its benefits, biases and disadvantages.

The Evidence of the Integration Impact

A review of the scientific literature identified 59 studies where information was introduced on the integration of vaccination activities with health activities from other programs (Table 1).[5] This review suggested that efforts have been made in several countries to integrate various types of health services with vaccination services. Most studies were located in Africa but two took place in Latin America, in particular in Peru, and Mexico. Other types of health services delivered with vaccination included projects on:

1. Family planning;
2. Intermittent preventive treatment in infants (IPTi);
3. Vitamin A supplementation;
4. Administration of anti-parasitic medicine;
5. Delivery of bed nets;
6. HIV tests and counseling;
7. Hearing screening;
8. Infant growth monitoring;
9. Drinking water interventions; and
10. Health education (for breastfeeding and nutrition).

Most of the programs studied comprise services for children or services impacting children, including the delivery of bed nets to families and activities to deliver drinking water to homes.

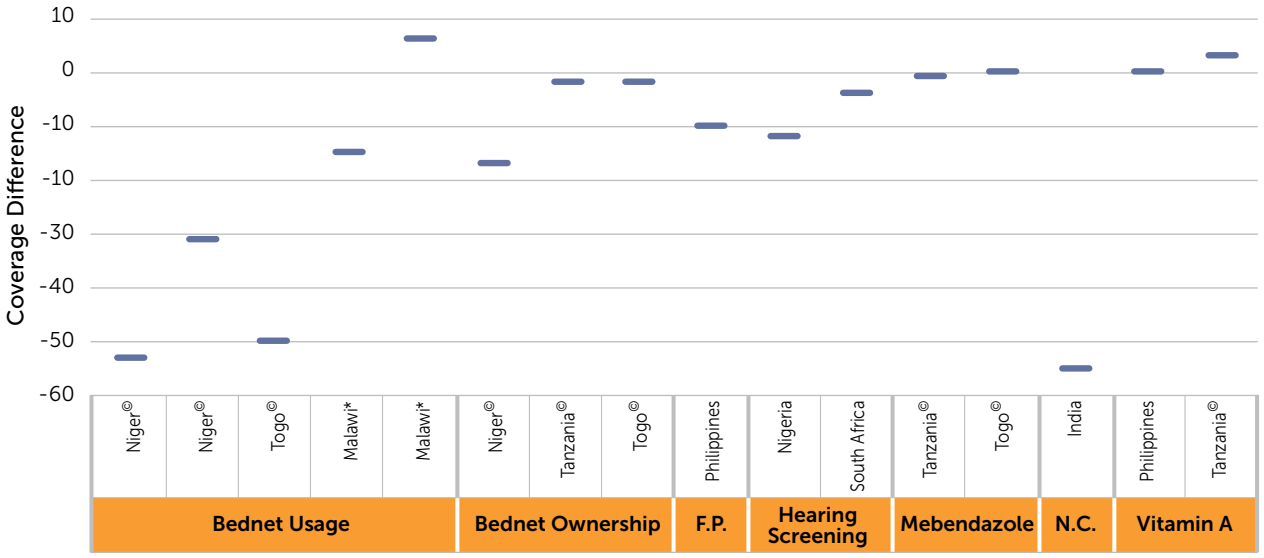
The analysis of evidence on the usefulness or effectiveness of the integration activities was challenging. Most of the studies identified in the review used different methodologies for integration; moreover, the studies had different approaches to define the concept of what to include in the integration. For example, in some of the studies the activities under other health services were delivered together with vaccination during the delivery of routine

Table 1. Study of Interventions Integrated with Immunization Services (n=59)

Intervention Integrated with Immunization	Countries
Family planning	Ethiopia (1991), Burundi (1993), India (1983; 2004), Ghana (2001), Rwanda (1992), Madagascar (2004)*
Intermittent preventive treatment in infants (IPTi)	Tanzania (2005), Ghana (2006), Madagascar (2006)*
Vitamin A supplementation	Indonesia (2001), Ghana (2002), India (2002), Peru (2002), Guinea-Bissau (1997), Ethiopia (2006)*
Administration of anti-parasitic medicine	Togo (2004)*, Zambia (2003)*, Mali (2005)*, Mexico (1993)*, Cameroon (2005)
Delivery of bednets	Togo (2005), Zambia (2003), Ghana (2001), Malawi (2005), Cameroon (2005)
HIV tests and counseling	Tanzania (2014), South Africa (2004; 2007), Zimbabwe (2001)
Hearing screening	Nigeria (2005), South Africa (2007)
Infant growth monitoring	India (1998), Philippines (1999), Ethiopia (2006), Madagascar (2006)*
Drinking water interventions	Kenya (2011)
Health education (breastfeeding, nutrition)	India (1998), Philippines (1999)

Note: *Indicates during an integrated campaign or during a Health Week; otherwise integration was through the routine program.

Figure 1. Is an integrated service able to attain the coverage of the immunization service? Difference between vaccination coverage minus coverage for the other service upon integration of immunization with other services.



Source: Reference #7. Note: ©Study completed in campaign setting, otherwise setting was routine services.
* In Malawi, two intervention locations were used with different coverage differences. F.P: Family Planning; N.C: Nutrition Counseling.

vaccination services while others were integrated during campaigns. A third of the studies identified related to a health activity delivered with the vaccination services on the same day and in the same place as the vaccination. In 15% of the studies, the integration did not entail the delivery of a health service, rather education on a specific topic during vaccination activities. In half of the studies, delivery of other health services was during vaccination campaigns or during a Health Week. Therefore, it is not feasible to compare them in terms of their design or to assess the best way to perform integration.

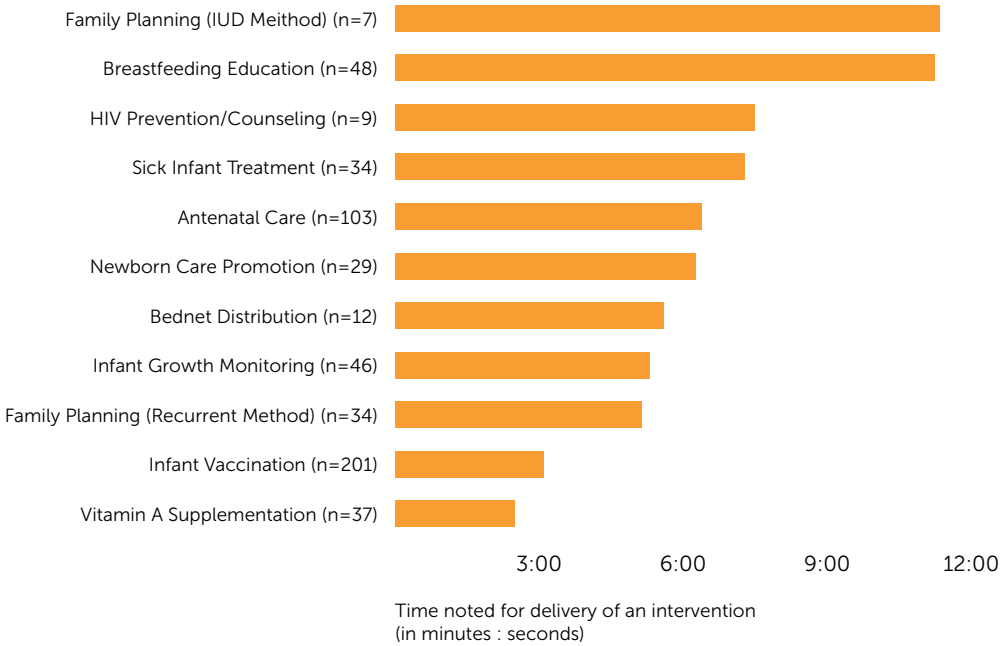
Unfortunately, only few studies identified were properly designed and most of them were observation studies. Only 10% had a control group and only 19% had information on economic costs and recourses. The highest concern was that very few studies reported on the impact of integration on the immunization services. For example, only 12% reported coverage levels before and after the integration activities. Due to these limitations it is not feasible to document an increase or a reduction of vaccination coverage when integrated with these studies. As a matter of fact, the study review demonstrated the importance and the need to have standardized measures for assessing not only the quality of the process to integrate services but also the impact of any integration.

The purpose or expectation is that with integration the coverage of both services will improve. The evidence, albeit not final, is suggestive of integration resulting in higher coverage levels whenever the service integrated with the vaccination is "simpler". Figure 1 illustrates the difference in coverage between vaccination and the other service upon integration of both services. For the seven services in the research, the "simpler" interventions (administration of vitamin A and/or anti-parasitic medicine) reached higher coverages than the interventions requiring behavioral

changes in individuals, such as the use of bed nets. Therefore the difference in coverage between vaccination and the other "simple" service was almost zero as compared to the other more complicated services. For example, the difference in coverage between vaccination and the administration of vitamin A was almost zero in two studies due to their coverage being similar. By comparison, the difference in coverage between vaccination and the use of bed nets was between -15 and up to -50 since vaccination coverage was much higher.

Another aspect of an impact evaluation for integration is that a vaccination visit is usually faster than a visit for other services.[6] Prior to deciding the integration of two or more services, the time required for their delivery needs to be considered, otherwise the times required for service delivery and the consequences for the other service need to be established as acceptable. Figure 2 illustrates the time differences for visits to receive various services. As illustrated, a vaccination visit lasts almost three minutes. However, a visit to the family planning or breastfeeding service lasts almost 12 minutes. Obviously the integration or combination of these services will increase the time required for administration of a vaccine in the schedule.

Figure 2. Differences in the Delivery Time of Different Health Services.
Ethiopia, Cameroon, Mali: Study on "Time Required for Service Implementation."



Source: Reference #6.

Benefits and Disadvantages of Integration

Measuring or establishing the impact of integrating some health services has been challenging, however, the abovementioned studies still have lessons learned on the benefits of integration. Some benefits have been mentioned in the studies but, unfortunately they have not been quantified. Nevertheless, the authors of various studies indicated that developing a new service as integrated was faster than developing it as an isolated service. Four of the integration studies for health activities noted a reduction in resource competition amongst the services when the activities were delivered during vaccination campaigns. Likewise, some authors noted that the delivery of a new service is likely to be faster when integrated with vaccination services rather than delivered for the first time as an isolated service.

