

**State of the art of new vaccines
Research & Development**

Initiative for Vaccine Research

World Health Organization

Geneva

April 2003

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Abbreviations

AIDS	acquired immunodeficiency syndrome
ARI	acute respiratory infection
BCG	bacillus Calmette-Guerin (vaccine)
BL	Burkitt's lymphoma
BMRC	British Medical Research Council
CDC	Centers for Disease Control
CDS	WHO communicable disease programme
CSP	circumsporozoite protein
CTL	cytotoxic lymphocyte
DALYs	disability adjusted life years
DCVM	developing country vaccine manufacturer
DHF	dengue hemorrhagic fever
DNA	desoxy ribonucleic acid
DOTS	directly observed treatment short course
DT	diphtheria toxoid
DTH	delayed-type hypersensitivity
EBNA	Epstein Barr virus nuclear antigen
EBV	Epstein Barr virus
ENSO	El Nino Southern Oscillation
EPI	Expanded Programme on Immunization
ETEC	enterotoxigenic <i>E. coli</i>
ERIG	equine rabies immunoglobulins
GAVI	Global Alliance for Vaccines and Immunization
GMP	good manufacturing practice
GSK	GlaxoSmithKline Laboratories
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HDCV	human fibroblast cell rabies vaccine
HEV	hepatitis E virus
Hib	<i>Haemophilus influenzae</i> type b
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HP	<i>Helicobacter pylori</i>
HPV	human papilloma virus
HRIG	human rabies immunoglobulins
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
IAVI	International AIDS Vaccine Initiative
ICGEB	International Center for Genetic Engineering and Biotechnology (India)
IFPMA	International Federation of Pharmaceutical Manufacturers Associations
IPV	inactivated poliomyelitis vaccine
IS	intussusception
IVI	International Vaccine Institute
IVR	Initiative for Vaccine Research
IVU	intravenous drug user
JE	Japanese encephalitis
LPS	lipopolysaccharides
LRI	lower tract respiratory infection
MAP	multiple antigen peptide
MDR-TB	multidrug-resistant tuberculosis
MHC	major histocompatibility complex
MIM	Multiple Initiative on Malaria
MMV	Medicines for Malaria Venture
MRI	Medical Research Institute (US Navy)
MVA	modified vaccinia Ankara

WHO/IVR

MVI	Malaria Vaccine Initiative
NID	national immunization day
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institute of Health
NLV	Norwalk-like virus
NPC	nasopharyngeal cancer
OMP	outer membrane protein
OPV	oral poliomyelitis vaccine
ORF	open reading frame
PATH	Programme for Appropriate Technology in Health
PCECV	chicken embryo cell rabies vaccine
PCR	polymerase chain reaction
PEDV	purified by ultracentrifugation rabies vaccine
PEP	post-exposure prophylaxis
PFP	purified F protein
PHKCV	primary Syrian hamster kidney cell rabies vaccine
PIV-3	Parainfluenza virus type 3
PS	polysaccharide
R&D	research and development
RMAB	rabies monoclonal antibody
RNA	ribonucleic acid
RSV	respiratory syncytial virus
RV	rotavirus
RVA	fetal rhesus cell rabies vaccine
SP	<i>Streptococcus pneumoniae</i>
STD	sexually transmitted disease
SVDP	Schistosomiasis Vaccine Development Programme
TB	tuberculosis
TBE	Tick-borne encephalitis
TDR	WHO tropical disease research programme
TPI	triose phosphate isomerase
TT	tetanus toxoid
UCLA	University of California Los Angeles
UNAIDS	Joint United Nations Programme for AIDS
UNDP	United Nations Development Programme
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
VDPV	vaccine-derived poliomyelitis virus
VLP	virus-like particle
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

Background

The world's poorest regions are still suffering a heavy toll of premature death and disability from infectious diseases for which vaccines do not exist or else need to be improved. Infectious diseases are still responsible for a third of all deaths, killing at least 15 million people a year. The health disparity between rich and poor countries results in average life spans of 77 and 52 years respectively. Deaths attributable to infectious diseases contribute most to the disparity¹. Of these, more than 5 million are children under five. Worse, at least two million children still die each year from diseases that could have been prevented by already existing vaccines. Every day 4 000 children are dying from diseases that could be avoided with low-cost and effective vaccines. On top of this high death toll, millions more children are suffering disability and illness because they have not been immunized; they experience the consequences of compromised education, socialisation, and economic contribution.