To be successful, the integration of several services should be accepted by the community, which is quite an important concept. However, this sometimes implies that both need to be reflected in human behavior. For example, if education on malaria prevention or the use of clean water was integrated with a vaccination visit, the impact would be determined by the actual use of bed nets and water-treatment by families. The success of family planning services is also dependent on a behavioral change amongst individuals. Therefore, the impact of integration is based not only on the effectiveness of the integration or the health activities but also on proper use by individuals.

Some authors noted several disadvantages and challenges associated with the efforts to integrate different health services. For example, reference was made to increased responsibilities and more work for health workers as well as to the need for more training and supervision amongst workers. These activities result in more economic costs and the need to have more resources. Workers also need more time to deliver mixed services and the wait time for a service may increase for a family. The time a family makes several visits to a health center to receive services should be balanced with the increased time used during the day to receive several services on the same day. The consequences for parents in connection with child care when there are several children during a visit should be taken into account. What is most manageable for a mother: to make several short visits with two or three children or just one long visit to receive the services?

Integration increases the time devoted to the responsibilities of the workers but also the quantity of messages a family receives. There is concern that the impact of health messages when combined may be less effective as compared to their individual communication. Also, the integration of some services may have unwanted consequences or result in stigma. In an African country, HIV services were integrated with immunization services and, as a result, the community was able to see who was receiving HIV treatment. As a consequence, the number of customers requesting HIV services decreased.

Conclusions

To conclude, to guarantee the successful integration of several health services, a properly working health system is required, with the required equipment and the resources and supplies needed.[7, 8] It should be noted that integration or combination of two weak services will not necessarily result in a strong system or service and it is not a "magic" solution.[9] One of the services at least should be strong prior to integration and expected to improve the delivery of the other health service. Nevertheless, simultaneously, managers should not overburden the health system or the vaccination program.

Several health services would now like to be integrated with vaccination services, and the international agencies recommend doing it but there is a real risk to end up with an overburdened vaccination system. We have learned the importance of managing expectations; integration is not a solution to all the problems faced by various health services. Strong planning, resources, and political support are needed. Its success depends on the context of integration, i.e., is it integrated with the routine program or a campaign? Monitoring and evaluation are key. To conclude, benefits are real and coverage may improve but integration is not a solution to every problem. Success depends on good planning.[9, 10]

Acknowledgement

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Module 4:
Vaccine Advocacy and Social Communication

SABIN
VACCINE INSTITUTE

Advocacy for Stronger Immunization Programs

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Introduction

Immunization is one of the most cost-effective health interventions available. It helps save 3 million lives each year. Nevertheless, it is estimated that at least 23 million children still do not have access to basic immunization services, especially in sub-Saharan Africa, Asia, and Latin America.[1] There is a large disparity between immunization rates in developing versus developed countries.[2] According to the World Health Organization (WHO), globally, 1 in 5 children still do not receive routine life-saving immunizations and about 3 million people still die each year from vaccine-preventable diseases.

To bring more equity to immunization access it is necessary to ensure that the time lag for the introduction of new and underutilized vaccines is reduced. Historically, vaccines have first been introduced in developed countries where the burden is lowest followed many years later by developing countries where the burden is highest.[3]

Recognizing that much still needed to be done, the Decade of Vaccines Collaboration was created in 2010. This effort focused resources and global political will to improve access to vaccines. The product of the Decade of Vaccines was the Global Vaccine Action Plan (GVAP) which was approved by the World Health Assembly in 2012. The GVAP is a framework to prevent millions of deaths by 2020 by bringing more equitable access to existing vaccines for people in all communities.[1] The GVAP objectives are guided by the principles of country ownership, shared responsibility, equity, integration, sustainability and innovation.

In a 2016 mid-year evaluation of the GVAP, the Strategic Advisory Group of Experts on Immunization (SAGE) reported that they remain gravely concerned about the progress towards the GVAP goals. SAGE called on all countries and immunization stakeholders to make strident efforts to catch up and achieve GVAP goals by 2020. They noted "the next four years present unprecedented opportunities for countries to leverage the attention and support that immunization receives and apply it for the benefit of people everywhere".[4] Advocacy at all levels can play an enormous role in advancing the attainment of these goals. Advocacy can do this by highlighting the benefits of immunization, extending equitable coverage, and helping reduce the time to the introduction of new and underutilized vaccines.

In 2001, GAVI in partnership with PATH, published the report entitled "Advocacy for Immunization: How to generate and maintain support for vaccination programs".[2] In 2010, the United Nations Children's Fund (UNICEF) published "Advocacy Toolkit — a guide to influencing decisions that improve children's lives".[5] These documents serve as the basis for this chapter and provide a comprehensive step by step guide to advocacy. This chapter takes the main lessons from these documents and attempts to simplify the advocacy process so that individuals are empowered to advocate for immunization.

What is Advocacy?

In the broader sense, advocacy is the gaining of support for a particular cause or policy that leads to change. Advocacy is implemented with the objective of securing support of key constituents, placing an issue high on political and development agendas. Advocacy is also an important tool that can support resource mobilization for disease prevention and control, foster political will and increase financial and other resources on a sustainable basis. Advocacy provides an opportunity to hold authorities accountable to ensure that pledges are fulfilled and results are achieved. Most importantly, advocacy leads to change.

Advocacy can be viewed as a set of targeted actions directed at raising awareness and bringing focus to a specific issue. It is a deliberate process, based on demonstrated evidence, to directly and indirectly influence decision makers, stakeholders, and relevant audiences to support and implement actions that contribute to the fulfilment of a goal. Advocacy can be considered a core process for addressing public health inequity and a useful tool to change public perceptions and attitudes, modify behaviors and mobilize human and financial resources.

Sprechmann summarizes that advocacy is essentially about three things:[6]

- Creating policies where they are needed when none exist,
- Reforming harmful or ineffective policies, and
- Ensuring good policies are implemented and enforced.

Advocacy efforts often focus on bringing about some sort of social or political change. Changes in policy and practice can usually be expressed as one of five types of change:[7] discursive change (changes in the words, narrative and concepts); procedural change (changes in the way things are done); attitudinal change (changes in attitudes towards other actors or their values and causes); content change (actual changes in the strategy or policy documents or budgets); behavioral change (permanent changes in the way individuals or organizations act or behave).

In global health, particularly in the context of supporting behavioral change, the terms: "advocacy," "communication" and "social mobilization" are often used interchangeably. This can happen, largely, because the three concepts are not mutually exclusive. Instead, they involve social processes that overlap. Advocacy is the gaining of support for a particular cause or policy. Communication is the means of connection between people using messages and channels. Social mobilization is the act of assembling people to increase knowledge and demand for a cause. While these concepts all play an important role in promoting immunization this chapter will solely focus on the advocacy process. Communications and social mobilization are addressed in the chapter: The role of health teams in achieving a successful vaccination campaign by Ana María Morales.

Lastly, advocacy can take many forms. Depending on the goal, advocacy can be called engagement, lobbying, public relations, policy development, awareness raising, empowerment, social mobilization, campaigning, media work and communications (Table 1).

Table 1.* Types of Advocacy

Advocacy involves ...	Particularly when it is geared to ...
Awareness raising, communications and media work	Deliver persuasive, evidence-based and solution-oriented messages to the public, decision-makers, stakeholders and those who influence them
Communication for behavior change	Create an enabling environment for effective implementation of policy changes to protect the rights of children and women, as well as to allow their voices to be heard at the highest level
Developing partnerships/ coalitions/ alliances	Generate organizational support and momentum behind issues, connect messengers with decision-makers, and utilize diversity to achieve common advocacy goals
Lobbying and negotiating	One-on-one discussions with decision-makers to influence them to change policy, practice or behavior
Campaigning	Create and mobilize the public around the advocacy issue, change perceptions, and build support to influence decision-makers and stakeholders
Research/ publications	Illustrate the underlying causes and solutions to a problem, and draw recommendations which can be addressed by decision-makers and stakeholders
Work with children and young people	Facilitate the creation of a platform for children and young people's voices to be heard and acted-on by decision-makers and stakeholders
Social mobilization	Engage multiple levels of society, including those who are marginalized, as allies and partners in overcoming barriers to implementation of programs to protect children and women
Conferences/events	Bring together a variety of stakeholders and decision-makers to highlight the causes and identify the solutions to the issue, with follow-up that includes concrete and immediate action

Source: *Adapted from Advocacy Toolkit — A guide to influencing decisions that improve children's lives[5]

Why Should Immunization Managers Advocate for Immunization?

Strong advocacy requires coordination and leadership as well as the capacity to work with multiple stakeholders. Immunization managers are responsible for all aspects of the immunization program. As such they have access to scientific information that may not be available to policy makers or the general population. This, combined with experience and health expertise, lends the necessary credibility and authority, to effectively advocate for the implementation and benefits of immunization.

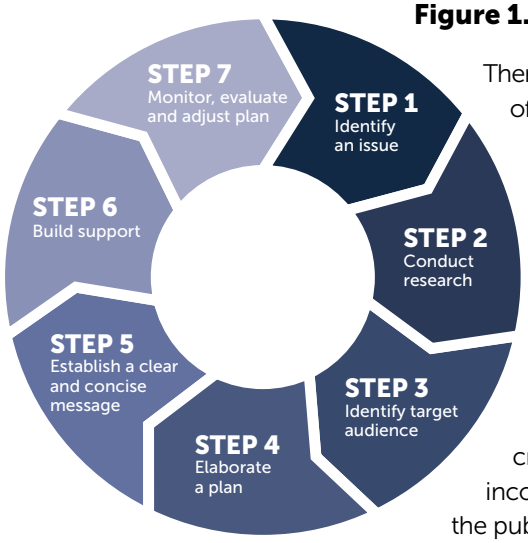
Advocacy is an important tool that can help sustain immunization programs and should be routinely used by immunization managers in support of their objectives. The advocacy process can support the immunization program at all stages. Before a new vaccine is introduced advocacy can help achieve political will and can help secure the necessary financial resources. After a vaccine is introduced continued advocacy efforts can help achieve and maintain coverage levels, community demand and can communicate the health gains and benefits achieved. Effective advocacy may increase the visibility of a topic and help facilitate dialogue. Advocacy can:

- Identify erroneous information,
- Disseminate impartial scientific evidence,
- Advise public policies,
- Connect people,
- Empower communities,
- Facilitate access to information, and
- Refute misinformation.

Creating a Strategy for Advocacy

The advocacy process is dynamic, with a constantly changing landscape. Therefore advocacy must be strategic and well-planned. There are many reasons why planning is indispensable. A robust plan can help minimize risks, make the best use of limited resources, maximize opportunities and align areas of work and organizational goals. Being systematic can highlight areas that require change such as organizational processes, systems, personnel, capacities and practices.[7] However, it is important to note that effective and far reaching advocacy must be grounded on credibility, research and the ability to assess and anticipate risks.[5]

Figure 1. Advocacy Cycle



There are several elements that can enable effective advocacy: the relevance of the cause; the power of the advocates which lends strength to the outreach; the thoroughness with which the advocates researched the issues; the state of the opposition; the climate of opinion about the issue in the community; the advocates' skill in using the advocacy tools available (including the media); the selection of effective strategies and tactics; the credibility of the advocates.

The advocacy enabling elements crafted into a plan will guide activities to the intended goal. Effective advocates follow a plan that includes: collecting information/research, identifying the audience, creating messages and convincing materials, creating a coalition, incorporating decision makers, elaborating a plan, informing and including the public and working with the media. Activities must be monitored for effectiveness and plans be adjusted when needed (Figure 1).

Step 1: Identify an Issue

As a first step it is important to identify the issue in which change would be beneficial. For vaccines and immunization that usually means a change in policy to allow for the introduction or expanded use of a product. However, the issue at hand could also be an increase in resources for immunizations, better community acceptance of immunizations or better disease surveillance programs for example.

Step 2: Conduct Research

Advocacy depends on strong analysis of the environment and its multiple influencers. Information and data gathering is the foundation for effective advocacy. It is during this phase that the available leadership, partners, resources and potential risks are identified. Good quality information provides the necessary base that will support the credibility of the advocate as well as gain the necessary trust of the relevant stakeholders. Therefore, enough time to conduct the appropriate level of research should be taken into consideration.

Gathering information relating to the burden of disease and vaccination are essential. Making the statistics as localized as possible are critical because people respond better to issues that affect them personally. Local and regional statistics

can be compared with national or global statistics to give dimensions to the problem. Additionally, any advocacy plan should draw from an existing communications plan or strategy already in place in the National Immunization Program (NIP)[8] as well as existing materials that can be adapted. Review of available resources within the NIP will help identify the gaps and needs of the program and define the best intervention to support the set objectives.

Part of research involves an evaluation of available resources. Resources are not only monetary, they can include people or organizations that are interested in the same issue or problem. Creating a list of resources that are available and that can be mobilized in order to achieve your objectives is an important step. Such resources can be groups, organizations or societies that you belong to, contacts in the media, government personnel and community groups.

Research also involves analyzing policies and practices. This includes understanding what policies are currently in place, how they are applied and how they could potentially impact the change that is being sought. Policies that affect vaccination should be reviewed and a determination of whether or not they are being correctly applied should be made. There is also a need to understand who makes the important policy decisions at the national and local levels as well as who is influencing these decisions.

During this phase a preliminary timeline should be developed. This timeline should take into consideration any other relevant NIP plans such as national strategies and multi-year plans, and how the advocacy plan fits within national planning cycles and existing communications work.

Step 3: Identify Target Audience

Once background research has been completed it becomes possible to identify the audiences that will need to be reached in order to effect the desired change. Identifying the target audience means understanding who must be engaged in order to effect change — those that have a direct influence and those that have an interest and/or can impact the process.

Identifying those audiences that need to be informed or influenced will allow for the tailoring of appropriate language and materials to be disseminated and assimilated. Target audiences usually fall under four broad categories that, though diverse, interact with and influence one another. An advocacy plan should target the below categories simultaneously in order to achieve the best results:[2]

- Potential partners such as aid organizations, government agencies, NGOs, researchers,
- Policy makers and decision makers that can influence vaccination,
- The general public, and
- Traditional and digital media.

Step 4: Elaborate a Plan

Based on the information gathered, it is possible to define the overarching goal and the objectives of the advocacy strategy. The elaboration of a plan will support the implementation of the advocacy activities and support the achievement of the desired outcome. The development of a plan starts with the decision on an overarching goal and is followed by drafting the supporting objectives.

Your goal should reflect your overall aim — what you hope to achieve in the long term. The objectives can be seen as milestones that define what the target is and what the plan hopes to achieve. Objectives should be specific and measurable. Their number should be feasible with what can be realistically accomplished under the set timeframe. The objectives should also serve to directly impact the change that you are trying to bring about.

In order to support the appropriate development of advocacy objectives, Lasher (2001) proposes specific criteria that should be considered and used as a guide. Not all objectives will meet all of the criteria listed below.[2]

- Do qualitative or quantitative data exist to show that achieving the objective will improve the situation?
- Is the objective achievable? Even with opposition?
- Will the objective gain the support of many people?
- Will you be able to raise money or other resources to support your work on the objective?
- Can you clearly identify key decision makers? What are their names or positions?
- Can the objective be achieved in a realistic time frame?
- Do you have the necessary alliances with key individuals or organizations to reach your objective?
- How will the objective help build new alliances with other agencies, NGOs, leaders, or stakeholders?
- Will the objective inspire more people to get involved?

Step 5: Establish a Clear and Concise Message

The essence of any advocacy activity is messaging. Without a clear and consistent message that resonates with your target audience, what you are saying will likely not resonate or be memorable. Your target audience deals with making several decisions each day, processing countless data points. In other words, what you are saying is competing with other messages, and your target audience may have limited time and selective attention. Therefore, using a format tailored to your audience is key to advancing relevant information.

The language used in the outreach messages should be simple and concise. It should be tailored to fit the audience and their level of understanding about the topic. Technical jargon should be avoided especially when talking to an audience that are not health experts. Ensure that language and tone are consistent with the message. A range of channels should be used to deliver the messages, including community volunteers and health workers, as well as the mass media.

Your messages will be informed by the research performed on audience, their environment, and the larger context of the topic. At the core of every message is:

1. **Content (what):** what will you say and how will you word it?
2. **Audience (who):** which is the most important audience or the audience you must reach most urgently? The group that you want to target to accomplish your goal? What do you want them to do with this information or content? Be very clear.
3. **Channel (how):** how will you share your content? Mass media: TV, public service announcements, etc.
4. **Timing (when):** ask yourself, is there a particular moment when your audience will be more easily engaged or more prone to act on your message? Is there a particular news event or change in the message's and audience's context that will facilitate communication?
5. **Metrics (did it work?):** did your message achieve your goal? If not, how can you improve or adjust it? This might require going back and better identifying how to express content, who your target audience is, how you best can reach them and when to do so.

Step 6: Build Support

Once you have created your plan it is important to mobilize and collaborate with key organizations and people, the community and the media. The power and influence of a coalition or group are greater than those of individuals working alone. Coalitions can lend credibility to a topic, share resources and influence decision makers effectively.

To start building a coalition you can, for example, use your own contacts, as well as approach national and subnational professional societies. An important and often overlooked ally is the media. Media can reach a broad number of people and help explain scientific topics in accessible language. Potential partners can also include policy makers and key opinion leaders, as well as members of the general public. Each partner can be engaged through a variety of channels (Table 2).

Table 2. Partner Engagement

Policy Makers and Key Opinion Leaders	General Public	Media
▪ Organize face to face meetings with policy makers and their staff ▪ Organize symposia and events ▪ Invite policy makers to visit immunization sessions, and other immunization activities ▪ Periodically communicate with them (through mail, email or phone)	▪ Community level meetings and workshops ▪ Advertisements and public service announcements ▪ National Immunization Days ▪ Campaigns letters ▪ Social Media: Twitter/Facebook (or other popular platforms)	▪ Journalist briefings ▪ Opinion articles in local newspapers ▪ Press releases

Step 7: Monitor, Evaluate and Adjust the Plan

Evaluating the impact of advocacy activities on immunization is complex. Multiple partners, people and organizations are working to advance regional and global immunization targets. Other forces (social, political or economic) are also effecting changes that are not always visible or easily measured. The task of determining what the plan has accomplished can be quite challenging.

A first approach to monitoring and evaluation (M&E) is to define appropriate indicators to measure the advocacy effort. Indicators summarize complex data into a logical form that enables comparisons of trends over time, as well as within and between settings and countries. Indicators facilitate the evaluation of policies and monitoring of progress in achieving goals.[9] There are several types of indicators but for the purpose of this chapter, process indicator and impact indicator are the most relevant. These can be defined as:[9]

- Process indicator: measures the effectiveness of activities being undertaken. They demonstrate how well a program has been implemented, with focus on the implementation stage.
- Impact indicator: measures how much of the observed change can be attributed to the plan. Impact indicators are more complex and therefore more difficult to measure. However, they are able to measure the long-term effect of interventions.

To better address the challenges of M&E, first focus on reporting the progress rather than the achievement of the overall goal. This approach highlights what has been attained even if the goal has yet to be accomplished. Within this scope there are elements of the plan that can and should be measured during implementation. Activities such as meetings and workshops, media coverage, expressions of public support and coalition-building can be assessed and measured somewhat easily.

In 2010, UNICEF published a companion toolkit which outlines the basic steps for the monitoring and evaluation of advocacy activities.[10] This toolkit is a good resource for more in-depth information on M&E for advocacy. The toolkit also presents examples of measurement indicators for advocacy activities some of which are shown below (Table 3).

The M&E process is an ongoing activity done in parallel with the advocacy activities. It should not occur solely at the end of an advocacy effort. The results from ongoing M&E efforts will help determine if adjustments to the plan are needed and how best to make them. The monitoring of activities allows for quick reaction and flexibility to take opportunities and anticipate and overcome new challenges.[11] Evaluations can also provide information on future needs and highlight where further efforts are needed.[12] It can support the redesign of programs by finding problems and weaknesses that need to be addressed. Lastly M&E informs the plan for continuity and self-sufficiency of an initiative.