The most effective way to reduce disease and death from infectious diseases is to vaccinate susceptible populations. Although highly effective vaccines are available against a number of pathogens, for other infectious diseases vaccines are either not completely protective or no vaccine is available. For these diseases, it is of crucial importance that vaccine R&D is considered as a priority. The present document represents an extensive analysis of the state of the art of vaccine R&D against infectious diseases of public health importance for which vaccines are either non-existent, or need substantive improvement. The first section is a critical review of the situation for each specific infectious disease in terms of epidemiological data, control strategies and vaccine R&D. The second section provides a table listing the main vaccine candidates for each disease, their state of development and the industrial or academic entities involved.

Section I – Disease-specific vaccine R&D-related issues

Diarrhoeal Diseases

The disease burden for diarrhoeal diseases is estimated at 62 451 000 DALYs in 2001 ([WHO](#), 2002). In some developing countries, children have more than 12 episodes of diarrhoea per year and diarrhoeal diseases account for 15-34% of all deaths. Conservative estimates place the death toll from diarrhoeal diseases at 4 to 6 million deaths per year, with most of these occurring in young children. The diversity of bacterial and viral infections that may cause diarrhoea complicates accurate surveillance and diagnosis, especially in developing countries with little or no access to modern laboratory procedures. The specific disease burden attributable to a particular infectious agent is particularly complex given the multiplicity of these agents, their serotypes, and depends largely on laboratory facilities in endemic areas, as illustrated by the study of the disease burden of shigella (see specific chapter)². If, in the long term, access to clean water, better hygiene, and improvement of sanitary measures would certainly have the greatest impact on diarrhoeal disease, then immunizations against specific diseases are the best hope for the short- and mid-term. This is particularly true for viral diseases such as rotavirus, present in both high and low hygiene level countries.

Rotavirus

Disease burden. Rotavirus (RV) is the leading cause of severe diarrhoeal disease and dehydration of infants in both developed and developing countries, with seasonal peaks according to the latitude and climate. In a 1985 review, RV were estimated to account for between 20% and 70% of hospitalized cases of diarrhoea and 20% of all diarrhoeal deaths in children less than 5 years of age worldwide³. Most of the studies indicate that symptomatic disease occurs between 3 months and 2 years of age with a peak incidence between 7 and 15 months (0.07 to 0.8 episodes per child per year)⁴. By age 3-4 years, virtually all children have had the disease. Based on 3.3 million deaths (2 million according to [WHO](#) for the year 2001) attributable to diarrhoea worldwide, 875 000 deaths (600 000 according to WHO) would be attributable to RV, primarily under 5 years of age, and that up to 85% of these deaths occur in countries defined as “low-income” according to the [World Bank](#) classification scheme^{5 6}. In epidemiological studies conducted in developing countries, RV accounted for a median of 8% of all diarrhoeal episodes, 28% of outpatients or clinic visits for diarrhoea, and 34% of hospitalizations of young children for diarrhoea^{7 8 9}. In Peru for example, RV is accountable for 384 000 cases, 64 000 clinic visits, 30 000 hospitalizations, and 1 600 deaths per year with a US\$ 2.6 million medical care

cost¹⁰. RV is responsible for 25% of the deaths associated with diarrhoea and responsible for 6% of all deaths in children less than 5 years of age¹¹.

Vaccines. RV is double-stranded RNA virus belonging to the family of *Reoviridae*. Two structural outer capsid proteins, VP7 (G protein) and VP4 (P protein) define the serotype of the virus and are the major antigens involved in virus neutralization^{12, 13}. G serotypes 1-4 & 9 and P genotypes P[4] and P[8] are predominant worldwide¹⁴. Among them, G1P[8] is the predominant strain, followed by G3P[8], G2P[4], and G4P[8]. G9 strains have been emerging since the late 1990s and in some regions are now the predominant strain. Additional serotypes (G5, 8, 10) and phenotypes (P[6], [9]) have been described in multiple countries and reviewed recently¹⁵. Rotavirus surveillance system networks have been constituted with the collaboration of the [US Centers for Disease Control and Prevention](#) and WHO to estimate the hospital-based disease burden of RV gastroenteritis in children less than 5 years of age, the baseline incidence of IS, and to constantly update the frequency and characteristics of circulating strain. This latter aspect is of tremendous importance for the development of a RV vaccine and for studying the possible vaccine selective pressure and the emergence of new strains in the vaccinated populations.