Table 3*. Examples of Measurement Indicators for Advocacy Activities

Activities	Process Indicators	Impact Indicators
Coalition and network building	Number of coalition members Number of coalition meetings held and attendance	Types of constituencies represented in the coalition Increased breadth of partners supporting the coalition Increased network engagement
Briefings/presentations	Number of briefings or presentations held Number of individuals attending briefings and presentations	Types of audiences reached through briefings or presentations Increased knowledge, awareness or skills on briefing/presentation topics
Relationship building with decision-makers	Number of meetings held with decision-makers	Change in legislation/policies Implementation of new legislation/policies
Partnerships or alliances	Number of collaborative actions taken between organizations New organizations signing on as collaborators	New or stronger organizational relationships developed New relationships with unlikely partners developed Better policy agenda alignment between collaborators
Media coverage	Number of media citations of advocate research or products Number of stories successfully placed in the media Number of advocate or trained spokesperson citations in the media	Increased volume and range of media coverage Increased visibility of the campaign message Increased awareness of campaign messages among selected groups (e.g., policymakers, general public, opinion leaders)
Digital or Internet based media/social media	Number and frequency of electronic messages sent Number of list subscribers A new website or web pages developed	Increased dissemination and communication of message/content among target audience Increased public involvement in an issue/campaign

Source: *Adapted from UNICEF Monitoring and Evaluation — Companion to the Advocacy Toolkit.[10]

Conclusion

Advocacy as a core component of an immunization program can help achieve coverage goals, mobilize the community and policy makers towards best immunization practices and support policy change that can lead to long term sustainability. Advocacy for immunization is vital for supporting robust programs and sustainable funding. Effective advocacy must be based on evidence, carefully planned, systematically implemented, monitored and adjusted as needed.

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The Role of Health Teams in Achieving a Successful Vaccination Campaign

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Introduction

Are vaccines dangerous? Since Edward Jenner first entered this field through inoculation, and later on when Louis Pasteur invented the first laboratory vaccine, there have always been critical voices second guessing their safety and efficacy.

The fear of the unknown and the potential side effects were part of the arguments used in the anti-vaccine campaigns at the beginning of the XIX century.[1]

In his work, *The cow-pack or the wonderful effects of the new inoculation,* in 1802, the English cartoonist James Gillray represented how sensitive this topic was for the citizenry (Figure 1). Later on, when England approved mandatory child vaccination in 1853 and imposed fines or even imprisonment on parents who refused to have their children vaccinated, new counterarguments emerged, such as whether this measure was a violation of the individual rights of people. In the early 1900's, the first anti-vaccine leagues were born in New England and New York.

Figure 1. Cartoon by James Gillray: The Cow-Pack or the Wonderful Effects of the New Inoculation (1802)

Source: British Museum

That debate is not very different from the existing one. When analyzing news headlines, in the various countries of the region, it is possible to see the resistance against vaccines (Figure 2). In particular, this is due to dissenting opinions amongst the political actors, opinion leaders and technicians in this area, who act as points of reference for the public.

Figure 2. Vaccination Headlines in the Press in Latin America

ENGLISH TRANSLATIONS

1. Robert de Niro controversially supports anti-vaccine documentary.
2. Audit reveals mistakes in Human Papillomavirus vaccination in RM
3. Scientist argues the existence of links between a vaccine and autism.
4. Cancellation of papilloma vaccines at schools due to rejection.
5. Ten communities ignored the recommendation to vaccinate pregnant women against whooping cough.
6. Chile will be the first country to pass a law excluding thimerosal.
7. More than 200 arrests in expired vaccine scandal in China.
8. Vaccines are dangerous.
9. Jim Carrey stirs controversy due to criticism against vaccines.

This resistance is influenced by various factors: the decrease in risk perception of diseases prevented by vaccines; overloaded immunization programs whose need has not always been properly communicated to the public; greater knowledge about side or adverse effects as well as accidents or errors in supply; pressure of opinion leaders; lobbying by anti-vaccination groups and mistrust toward pharmaceutical companies.

The media and social networks play a remarkable role in this situation, acting as a sounding box, sometimes by disseminating information of dubious or contradictory origin which impacts the decision-making process of the population.

All these elements contribute to a certain apprehension toward the health system, represented by suppliers and policy makers.

In 2013, an example of the impact of anti-vaccination lobbying campaigns was seen in Chile with the passing of the law prohibiting thimerosal as a preservative in vaccines. The influence of these sectors resulted in a group of parliamentarians from different political paths to present a bill that was approved by Congress, notwithstanding the fact that stewardship around this issue belongs to the Ministry of Health. The initiative was voted on without consideration for the concerns raised by the Pan American Health Organization (PAHO) and the scientific associations.[2] The law had to be vetoed by the President of the Republic; however, it led to a loss of confidence in the national immunization program. This led to a decrease in coverage figures and, in many cases there were legal disputes between parents who refused to have their children vaccinated and the health services, which had to be settled in court.

Another well-known example includes recent events in Colombia with the introduction of the human papillomavirus (HPV) vaccine. Opposing population groups, including support by religious sectors and opinion leaders, argued unfoundedly that HPV vaccination in pre-adolescent girls would imply some sort of permissiveness for early sexual initiation, which became a flag for their fight. This resulted in improper coverage amongst the target group. Similar situations were replicated later on in other countries of the region.

A dominant factor contributing to this issue is the low-risk perception associated with vaccine-preventable diseases, a situation that is shared in several countries of the Americas. In particular, this issue stands out within sectors of the population that have the highest level of education or income, and have ceased to vaccinate their children because they do not consider it a preventive action, such as the case with the bacille Calmette-Guérin (BCG) vaccine. This is an alarming trend in a region where tuberculosis is still an existing public health problem, and where the lack of acquired immunity from the vaccine may lead to more severe occurrences of the disease.

The Art of Persuasion

Trust in the immunization programs is not infinite. The successful actions carried out in recent decades to control diseases such as smallpox or poliomyelitis are no longer sufficient as prevention policies.

Consequently, influenza vaccination coverage in adults over 65 years, in most countries, and in particular, in the most developed countries, does not reach 80%, as reported in the 2015 Health Indicators of the Organization for Economic Cooperation and Development (OECD). The success of a campaign depends in part on the level of protection attained for its vulnerable population.

For individuals to be willing to be vaccinated, they need to understand that the benefit will be greater than the cost of vaccine administration. Cost is not understood in monetary terms since the vaccines included in the immunization programs from the various LAC countries are usually provided free of cost. Rather, cost is viewed in terms of time spent on transfer, waiting, pain, some adverse effect, and/or a small scar on the arm.

How can this objective of vaccine uptake be attained? Based on the cultural changes that happened over recent decades, the response is for people to not only be convinced of the value of vaccines for them or their children, but also inclined to opt freely for vaccination.

Currently, there is a greater demand for information. Communities demand trustworthy and specialized evidence regarding vaccines. There is greater social control on State policies and this transcends borders. For the immunization programs this accounts for a true cultural revolution because there is a need to shift the paradigm used over recent years, mainly to change the way public health communication takes place.

Failing to address this demand for more information has very high costs and immediate consequences for the authorities or the officials who have a public role to fulfill.

It is essential for health sector workers to communicate effectively. Whenever problems are not anticipated, there is no capacity to control them. The health of the population is strictly related to access and use of information. However, information is not always available or it is not available in the proper form or of high quality. Examples from the situation with the HPV vaccine in Colombia are telling.

The most common mistakes made by the technical teams from the health sector entail assuming that "individuals know" the importance of vaccination while health illiteracy amongst the members of our populations is high, in particular amongst the groups with the lowest school attendance.[3]

The other mistaken assumption is that whatever worked in other countries is perfectly applicable to the local situation, without considering the distinct idiosyncrasies at the local level. For example, the inclusion of a new vaccine in an immunization plan, such as the HPV vaccine, without previously assessing all local scenarios is setting up for failure. Cultural resistance by the most conservative groups of the population, the religious sectors, the indigenous peoples, the anti-vaccination organizations, the youth who are the target population for vaccination or even the offensive by the pharmaceutical industry itself, must be considered in advance.

If there is no rapid response capacity by the technical teams or the authorities, uncertainty and disinformation will influence the opinion of citizens and regaining public trust becomes extremely difficult. Typically after such situations, vaccination coverage does not reach the expected levels to guarantee the protection of the target population, as well as the cost-effectiveness of the measure.

Paradigm Shift

It is erroneously believed that the population should be informed or educated through communications campaigns with a spot on television, phrases on radio programs or information handouts. These approaches are all merely instruments of a global strategy.

A paradigm shift involves abandoning that school of thinking and reformulating the way things are done. It should be understood that it is not only a matter of knowledge but also beliefs. The communications strategy should then be focused on stimulating, listening, learning and transferring responsibility for self-care to the public.

The success of a vaccination campaign is conditioned by the responsibility people undertake to care for their own health, which is strongly determined by the educational level they have attained and that of their family environment. This is where there can be a significant inequality gap.

Individuals with low educational levels or poor health literacy are at greater risk of mortality, visit emergency rooms more frequently due to a decompensation or severe impairment of their health (a condition that may be irreversible in some cases) and have a higher-than-average hospitalization rate. Preventive care, such as vaccinations or tests, is less frequent in these individuals.[4]

Therefore, there is a need to create opportunities for education. In the area of health care, teams of practitioners make efforts to educate the population through promotional activities or during medical visits. However, this is not always compatible with the demands of their work and the fulfillment of the health goals imposed on them. Often times, targeting coverage indicators rather than education or health care quality indicators becomes the priority.

The communications strategy for a vaccination campaign should be complemented with the following:

- Review the situation before introducing a new vaccine into the immunization plan or changing a pre-established schedule.
- Identify the problems and implement actions to anticipate an eventual challenge by other stakeholders opposing the measure. Within this context, assess the level of prejudices in connection with the vaccines within a specific population. Information disseminated on the radio, television and social networks reaches everyone. It is naive to think that detractors of the vaccine have not done their homework.
- Define a work strategy with constant communication strategies to create conditions fostering a policy change and a constant assessment of the actions.
- Identify strategic allies or partners to technically endorse the work being developed, such as international agencies (WHO, PAHO, CDC, other Ministries of Health of the Region), scientific associations, professional associations, civil society organizations or other public or private agents, to have them participate in the campaigns and speak independently.
- Design the health network so that every technical team manages the same information promptly; determine the tasks to be completed by the authorities and technical officials on the field.
- Design an activity program. The launch of a vaccination campaign is not sufficient. A set of actions to be sequentially implemented is required. Hold a press conference to provide context for the significance of vaccination and show cases of people impacted by the disease or the adverse effects; visit various health centers to verify immunization coverage and focus the message on the groups that are lagging the most; add credible spokespersons supportive of the initiative to the campaign.
- Promote strategies that bring vaccination closer to people, through discussions at schools or other educational institutions, grassroots organizations, elderly care centers or patient associations for individuals to ask questions and have their doubts answered.
- Vaccination on the field. Reach out to the target group rather than wait for the target group to go to the health clinic. Depending on the intended target population, this can be achieved through mobile vaccination units, visits to neonatal wards, preschools, schools or elderly care centers.
- Educate and constantly deliver information not only through the national media outlets but also through the community media outlets to reach out to the public. Provide communications material for them to use, promote interviews with technical spokespersons as part of mass programs on radio stations; and develop key messages that can be replicated on social networks and web pages.

Conclusion

To sum up, maintaining a population protected against preventable diseases for which effective vaccines have been developed requires a profound change in the way things are done. Effective communication and leadership amongst workers in the immunization programs are the most important. Work in the field is an inescapable requirement as well as the knowledge of the population and their acceptance level of the immunization plan. Prior to the vaccination period, the strategies described in this chapter should be developed to excite, convince, and relate to community leaders. Each and every one is different from the next.

When communicating, the following is important to keep in mind:

- Concerns expressed by individuals need to be addressed in form and substance, and this entails active listening.
- Use straightforward language.
- The message being conveyed needs to be straightforward and clear. Define key phrases for people to remember, in particular, the importance of protecting the family.
- The spokesperson needs to be credible, empathetic, and honest when faced with an adverse situation. Challenges need to be acknowledged.

A poorly-planned vaccination campaign- without awareness for the cultural environment or strong local leadership- may risk failing to reach minimum coverage levels. A failed program will not only lead to direct losses in personnel time, cost of vaccines and supplies, but also a great indirect cost: the emergence of cases of the disease intended to be prevented, discredit for the health authority and uncertainty about the program as a whole.

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Module 5:
Looking Ahead

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Achievements and Future Challenges in the Prevention of Pneumococcal Diseases in Latin America and the Caribbean

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Introduction

In December 2015, coinciding with the fifth anniversary of the extended-spectrum pneumococcal conjugate vaccines (PCVs), the global immunization map indicated that more than 95% of the children born in Latin America and the Caribbean (LAC) had free access to these technologies through the routine immunization programs in their home countries (Figure 1). This milestone in itself deserves an outstanding mark in the history of immunizations in the Americas, considering the great projections in public health for PCV, the scope of the national investment involved, as well as the delay registered in earlier decades in this same region to offer equal access opportunities to other vaccines that emerged after the creation of the Expanded Program on Immunization (EPI).

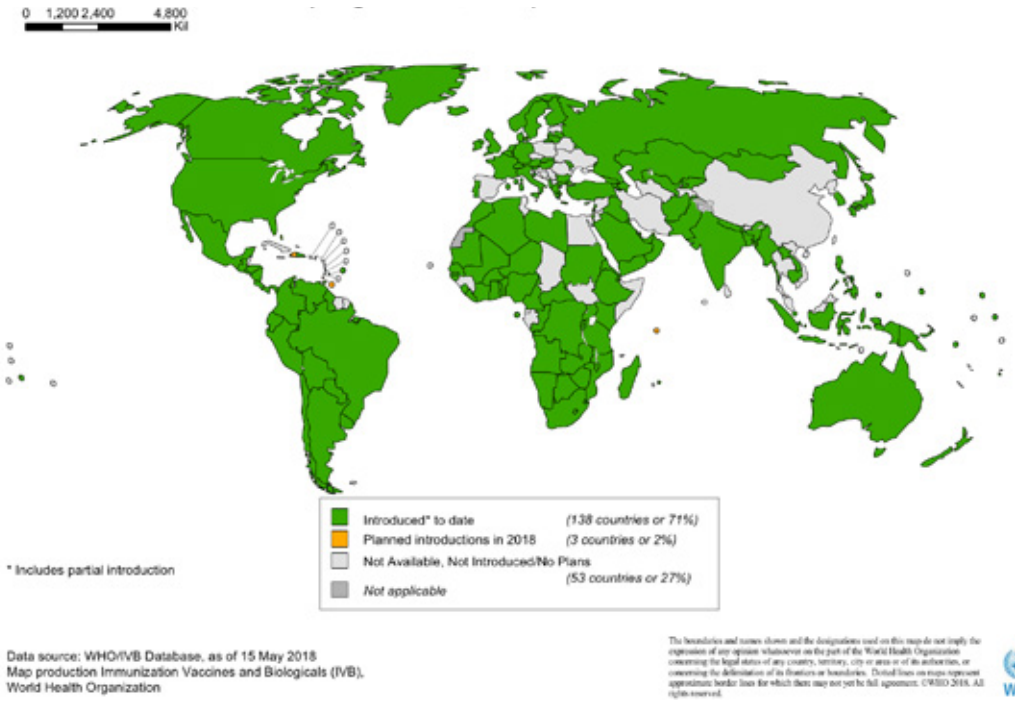
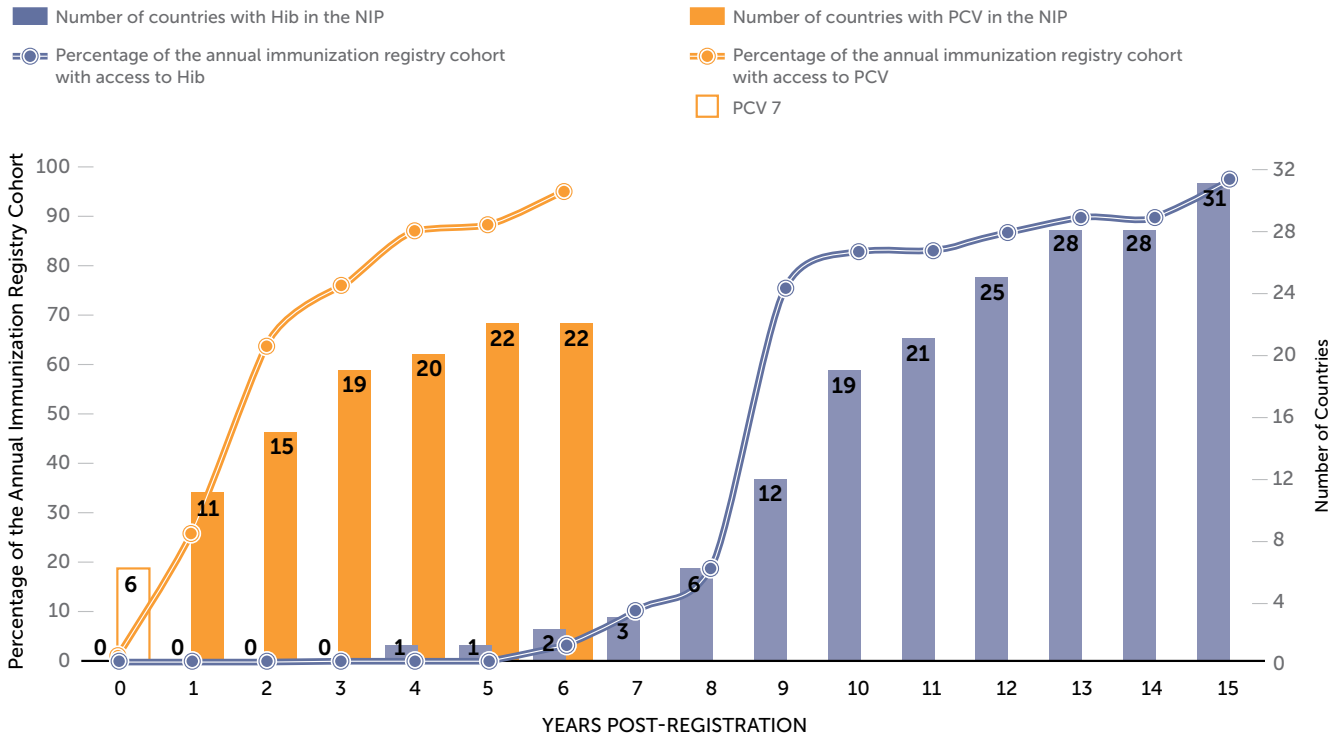


Figure 1. Status of Introduction for Pneumococcal Conjugate Vaccines in the National Immunization Programs, as of May 2018

Figure 2. Progression of the Implementation of Conjugate Vaccines Against *H. influenza type b* (Hib) and *S. pneumoniae* (PCV) in the National Immunization Programs of Latin America and the Caribbean



International Vaccine Access Center (IVAC), Johns Hopkins Bloomberg School of Public Health. Vaccine Information and Epidemiology Window (VIEW-hub) Global Vaccine Introduction Report (May, 2016). http://www.jhsph.edu/research/centers-and-institutes/ivac/view-hub/. Accessed May 2, 2016.

Indeed, the rapid progress made by the PCVs in LAC, starting in 2010 to date, would not have been possible as a mere coincidence of the decisions adopted within the various countries and neither can it be explained as a subsequent imitation of such decisions, based on the experiences reported by the frontrunners in the implementation of national immunization programs. A recent example of this is *Haemophilus influenzae* type b conjugate vaccine.[1] Despite its categorical and almost immediate success in developed countries, five years elapsed before this technology was first adopted in Latin America, and another 10 years elapsed to achieve progress comparable to the one recorded with the second-generation PCVs, in the first five years of existence in the market (Figure 2).