Two RV vaccines are currently licensed. In the USA, a tetravalent rhesus-human reassortant vaccine developed by [NIH](#) was licensed in 1998 by [Wyeth](#) (Rotashield[®]) and recommended for routine immunization of US infants at 2, 4, and 6 months, and administered to more than 900 000 children. An increased frequency of vaccine-associated intussusceptions (IS) was however demonstrated, leading to the withdrawal of the vaccine from the market in 1999. The risk of IS appears to be limited to the two weeks following the first two doses of vaccine and is highest during the period 3-7 days following the first dose of vaccine¹⁶. The efficacy estimates from several efficacy trials were approximately 50-60% against all cases of RV diarrhoea, and 70-90% against severe RV disease, such as dehydrating diarrhoea and hospitalizations. Unfortunately, the vaccine tested concomitantly in Asia (Bangladesh and India) and Africa (Ghana and South Africa) could not be evaluated in terms of risk-benefit for children (intussusception incidence vs. efficacy and hospitalizations and deaths prevented), the trials being stopped. The use of this vaccine in developing countries is therefore highly unlikely. The second licensed vaccine is a lamb-derived monovalent (group A, subgroup I, G10/P[12]) live-attenuated 3-oral dose vaccine, developed by the Lanzhou Institute of Biomedical Products, China) in collaboration with [IVI](#), licensed only in China. Sixty one percent of the vaccine recipients develop neutralizing antibody responses to the vaccine strain. It has not been included in the routine program of childhood immunizations¹⁷. The vaccine efficacy is not known since it was not tested against placebo. No controlled phase III of this vaccine was conducted until full completion.

[GAVI](#) has selected the development of a rotavirus vaccine as one of its three priority R&D projects. Several new vaccine approaches are currently being pursued¹⁸:

- A human-derived monovalent (G1/P8) live-attenuated 2-3 oral dose vaccine developed by [Avant Immunotherapeutics](#) and licensed to [GlaxoSmithKline](#), who slightly modified the vaccine strain. The vaccine is being tested in Latin America and Asia in phase III trials and has undergone testing in Europe, the US, South Africa and Bangladesh.
- A bovine-human reassortant pentavalent (G1, G2, G3, G4 and P[8]) live-attenuated 3 oral dose vaccine, developed by Merck. This vaccine programme is currently in late phase III and has entered development in Central and South America. Large efficacy trials are yet to be planned in developing countries.
- A human neonatal G3P[6] strain-derived vaccine developed by Dr. Ruth Bishop (Parkville, Victoria, Australia). However, 50% of vaccine recipients and placebos developed a rotavirus disease during the study and did not develop a serological immune response. It is intended to conduct further clinical testing at a higher viral concentration.
- Two human-bovian naturally occurring neonatal-derived strains (116E and I321) are under development in India
- A killed injectable approach is still in discussion

It remains unknown whether IS will be associated with other RV vaccines. Future field efficacy trials will monitor IS in the vaccine recipients and placebo groups, as well as the serotypes and genotypes of viruses recovered from stools in both groups that may result from selective vaccine pressure. Baseline data on rates of IS will be useful to assess safety of the vaccine following introduction. Several alternative vaccine approaches have been proposed to avoid IS. They include 1) non-oral vaccines,

either vaccine-like particles administered intranasally or injectable inactivated vaccines, 2) nasal administration of live vaccines, 3) live vaccines other than rhesus-based vaccines (bovine or human-based), 4) altered rhesus vaccines (low titer), and 5) administration of the vaccine in the neonatal period since natural IS is rare in this age-group and bovine-derived vaccines have been found to be safely administered in this age group.

Enterotoxigenic Escherichia coli

Disease burden. Among children aged <5 years in the developing world, the annual burden of diarrhoea is estimated to be 1.5 billion episodes, accounting for 3 million deaths. In community-based studies of children in these settings, *enterotoxigenic Escherichia coli* (ETEC) is the most frequently isolated enteropathogen, accounting for approximately 210 million diarrhoea episodes and approximately 380 000 deaths annually. The peak of incidence of ETEC diarrhoea in these settings occurs in the first two years of life, with a declining incidence with age thereafter. Although ETEC is usually thought of as a childhood disease, due to its substantially higher incidence in early childhood than in older age groups, surveillance of hospitalised cases of ETEC diarrhoea has shown that almost half of such cases occur in persons aged >10 years, due to the large denominator population at risk in these older age groups. In children, the tendency of ETEC to cause dehydrating diarrhoea is less (approximately 5% of episodes) than that of rotavirus (approximately 36% of episodes). However, because the incidence of ETEC diarrhoea is considerably more common than rotavirus diarrhoea in children, the absolute number of dehydrating diarrhoea episodes due to ETEC is around 70% of the number of such episodes due to rotavirus. In addition, unlike rotavirus, ETEC diarrhoea in children has been associated with subsequent growth faltering^{19 20 21 22}.

Vaccines. Natural history studies of ETEC infections in children in developing countries suggest that these infections are immunizing, as reflected by declining rates of ETEC diarrhoea with age, lower ratios of symptomatic to asymptomatic ETEC infections with increasing age, and the protective relationships between initial ETEC infections and subsequent infections that have similar toxin and/or colonization factor phenotypes. These data therefore suggest that immunization against ETEC early in life may well be an effective preventive strategy. New ETEC vaccines for use in developing country populations should be targeted for incorporation into the existing schedule of immunization of the Expanded Programme on Immunization (EPI). Travellers from industrialized to developing country settings – including military troops on deployment – constitute another target population for vaccination against ETEC. Studies of travellers to Africa, Asia and Latin America suggest that the risk of diarrhoea during brief trips is about 50%, with most episodes occurring during the first week after arrival. ETEC has been isolated as the cause of about 35% of such diarrhoea episodes, and is the most common cause of traveller's diarrhoea. Although a variety of non-vaccine strategies exist to prevent traveller's diarrhoea, including fastidious attention to diet and intake of certain antibiotics, non-compliance commonly vitiates the effectiveness of all of these strategies, and side-effects constitute a particular drawback for antibiotics²³.

Due to the antigenic similarity of the B subunits of cholera toxin and ETEC heat-labile toxin (LT), a recombinant toxin-killed whole cell (rBS-WC) cholera vaccine was tested in Finnish tourists visiting Morocco. Vaccination prevented 23% of all diarrhoea episodes and 52% of episodes due to ETEC. Several approaches have been pursued to create killed vaccines including vaccines consisting of purified colonization factors, vaccines consisting of LT-only or LT-ST toxoid, and edible transgenic plant vaccines that express cholera toxin B subunit. The most successful approach, developed by investigators at the University of Göteborg (Sweden), is based on a cholera toxin rBS combined with 5 strains of formalin-killed ETEC cells that collectively express the colonization factors of greatest epidemiological importance in developing countries. Phase II studies of 2 dose regimens of this vaccine have been conducted in Bangladesh, Egypt, Israel, Nicaragua, the United States and Europe. These studies have found the vaccine to be safe and immunogenic, as manifested by induction of mucosal antibody responses to rBS and the CFA components of the vaccine. A pilot efficacy trial of this vaccine in European tourists travelling to developing country destinations found the vaccine to confer about 80% protection against ST-ETEC diarrhoea (the only toxin phenotype detected in this study), although small numbers of outcome events precluded statistically precise estimates of efficacy. Phase III trials of vaccine efficacy are ongoing in United States travellers to Latin America, European travellers to Kenya, Israeli military recruits, and Egyptian infants and young children.

The live vaccine approach is being pursued by investigators at the Center for Vaccine Development (CVD) at the University of Maryland (USA). Their vaccine development strategy is to use live attenuated *Shigella* organisms as vectors for expression of ETEC fimbrial and LT antigens. Such constructs might thereby protect against both *Shigella* and ETEC. Other approaches include colonization factor antigens encapsulated in biodegradable microspheres, developed by the CVD and tested in Phase I trials, and the expression of *E. coli* LT-B in tobacco, potatoes, tomatoes, and bananas. DNA immunizations are still in preclinical development²⁴. In addition, two companies in the USA, [Antex Biologics](#) and [Microscience](#), are both developing ETEC vaccines. Antex's product, Activax²⁵, is a combined multivalent vaccine for the prevention of traveler's diarrhoeal diseases containing protective antigens against *Campylobacter*, *Shigella* and ETEC. Microscience's technology is based on the utilization of their *spi*-VEC oral live attenuated typhoid vaccine²⁶ delivering ETEC antigens. Lastly, a new delivery technology, the transcutaneous immunization patch (containing CS6-LT) has been successfully tested in humans for ETEC vaccine²⁷.

Shigella

Disease burden. Shigellosis is endemic throughout the world. Worldwide there are approximately 164.7 million cases, of which 163.2 million in developing countries and 1.5 million in industrialized countries. Each year 1.1 million people are estimated to die from *Shigella* infection and 580 000 cases of shigellosis are reported among travellers from industrialized countries. A total of 69% of all episodes and 61% of all deaths attributable to shigellosis involve children less than 5 years of age²⁸. Since the late 1960s pandemic waves of *Shigella* dysentery (diarrhoea containing blood) have hit Central America, South and Southeast Asia and sub-Saharan Africa, often striking areas of political upheaval and natural disaster. During the 1994 genocide in Rwanda between 500 000 and 800 000 Rwandan refugees fled into the North Kivu region of Zaire. In the first month alone, approximately 20 000 people died from dysentery caused by a strain of *Shigella* that was resistant to all commonly used antibiotics. The combination of *Shigella* infections and HIV epidemics has had serious consequences, HIV speeding the spread of *Shigella* among HIV-positive groups with compromised immunity. *Shigella* infection also occurs in industrialized countries, particularly where there is poor hygiene, and among soldiers and travellers to the developing world.