The promising prevention scenario for pneumococcal diseases that we currently observe is the product of a prolonged joint effort in the region that set the stage for decision-making in the countries as soon as the first clinical trials for PCV reported successful results. This unprecedented process covered initiatives directed, *inter alia*, to obtain the evidence base necessary for decision-making, to strengthen the technical competences for the national introduction of the PCVs in the national immunization programs and to raise public awareness on the importance of pneumococcal diseases and their prevention.[2]

This paper revisits the most salient milestones of the preparatory work conducted for the implementation of the PCVs, underscores externalities in the LAC national immunization programs, and identifies the upcoming challenges for all of the countries in the region to continue to make progress in the control of diseases caused by *Streptococcus pneumoniae* and future vaccine-preventable diseases.

SIREVA Initiative for the Surveillance of the *S. Pneumoniae* Serotypes Responsible for Invasive Infections

In the early 90s, the relative frequency of the *S. pneumoniae* serotypes responsible for invasive infections in the LAC countries was practically unknown. Even when pneumococcal diseases were a familiar problem for doctors and microbiologists, absent this critical information, no country in the region was in a position to discuss potential local projections of the candidate PCVs under development then.

The Network Surveillance System for Bacterial Agents Responsible for Pneumonia and Meningitis (SIREVA) implemented by the Pan-American Health Organization (PAHO) was key to characterizing the microbiology of acute pneumococcal diseases and clearing legitimate uncertainties around the potential effectiveness of the PCVs in children of the region, given their antigen-based formulation focused primarily on the most prevalent serotypes in developed countries.

The SIREVA Laboratory Network contributed solid and conclusive information on the *S. pneumoniae* serotypes responsible for invasive infections in children in LAC, long before and after the arrival of the first PCV, with a broad geographical base and strict quality control procedures.[3-5] SIREVA became the model for similar initiatives in other regions and continues to be praised based on the success obtained in its specific objective.[6-7] Likewise, SIREVA promoters and collaborators deserve recognition for training several professionals and technical staff, transferring new technologies and implementing standardized procedures for bacteriological and molecular diagnosis of *S. pneumoniae*, *H. influenzae* and *N. meningitidis*, in addition to the active communication and feedback provided on the information gathered by the network toward the base of the national surveillance systems.

The SIREVA initiative underscores international cooperation as an example that can foster local efforts to provide a timely response to questions that exceed the capabilities of the isolated surveillance systems in each country. The experience in developed countries indicates that the follow up of infections caused by *S. pneumoniae* and other vaccine-preventable encapsulated bacteria in vaccinated populations poses difficulties even greater than the ones recorded pre-vaccine. The role SIREVA will play in years to come is envisioned to be as relevant as or more relevant than in the past.

Systematic Analysis of Epidemiological Evidence

Incidence and burden of disease are currently essential data to support the policies on vaccine use, prioritize health resources within the countries and the international aid organizations and also to assess the impact of immunization programs.[8] The lack of quantitative epidemiological information has been identified repeatedly as a significant hurdle to the implementation of new vaccines, in particular in middle-income countries where program financing depends exclusively on the national budget.

PCV clinical development prompted a fast deployment of epidemiological, prospective, and retrospective studies with the purpose of gathering evidence to inform future decisions on financing and implementation in various countries and at the international level. Most certainly, the most decisive arguments for the course of decision-making in LAC countries were derived from the analysis of the regional burden of pneumococcal diseases in children younger than 5, led by PAHO and the Sabin Vaccine Institute,[9] and the study on global burden, performed by the World Health Organization (WHO) and PneumoADIP.[10] The two studies, performed by different teams of experts and with different methodological approaches, concluded that *S. pneumoniae* is the vaccine-preventable agent responsible for the highest number of deaths and severe diseases in the population younger than 5. Additionally, the team of experts convened by PAHO-Sabin performed an economic analysis of the PCVs from the regional perspective.[11] This cost-effectiveness analysis estimated that the routine use of second-generation PCV in LAC would avoid 9,500 deaths and more than 850,000 annual cases of pneumococcal disease in children younger than 5, and that such measures would more than satisfy the cost-effectiveness criterion recommended for investments in public health, within a wide range of vaccine prices.[11] Finally, both studies contributed benchmark estimates of incidence and fatality for the main clinical forms of pneumococcal infection, which may be used as input in the analysis processes within each country.

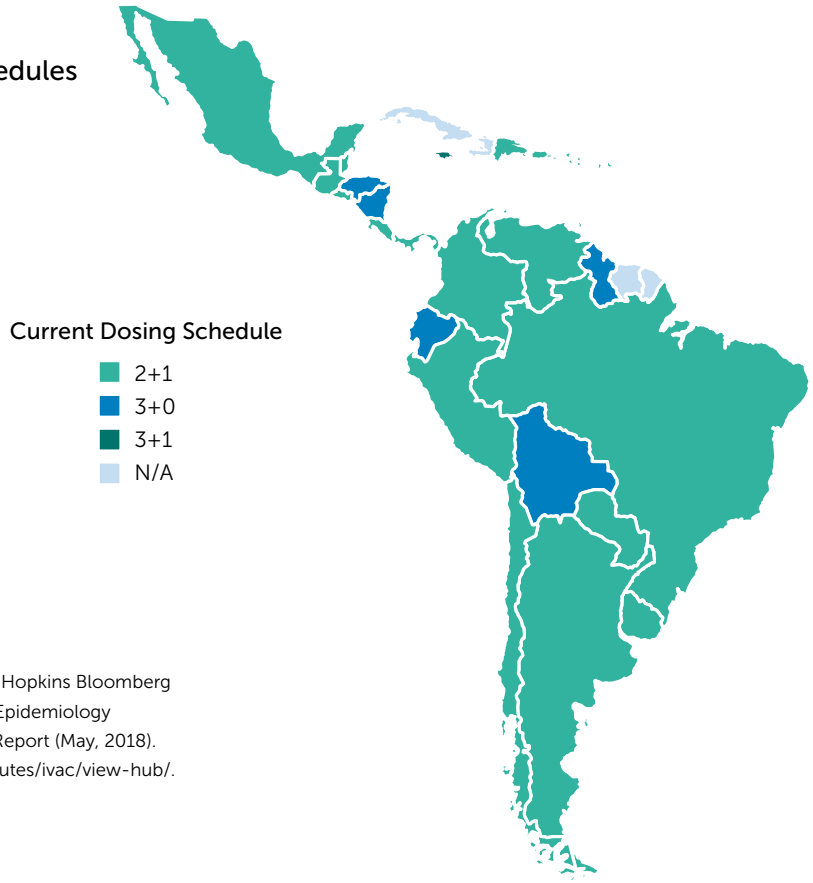
Table 1 summarizes the main results of the analysis on the regional burden of the pneumococcal diseases by PAHO-Sabin, together with the data provided for the same subregion and the set of countries of the Americas, in the study on global burden. The table also shows some specificities that could explain the differences in rates and estimated cases in each study.

Beyond its informational value for decision-making in the countries, the two studies on the burden of the pneumococcal disease in LAC highlighted the weaknesses of the epidemiological evidence in the region. Indeed, after extensive searches in the medical literature and direct consultations with researchers and personnel from the surveillance system in the countries, both systematic reviews found less than a dozen studies with data to populate their respective models on burden of disease, most of them from a few countries in the region (Table 1).

Table 1. Estimates of Annual Incidence and Burden of Disease for Pneumococcal Diseases in Children < 5 years in LAC, as of Two Independent Systematic Reviews

	PAHO-Sabin (Ref. 9) LAC	WHO-PneumoADIP (Ref.10) LAC	WHO-PneumoADIP (Ref.6) The Americas
PNEUMOCOCCAL MENINGITIS			
Annual cases	3,918	8,400 (6,000–11,500)	9,500 (6,800–13,000)
Incx10^5	7.9 (3.2–11.5)	Unreported	12 (9–17)
OTHER INVASIVE PNEUMOCOCCAL DISEASES			
Annual cases	1,200 (900–1,500)	Unreported	55,400 (39,800–75,400)
Incx10^5	32.3 (31.5–33.1)	Unreported	71 (51–97)
PNEUMOCOCCAL PNEUMONIA			
Annual cases	327,225	595,000 (463,000–741,000)	648,000 (505,000–807,000)
Incx10^5	~ 674 (586–857)	Unreported	836 (651–1,040)
DEATHS CAUSED BY PNEUMOCOCCAL DISEASES			
Current number	18,068 (12,000–28,000)	33,000 (23,000–39,000)	33,100 (23,600–39,500)
Incx10^5		Unreported	43 (30–51)
METHODS			
Population: Source (N)	Year 2005 cohort (11,700,500)	UN, 1 to 59 months, year 2000 (77,548,765)	
Review period	1990–2006	1980–2005	
Fatality adjustment	—	HIV and access to treatment	
Countries with incidence data for pneumonia	Argentina, Brazil, Uruguay, Chile, Cuba, Guatemala, Dominican Rep.		
Countries with incidence data for meningitis	Argentina, Cuba, Chile, Brazil, Guatemala		
Countries with incidence data for other invasive diseases	Argentina, Chile		
Countries with fatality data for some of the disorders	Argentina, Brazil, Costa Rica, Uruguay, Chile, Cuba, Guatemala, Dominican Rep., Peru		

Figure 3. PCV Immunization Schedules Used in the Routine Programs of the Region of the Americas



International Vaccine Access Center (IVAC), Johns Hopkins Bloomberg School of Public Health. Vaccine information and Epidemiology Window (VIEW-hub) Global Vaccine Introduction Report (May, 2018). http://www.jhsph.edu/research/centers-and-institutes/ivac/view-hub/.