Three types of shigella are responsible for bacillary dysentery: *S. sonnei* is classically predominant in developed countries (5% of infections in developing countries and 77% in industrialized countries) although it seems to become predominant in Thailand (71%)²⁹ as compared to previous years³⁰, a phenomenon probably linked to the level of development of the country. *S. flexneri* type 2a is predominant in developing countries (60%), resistant to ampicillin (but still sensitive to quinolone). Recently, the trend in the *S. flexneri* serotypes has been altered in Bangladesh where *S. flexneri* 2b dominates³¹. *S. dysenteriae* type 1 (Sd1) is the only cause of epidemic dysentery. Approximately 5-15% of Sd1 cases are fatal. Sd1 has caused epidemics of dysentery throughout the world. It caused a 4-year epidemic in Central America beginning in 1968 that resulted in more than 500 000 cases and at least 20 000 deaths. No epidemics have occurred in the region since then, but Sd1 continues to occur sporadically in the Western hemisphere. In Africa, epidemic dysentery due to Sd1 appeared in eastern Zaire in 1979 and has subsequently been confirmed in many other African countries. A major obstacle to the control of Sd1 is its resistance to many antimicrobial drugs.

Vaccines. The candidate shigellosis vaccines currently in advanced development include non-living vaccines:

- A parenteral conjugate vaccine consisting of *S. sonnei* detoxified LPS linked to a *Pseudomonas aeruginosa* carrier protein (O-rEPA), developed by [NIH](#), has been tested successfully in Phase III trials;
- A parenteral nuclear protein/ribosomal vaccine approach, developed by [IVI](#) and [WRAIR](#), still in preclinical stage;
- A nasally administered *Shigella*-proteosome vaccine consisting of *Shigella* LPS non-covalently linked to micelles from the outer membrane protein of group B *Neisseria meningitidis*.

Progress has been made with two candidate live oral vaccines in clinical trials:

- A live attenuated *S. flexneri* 2a strain SC602 ([Pasteur Institute, Paris](#)), tested in adults in the USA and in adults and children in Bangladesh in collaboration with The Walter Reed Army Institute of Research ([WRAIR](#)) and [IVI](#);
- A live attenuated *S. flexneri* 2a strain CVD 1207 ([Center for Vaccine Development](#), University of Maryland).

In addition, [Antex \(USA\)](#) is developing both a Shigella inactivated whole cell vaccine and a Traveler's Diarrhoea Activax³² product containing antigens from *Campylobacter*, *Shigella* and ETEC. These candidate vaccines should soon enter clinical testing.

Cholera

Disease burden. During 2001, 58 countries officially notified WHO of a total of 184 311 cases (one third more than in 2000) and 2 728 deaths. The reported overall case-fatality rate (CFR) has dropped to 1.48% with regards to the 3.6% reported in 2000. This absolute decline in CFR reflects contrasting realities, as CFR for South Africa is very low (0.22%) whereas rates of up to 30% have been observed in other parts of Africa. With a total of 173 359 cases, Africa accounted for 94% of the global total. Asia reported a total of 10 340 cases, which represents a small decrease compared to 2000. However, globally, the actual figures are likely to be higher, owing to the under-reporting and other limitations of surveillance systems. The year 2001 was marked by major outbreaks of cholera in several African subregions³³.

Vibrio cholerae is the infectious agent responsible for cholera. Only *Vibrio cholerae* serogroup O1 and serogroup O139 are known to cause epidemics of cholera. Isolates of *Vibrio cholerae* serogroup O1 are classified into two biotypes, El Tor and classical, on the basis of several phenotypic characteristics. Currently, the El Tor biotype is responsible for virtually all of the cholera cases throughout the world, and classical isolates are not encountered outside of Bangladesh. In addition, *V. cholerae* O1 is classified into two serotypes, Inaba and Ogawa, based on agglutination in antiserum. A possible third serotype, Hikojima, has been described, but it is very rare. Immunity due to previous *Vibrio cholerae* infection is serogroup specific. There are other serogroups of *Vibrio cholerae*, which can cause isolated cases of watery diarrhoea, but they do not cause epidemics (O5, O37). The current seventh pandemic of cholera is due to *Vibrio cholerae* O1 that has been reported from all regions of the world.

Vaccines. The old killed injectable whole-cell vaccine was efficient in 50% of the cases and for no longer than 6 months. To date, 3 oral cholera vaccines are available, which have been shown to be safe, immunogenic and effective. These vaccines have been licensed in some countries and are mainly used by travellers. Oral cholera vaccines are now under consideration for use in public health. As mentioned above, several countries have already vaccinated populations considered to be at high risk from a cholera outbreak (including Mayotte Island and Micronesia Island).

One vaccine consists of heat- or formalin-killed whole-cell *V. cholerae* O1, Inaba and Ogawa serotypes, classical and El Tor biotypes with purified recombinant B-subunit of cholera toxoid (WC/rBS), (SBL Sweden, now PowderJect). Field trials in Bangladesh, and Peru have shown that this vaccine is safe and confers 85-90% protection during 6 months in all age groups after administration of 2 doses, one or two weeks apart. In Bangladesh, protection declined rapidly after 6 months in young children, but was still about 60% in older children and adults after 2 years. The vaccine was also used successfully for mass vaccination in a refugee camp in Uganda. Since protection is achieved one week after the second dose, the study showed that a minimum of 4-5 weeks was needed to achieve protection of a refugee camp³⁴. As a result of technology transfer, a variant of the WC/rB vaccine containing no recombinant B-subunit has been produced and tested in Viet Nam. It is administered in 2 doses, 1 week apart. A field trial conducted in 1992-1993 in Viet Nam (Nha-Trang) showed an efficacy of 66% against El Tor at 8 months in all age groups. The vaccine is licensed only in Viet Nam and is currently also being produced in Indonesia. A whole-cell bivalent O1 and O139 oral vaccine without CTB developed in Viet Nam was shown to be safe and very immunogenic in both adults and children³⁵. This bivalent vaccine is actually the only existing vaccine against O139 serogroup infection. Other oral live attenuated O139 cholera vaccines are currently under development.

Another oral vaccine consists of a live attenuated genetically modified *V. cholerae* O1 strain (CVD 103-HgR), developed by [Bernu Biotech](#) (Switzerland). Placebo-controlled trials in a number of countries in South America and Asia have shown the safety and immunogenicity of a single dose of CVD 103-HgR. The vaccine is currently licensed in some industrialized countries. The efficacy of this vaccine has been investigated in adult volunteers in the United States, where it has been found that a single dose of this oral vaccine confers high protection (up to 90%) against moderate and severe cholera following a challenge with *V. cholerae* O1 of either El Tor or classical biotype given 3 months after administration. The overall protective efficacy against El Tor cholera of any severity (i.e.

including mild cases) was 80%. A large field trial performed in Indonesia has not shown convincing protection in a population exposed to cholera a long time after vaccination.

Another live attenuated vaccine developed in Cuba (one oral dose) has been tested in Phase I. A parenteral O-antigen-conjugated vaccine ([Pasteur Institute Paris](#), [NIH](#)) was tested in Phase I in the US and is still in preclinical development at the Institut Pasteur. The [University Putra Malaysia](#) and the Malaysia National Biotechnology Directorate are developing a naked DNA cholera vaccine derived from a local isolate, to be administered by intramuscular injection. The work is still at a pre-clinical stage³⁶. In addition, [Avant Immunotherapeutics](#) (USA) and [BioSidus S.A.](#) (Argentina) are currently testing in Phase II trials a live oral recombinant vaccine strain ([see announcement](#)).

WHO is currently investigating the use of oral cholera vaccines as an additional tool to traditional control measures. Conventional recommendations focusing upon basic sanitary and hygiene measures are efficient when properly applied, but it is also recognized that they are often difficult to implement fully. In May 1999 WHO convened a meeting of experts which, in light of the progress made in development and evaluation of oral cholera vaccines since 1995 and new data available on feasibility and accountability relating to these vaccines, recommended considering the use of the oral WC/rB cholera vaccine among the tools to prevent cholera in populations believed to be at risk of a cholera epidemic within 6 months, and not experiencing a current epidemic. Such high-risk populations may include, but are not limited to, refugees and urban slum residents. Currently WHO is investigating the role of mass vaccination as a public health strategy for populations at high risk in order to contain as well as prevent outbreaks. Issues being addressed include logistics, costs, financing, vaccine production capacities and other criteria. Consultation is expected to establish guidelines for the use of oral cholera vaccination in cholera control activities.

In December 2002, WHO convened a panel of experts to discuss the potential use of cholera vaccines in public health.