Strengthening Technical Competencies and New Tools for Complex Decision-Making: the PROVAC Initiative

S. pneumoniae is a classic human pathogen well known to doctors and microbiologists across the world. Its clinical manifestations are present day in day out in the medical practice at all levels of care and the medical community has been demanding for decades effective vaccines against it, even more so since the emergence of antimicrobial-resistant strains. In spite of the high level of alert on the importance of pneumococcal diseases within the medical community, paradoxically, the 7-valent PCV was not immediately accepted by the public health authorities in developed countries. Instead, this costly technology gave rise to a new paradigm for the discussion of public immunization policies in the United States and other high-income countries including: the technical features of the new product, the burden of potentially preventable morbidity and mortality and the operational implications of their implementation in the routine programs.[12-15]

The need to introduce economic considerations in decision-making for the implementation of new vaccines in LAC countries started to be recognized well before the advent of the PCVs, with the Hib-conjugate vaccine and several others that emerged in the 80s and 90s.[16] Over the last decade, two new rotavirus vaccines entered the market within a short interval; two second-generation PCVs; two human papillomavirus (HPV) vaccines; one conjugate vaccine against *N. meningitis* A, C, Y and W-135 and another against Group B meningococcus, all of them with the potential to prevent deaths and acute morbidity during childhood or in later stages of the life cycle. Given the various options, cost-effectiveness analyses acquired an urgent and peremptory character for public health authorities in LAC countries.

The ProVac Initiative by PAHO was conceived with the purpose of promoting the introduction of new vaccines into the national immunization programs in LAC, through the development of technical competencies and the strengthening of procedures for evidence-based decision-making.[17] ProVac was adopted through a resolution by PAHO's Directing Council in late 2006, upon a period full of complex decisions to be made by the public health authorities in the member countries of the Organization.

For five consecutive years, promoters and collaborating partners of ProVac offered training workshops for evidence-based decision-making to officials from the public health sector and the immunization programs of 25 countries in the region. The workshops were mainly focused on the use of economic evaluations for the rational implementation of PCV, HPV, and rotavirus. Additionally, the ProVac team advised on the execution of 21 cost-effectiveness studies for the same vaccines by the multidisciplinary teams created within the various countries.[18,19]

Certainly, working with the ProVac Initiative on the analysis processes of the evidence around rotavirus, HPV and *S. pneumoniae* vaccines was a valuable endorsement for the public health authorities in the countries, as part of their role to establish priorities for the implementation of these vaccines in the national programs.[20]

Future Challenges

The gigantic technical and financial efforts to implement the routine use of PCV in developing countries have been stimulated by the evidence that identifies *S. pneumoniae* as the main vaccine-preventable cause of acute mortality and morbidity in children younger than 5, knowing beforehand that the 10 and 13-valent vaccines currently available have the potential to prevent an important part but, by no means, the whole burden of disease caused by this pathogen. In the time remaining to the registration of PCV with a wider antigen spectrum or other vaccines based on common antigens, the authorities responsible for the vaccination policies in the countries and at the international level should undertake the tasks typical of any new program and, at the same time, should thoroughly document the microbiology, clinical history, and epidemiology of the pneumococcal infections in the vaccinated populations to inform future discussions on the reasonableness of the vaccines currently under clinical development. In the short term, there are two specific tasks in LAC, which are inescapable for the countries and cross cutting to the region as a whole: to optimize the performance of the current programs and to document advances in the control of the target problem.

Immunization programs with PCV in LAC and other developing regions are aligned with the recommendation given by WHO-PAHO in April 2012, intended mainly to prevent the clinical forms of pneumococcal infection associated with a higher risk of death and severe morbidity in the population younger than 5; in particular, invasive infections and pneumonias. For the priority objective and based on the evidence available toward late 2011, the current recommendation endorses the undistinguishable use of the 10 and 13-valent vaccines, in vaccination schedules comprising three primary doses during the first six months of life (3+0), or two primary doses during the first six months plus a booster before 15 months (2+1), in addition to the 4 dose-schedules originally authorized by the regulatory agencies (3+1). The implicit flexibilities of these guidelines have opened the door to various modes of PCV administration within the routine childhood immunization programs globally (Figure 3), and also a series of questions on the relative advantages of the various options, in particular in terms of cost-effectiveness and impact on the general burden of pneumococcal diseases in the developing countries. The general scenario of the national immunization programs in LAC offers an exceptional opportunity to analyze these questions and optimize the performance of the PCV in the countries, through close monitoring and joint discussion of the experiences in the subpopulations with various modes to use PCVs.

The other imminent task for the national program managers is to document the benefit for the public health sector of the fiscal resources assigned to the implementation of PCVs, starting with the priority purpose of investments carried out so far, i.e., the control of invasive infections, pneumonias, and deaths caused or attributed to *S. pneumoniae* in children under 5. This task is inherent to the accountability of the current programs and its results will be essential to justify budgetary expansions for vaccine prevention of pneumococcal diseases in the future, for children or individuals from other age groups.

Promoters and collaborators of the three regional initiatives mentioned in this paper have conducted their own assessment of the work developed and shared the lessons learned. In a cross cutting fashion, the three experiences have underscored that the main weakness of the results lies in the lack or shortage of population-based information. With the PCVs already added to the routine childhood immunization programs, these messages require immediate attention and urgent actions by the national public health authorities, with the purpose of improving the official surveillance systems and reporting of infections caused by *S. pneumoniae* based on the demands involved in the monitoring and accountability of the recently-created vaccination programs. Otherwise, we would need to abandon the path followed to reach the current milestone and would seriously undermine the possibility of future progress in the control of pneumococcal diseases through the rational use of vaccines.

Conclusions

Currently, the scenario of vaccine-preventable diseases in LAC is diametrically opposed to the one prevailing 45 years ago. The priority agents are not necessarily the same in the entire region and the vaccination strategies may require inter-country and intra-country adjustments. Therefore, prioritizing investments, operational strategies and program control are currently the primary and undeniable responsibility of the national authorities. Additionally, the economic development of the region has led to the wane of international economic aid for vaccines and immunizations; the financing of the programs in most of the LAC countries is currently derived completely from the taxes collected by the States and, therefore, the first target of accountability is the citizenry itself.

The reader may infer that the control of vaccine-preventable diseases in LAC currently depends solely on the availability of efficient vaccines, the political will of the authorities and the competence of the technical teams within each country, to manage them efficiently and safely for the people at risk. Indeed, field workers will know that the current scenario contains a great number of complex challenges and questions that rarely will find a complete and timely response within each country. All in all, in the era of National Immunization Programs, the technical collaboration amongst the countries and the regional leadership for effective collaboration continue to be critical factors for success in the control of vaccine-preventable diseases, as they were at the beginning and throughout the Expanded Program on Immunization.

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Perspectives on the Future of Global Eradication of Polio, Measles, Rubella, and Congenital Rubella Syndrome

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Introduction

In 1985, the Pan American Health Organization (PAHO) with its member states and committed partners embarked on an initiative to eradicate polio by 1990.[1] Funds from USAID and other partners such as Rotary International arrived in 1987.[2] The Region, under the leadership of Dr. Ciro de Quadros, embarked on an ambitious plan of action to interrupt transmission on time three years later. The last case occurred on 23 August 1990, eight months, 23 days past the original target.[3]

While polio was being eradicated, countries of the Americas also worked diligently to define the disease burden of measles.[4] Many countries simultaneously administered polio vaccine to interrupt wild poliovirus transmission, and measles vaccine to prevent the large outbreaks of measles that they were encountering in the late 1980s and early 1990s. As a consequence of these efforts, the day polio eradication was certified in 1994, the Region embarked on the elimination of measles by 2000.[5,6] The last case of endemic measles at that time occurred in November 2002 in Venezuela.[7] Similarly, in September 2003 the Region launched the elimination of rubella and congenital rubella syndrome (CRS) by 2010.[8]

In early 2017 at the time of this writing, the world has never been closer to achieving the global polio eradication goal.[9] Five of the six World Health Organization (WHO) regions have measles elimination targets, and only three have rubella and CRS elimination targets. The Global Vaccine Action Plan (GVAP) calls for measles elimination by 2020 in five of six WHO regions.[10] Progress is grossly insufficient to come close to the GVAP aspirations.[11] The purpose of this chapter is to share perspectives on the future of the eradication of polio, measles, rubella, and congenital rubella syndrome (CRS), and potential actions required in order to achieve the targets.

Polio Pitfalls

For years, the global polio eradication initiative faced devastating set-backs, each with the potential to completely dismantle the program and commitment of governments and key partners. First, the global commitment was never fully recognized until 1998, two years before the global target. Success to that point, had been regionally driven, first in the Americas, followed by the Western Pacific Region (WPRO), and then the European Region (EURO). To work under conditions with insufficient global commitment was overwhelming for many Regions.[12]

However, India successfully eradicated wild poliovirus type 2 in 1999.[13] The last wild poliovirus type 2 in the world was reported in western Uttar Pradesh, although at the time no one fully realized the substantial milestone that had occurred. By the end of 2000 which was the original global target date, the number of polio cases reported in India was at an all-time low.[14] Prior, India was the country that typically reported two thirds of the world's polio cases every year. At the same time polio in Africa, Pakistan, and Afghanistan continued to paralyze thousands of children.

However, a setback in India occurred in 2001 when the polio leaders decided to downsize the number of mass polio vaccination campaigns required to stop transmission. Polio came roaring back exceeding more than 1600 cases in 2002 in Uttar Pradesh, India, alone.[15] To complicate matters, program leadership decided to conduct campaigns alternating between monovalent doses of oral polio vaccine (OPV) type 1 and type 3. Predictably, sequential outbreaks of wild poliovirus type 1 and type 3 occurred as a consequence of emerging pools accumulating from the sequential monovalent strategy. During the year OPV1 vaccine was not being used, wild poliovirus type 1 outbreaks occurred, and the same happened for outbreaks of wild poliovirus type 3.[16]

Almost simultaneously in Africa, the national immunization days required to maintain population immunity were canceled in African polio free countries largely because of insufficient funding and inadequate foresight. The decade of 2000 saw Nigeria continuing to export wild polioviruses to other recently free countries that were no longer protected with their national immunization days.[17] Religious and other community leaders in Nigeria had yet to be convinced that polio eradication was good for their children and their communities. The exportations led to large outbreaks that spread extensively in previously declared polio free areas such as Southern Sudan, Yemen, and the Horn of Africa. It was in these countries that vaccinators had previously been killed while conducting their duties to achieve a polio free status.[18]

In retrospect it is quite remarkable that the program survived to fight another day, largely due to the credit of the Bill and Melinda Gates Foundation (BMGF) and unrelenting commitment of governments and other key partners such as the Centers for Disease Control and Prevention (CDC) and Rotary International. In particular, BMGF started committing the necessary resources that would not infringe upon the extent to which the necessary strategies were required, such as the size or number of mass polio vaccination campaigns, to carry the program over the finish line. Today, the world is as close as it has ever been to achieving the target. By the end of 2016, only 35 wild poliovirus cases were reported globally and only four cases due to circulating vaccine-derived polioviruses (cVDPV). But, as alluded to above, the world has been on the brink of success several times and hopefully the historical lessons will be applied and mistakes will not be repeated.