Typhoid

Disease burden. The very conservative annual number of typhoid cases for the year 2000 is estimated at 17 million with 600 000 deaths. In virtually all endemic areas, the incidence of typhoid fever is highest in children 5-19 years of age, even if some reports mention an earlier age. The number of sporadic cases of typhoid fever has remained relatively constant in the industrialized world, and with the advent of proper sanitary facilities, has been virtually eliminated in many areas. Most cases in developed countries are imported from endemic countries. Typhoid fever is characterized by the sudden onset of sustained fever, severe headache, nausea, severe loss of appetite, constipation or sometimes diarrhoea. Severe forms have been described with mental dullness and meningitis. Case-fatality rates of 10% can be reduced to less than 1% with appropriate antibiotic therapy. However, strains resistant to chloramphenicol and other recommended antibiotics (like ampicillin, cotrimoxazole or even ciprofloxacin) have become prevalent in several areas of the world. Multidrug resistant strains have been reported from Asia, the Middle East and Latin America. Paratyphoid fever can be caused by any of three variations or bioserotypes of *S. enteritidis paratyphi* A, B and C. It is similar in its symptoms to typhoid fever, but tends to be milder, with a much lower fatality rate.

Typhoid fever is caused by *Salmonella typhi*, the typhoid bacillus. At present, there are 107 different strains of the bacteria. Typhoid fever is transmitted by food and water contaminated by the faeces and urine of patients and carriers. Polluted water is the most common source of typhoid. In addition, shellfish taken from sewage contaminated beds, vegetables fertilized by nightsoil and eaten raw, contaminated milk and milk products have been shown as a source of infection. People can transmit the disease as long as the bacteria remain in their system; most people are infectious prior to and during the first week of convalescence. About 10% of untreated patients will discharge bacteria for up to three months; 2-5% of untreated patients will become permanent carriers. *S. paratyphi* is becoming predominant in some provinces of China and increasing numbers of cases of *S. paratyphi* are being reported from Pakistan.

Vaccines. Although licensed, killed parenteral whole-cell vaccines are not well tolerated. A linear homopolymer of galacturonic acid or Vi antigen vaccine developed in collaboration with [Institute Pasteur](#) (Paris), showed a 72-80% efficacy after a single parenteral dose and is licensed in more than 92 countries in Africa, Asia, Europe, Australia and the Americas. The live attenuated strain Ty21a is

licensed in 56 countries in Asia, Africa, Europe and the Americas. A head-to-head comparison of Ty21a and Vi has been proposed by WHO in order to make future recommendations for countries severely affected by typhoid. Three new candidate vaccines are currently in late stage development: a Vi-EPA (conjugated to the exoprotein A from *Pseudomonas aeruginosa*) was evaluated in Phase III in the Mekong Delta ([NIH](#)), providing more than 91% protection; a Ty800 live attenuated oral vaccine (*phoP*, *phoQ* genes deletion) (Phase I completed, [Avant Immunotherapeutics](#)); ACAM 948-CVD live attenuated oral vaccine (*aroC*, *aroD*, *htrA* gene deletion) (Phase I completed, [Acambis/Berna](#)). In addition, [Microscience](#) (USA) is currently developing another live oral vaccine based on an attenuated *S. typhi* (Phase I/II in 2002).

Caliciviruses

Disease burden. The role of caliciviruses was unrecognized and under-appreciated because diagnostics were not commonly available or used. The application of new molecular diagnosis tools has shown that human caliciviruses (HuCV) consist of two genera: Sapoviruses or Noroviruses, (previously referred to as the Norwalk family of viruses or small round structured viruses) to be the most common cause of gastroenteritis outbreaks in the United States, and they may emerge as a common cause of sporadic cases of acute gastroenteritis among both children and adults. The implicated vehicles of infection are water, shellfish, and food contaminated both at their source and by food handlers. HuCV was the leading pathogen in 11% of cases of diarrhoeal episodes. A prospective population-based cohort study with a nested case-control study was conducted to estimate the incidence of gastroenteritis and the associated pathogens in the general Dutch population³⁷. HuCVs appear to be the second most common agent of severe diarrhoea in children after rotavirus and the most common cause of outbreaks of acute gastroenteritis, including those that are foodborne³⁸. Their role in developing countries remains to be established. However, in Bangladesh³⁹, and in South Africa⁴⁰, most children acquired serum antibodies to the Norwalk agent very early in life, which suggests that HuCVs may also play a preeminent role in diarrhoeal diseases in children from developing countries. HuCVs accounted for a 20% etiologic share of all episodes of acute gastroenteritis in prospectively followed children between 2 months and 2 years of age in Finland⁴¹. In Japan, among 95 hospitalized children with acute gastroenteritis, rotavirus A was detected in 47% of them followed by HuCV in 18%⁴². In Beijing, China, infants had a seroprevalence rate of 99% for HuCV. The lowest seroprevalence (41%) was at 7-11 months of age. A sharp increase in seroprevalence occurred in early childhood, with 65% at one, 85% at three, and 100% at 8-9 years of age⁴³. The development of modern diagnosis tools such as PCR and disease burden studies on calicivirus-induced diarrhoea are urgently needed, especially in developing countries.