Polio Endgame

The eradication of polio is really the eradication of 2 viruses, the wild type poliovirus and the oral polio vaccine virus.[19] Vaccine virus must also be eradicated because of its associated risks of vaccine paralytic poliomyelitis (VAPP), cVDPVs, and vaccine-derived polioviruses in immunocompromised individuals (iVDPV). If OPV use were to continue after wild poliovirus is eradicated in the world, then these risks would continue. Therefore, the cessation of OPV use must be addressed in the polio endgame strategy. Since wild poliovirus type 2 transmission was stopped in 1999, the OPV cessation process will be conducted globally in a phased manner. Such an effort was done in April 2016 by switching from trivalent OPV (tOPV) to bivalent OPV (bOPV) product that does not contain vaccine poliovirus type 2.[20]

The switch to bOPV use required efficient and rapid global coordination and synchronization of activities. Ideally, the killed vaccine (IPV), which has none of the above described risks, would have been introduced 6 months before the start of bOPV, in order to continue to provide children with type 2 protection to mitigate the small risk of reemergence and spread of cVDPV2, or some other unexpected event. IPV would also boost mucosal immunity to those children previously vaccinated with tOPV.[21]

PAHO conducted an aggressive planning process to ensure that no country was left behind.[22] The PAHO region has remained polio free for approximately 25 years, at a cost that is actually quite substantial to individual countries if one considers the cost of surveillance and maintaining a trained work force. Unfortunately, the supply of IPV for PAHO's Revolving Fund is limited to only one provider. Going forward supply issues will need to be urgently addressed. Because of multiple providers, the supply for bOPV looks more reassuring for PAHO member countries.

The cessation of the type 1 and 3 components of bOPV will be linked to the global eradication of the corresponding wild types. The work ahead at the time of this writing must be critically focused on stopping transmission of wild polioviruses existing in Afghanistan and Pakistan. The challenges in these countries because of security issues are enormous. From 2012-2014, 74 vaccinators were killed in Pakistan, including 41 in 2014.[18,23] The government across all sectors, including military, are engaged. Military involvement in India led to smallpox eradication in the last most difficult areas to work. Parts of Afghanistan and Pakistan remain unreachable because of continued concerns about security.

An often overlooked risk are ongoing outbreaks of cVDPVs. Experts argue that these outbreaks are short-lived and more amenable to mass polio vaccination campaigns to stop their transmission. The data suggest that cVDPV is more responsive to OPV, compared to other circulating wild polioviruses.[24] However, cVDPV does cause paralysis and can be transmitted from child to child causing unnecessary outbreaks. In Nigeria, cVDPV2 has persisted for several years.[25] Transmission may be easier to interrupt, but persistent circulation of cVDPVs signify a critical error in the immunization system. Such cVDPV-caused outbreaks only occur in areas where OPV coverage has been very low.

One key programmatic challenge is to sustain the intensity of the end game efforts in spite of the fact that the polio eradication target date has been changed a number of times. Global interruption of transmission of wild poliovirus, as well as cVDPVs, did not occur at the end of 2015, the last selected target date. Eradication will occur, but the question is when. It may be challenging even in 2017. In the Americas, the last case was reported in 1991 in Peru, but 8 other cases were reported earlier in Colombia that year.[3] In 1990, 18 cases were reported; and in 1989, 24 cases. The program did not go from 24 cases that occurred in 1989 to zero overnight. Recognizing the tremendous amount of work ahead in Afghanistan and Pakistan, partners and governments must "toe the line". Additionally,

political commitment must remain even past the last wild case in order to carry out the three year work plan required to certify the target has been achieved. Funding these efforts must also continue.

The risk of the emergence of cVDPV relates to every country's immunity profile. Where OPV was recently used with poor coverage results, cVDPV emergence remains a risk. The routine immunization program performance is more important than ever. To that end, resources from polio should be transitioned to support the strengthening of routine immunization and other services.[26]

Importantly, countries in the Americas will need to continue to maintain their guard against poliovirus importations and the emergence of any outbreak due to cVDPV until the world has reached the ultimate target of the eradication of wild and vaccine polioviruses. This issue will continue to challenge our resolve in the years to come.

Measles, Rubella, and Congenital Rubella Syndrome Opportunities

The opportunity to transition the assets of global polio eradication initiative to strengthen health systems, while eradicating measles and rubella, introducing new vaccines, and finishing the job with polio, is an ideal opportunity for the world to seize. The experience in the Americas demonstrates that it can be done, and GVAP provides an important roadmap for the world to use.[27] WHO has been very proactive to develop a toolkit intended to help countries plan for these new challenges. Documenting lessons learned at the country level will be important to help other countries benefit from these experiences.

Current polio field staff number >30,000 globally, most of whom are actually local volunteers. The equivalent number for measles and rubella is >130. Most of the polio field staff are already spending almost half of their time on routine immunization, measles-rubella, and new vaccine introductions. The switch already happened.[27] The potential for continued impact on reaching polio certification while eliminating measles and rubella is extraordinary. The areas of expertise that polio field staff provide other immunization services include: leadership and management oversight, policy and strategy development, planning, implementation and vaccine delivery, monitoring and evaluation, communications and community engagement, disease surveillance and data analysis and use for action, capacity building, and partnership coordination. Arguably, there is no better package of expertise to confront the GVAP targets, including the elimination of measles and rubella. The feasibility of global measles and rubella eradication has been assessed.[28] One key guiding principle that programs have learned over and over again, is that simultaneous actions can be implemented. For example, more than one antigen can be provided in a vaccination campaign.[29] This was demonstrated in Africa more than 40 years ago. When rubella is linked to measles elimination, achieving the goal results in the elimination of two infections, and one major disabling syndrome (CRS).

The global case needs to be packaged and communicated more strongly. The experience in the Americas consistently demonstrates that the elimination of CRS results in cost savings.[30,31] Very few political leaders or ministers of health will turn down a cost savings benefit of eliminating a debilitating disease, particularly if supported by a core partnership. The last case of CRS in the Americas was in 2009, in advance of the 2010 target.[7] It is not only cost savings, but very feasible even in a country as poor and challenged as Haiti.

One compelling argument for accelerating measles-rubella elimination is the cost of containing measles outbreaks. The import-related outbreak that spread nationwide in Ecuador in 2011-2012 cost the country

approximately 8.5 million US dollars to contain.[32] Infectious diseases are only a plane ride away, so a nation's capacity to respond to measles will also be a litmus test on how well that particular country will respond to any emerging threat, such as new viral strains of influenza.[33] Interestingly, we have learned that conducting one rubella mass vaccination campaign with measles-rubella containing vaccine, targeting all citizens, both men and women, aged <40 years will lead to the elimination of CRS. A one-time intervention leading to the elimination of a condition, in this case CRS, is unprecedented in global health. In addition, the population immunity provided to the expanded age groups also benefits measles prevention and control.[33] The older aged immunity gaps that result from childhood vaccinations are covered in the rubella mass vaccination campaign. The arguments to accelerate measles and rubella elimination are compelling.

Unfortunately, WHO estimates that >50% of the world's children are currently not vaccinated against rubella.[34] Any strategy that uses a single antigen measles vaccine anywhere in the world should be evaluated very thoroughly through an ethical lens. The hypothetical paradoxical response that including rubella antigen in the routine immunization program would increase the risk of CRS born infants has been dispelled by the wealth of global experience and data accumulated thus far. The world has a moral mandate to insure all children are protected from the devastating consequences of rubella, at only marginal incremental program costs.

In summary, like with polio, countries in the Americas will need to continue to maintain their guard against measles and rubella virus importations and the emergence of related outbreaks. The vast experience in the Americas dealing with measles importations every year reflects the very fact that this virus is the most infectious on the planet. This must reinforce our resolve to maintain high-quality surveillance and high levels of coverage. Follow-up MR campaigns conducted every 4 years are part of the elimination strategy and really should be maintained to ensure adequate population immunity is provided to prevent wide spread transmission when measles and rubella importations occur. Finally, immunization managers in the Americas should look for every opportunity to share their experience in other parts of the world.

Conclusion

Polio eradication will likely happen despite substantial challenges remaining, not to mention supply of IPV, the risk of cVDPV, the persistence of the wild poliovirus transmission in the remaining endemic areas, and immunity gaps. Progress has never been so close to achieving the goal. The transition of polio resources and assets to best practice opportunities like strengthening health systems, achieving universal immunization coverage and the elimination of measles and rubella, is a tremendous global opportunity to maximize the benefits of vaccines, and encompasses all the GVAP strategic objectives. A fundamental best practice is always building on the success of previous achievements, leading to no more polio, no more inequities, no more measles, rubella and CRS. The vision to accomplish these targets must also embrace the need for countries to increase ownership of their national programs by expanding the fiscal space of their own national budget allocations in order to guarantee the child's right to vaccine protection. There is arguably no better way to sustain national programs than by becoming less donor dependent. To that end, other regions of the world would certainly benefit from a Revolving Fund-like mechanism to assure them a safe, more affordable supply of vaccines, especially the newer candidates that come at a much higher cost. Otherwise, countries may continue to struggle with the increasingly challenging issues of national sustainability and country ownership. However, these are challenges that have solutions. Indeed, even the countries of the Americas should never take their Revolving Fund for granted. The current pricing challenges of new vaccines is a call for them to remain committed to the same regional solidarity in the future that led to so many successes in the past. Finally, in the Americas we cannot lose sight of the fact that now more than ever we cannot drop our guard on sustaining the region free of endemic polio, measles, rubella, and CRS.

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