Vaccines. Within the last decade molecular analyses of the genome of Norwalk-like viruses (NLVs) have confirmed that this important group of infectious agents belongs to the *Caliciviridae* family. HuCVs have a positive-sense, single-stranded RNA genome now well studied⁴⁴. A vaccine against NLV infection may become soon a public health priority, following rotavirus vaccines.

Norwalk virus capsid protein, assembled into virus-like particles, was used as a test antigen, to determine whether immune responses could be generated in volunteers who ingested transgenic potatoes. Healthy adult volunteers received 2 or 3 doses of transgenic potato or 3 doses of wild-type potato ([CVD](#), University of Maryland, USA). Most of the volunteers who ingested transgenic potatoes developed significant increases in the numbers of specific IgA antibody-secreting cells, 20% developed specific serum IgG, and 30% specific stool IgA.⁴⁵ Adults orally immunized with rNVL VLPs developed serum IgG dose-dependent responses, and most of the volunteers responded after the first rNVL VLP dose, with no increase in serum IgG titer after the second dose⁴⁶.

Acute Respiratory Infections

The disease burden for Acute Respiratory Infections (ARI) is estimated at 94 037 000 DALYs ([WHO, 2002](#)) and 3.9 million deaths ([WHO, 2002](#)). ARI are among the leading causes of death in children under 5 years but diagnosis and attribution are difficult and uncertain. A further complication is that community studies of childhood mortality depend largely on verbal autopsies, which can be very unreliable for the diagnosis of ARI. Another difficulty is that ARI are often associated with other life-threatening diseases such as measles⁴⁷. A study reports 62% of all deaths are attributable to ARI but most of these were associated with measles. When measles deaths are excluded the proportion falls to

24%⁴⁸. Better estimates of burden of childhood pneumonia are needed and should be given high priority. A recent meta-analysis study demonstrates that throughout the world 1.9 million (95% CI 1.6-2.2 million) children died from ARI in 2000, 70% of them in Africa and Southeast Asia. The proportion of deaths directly attributable to ARI declines from 23% to 18% and then 15% as under-5 mortality declines from 50 to 20 and then to 10/1000 per year⁴⁹.

The main etiological agents responsible for ARI in children include *Streptococcus pneumoniae* (SP), *Haemophilus influenzae* (Hi), respiratory syncytial virus (RSV), and Para Influenza Virus type 3 (PIV-3). Other infectious agents with lower prevalence include chlamydia, mycoplasma, and legionella. However, the agent-specific disease burden and the relative prevalence of the three major agents in ARI should deserve further studies. This is particularly true for Hib for which a safe and efficient vaccine is already available and licensed worldwide⁵⁰.

Bacterial Respiratory Infections

Streptococcus pneumoniae

Disease burden. Infections caused by pneumococci are a major cause of morbidity and mortality all over the world. Pneumonia, febrile bacteremia and meningitis are the most common manifestations of severe pneumococcal disease, whereas middle-ear infection, sinusitis or recurrent bronchitis represent less severe, but considerably more common manifestations. Thus, in the United States alone, 7 million cases of otitis media are attributed to pneumococci each year. Although all age groups may be affected, the highest rate of pneumococcal disease occurs in young children and in the elderly population. In addition, persons suffering from a wide range of chronic conditions and immune deficiencies are at increased risk. In developing countries infants aged under 3 months are also at risk, especially for pneumococcal meningitis. In spite of the importance of pneumococcal disease, there is a scarcity of information on disease burden, particularly from developing countries. This is partly due to the inherent problem of obtaining an etiological diagnosis in cases of pneumonia. However, based on available data, acute respiratory infections kill an estimated 1.9 million children aged 5 years and under annually. The pneumococcus is estimated to cause over 1 million of these deaths⁵¹, most of which occur in developing countries, where the pneumococcus is probably the most important pathogen of early infancy. In Europe and the United States, pneumococcal pneumonia is the most common community-acquired bacterial pneumonia, estimated to affect approximately 100 per 100 000 adults each year. The corresponding figures for febrile bacteremia and meningitis are 15-19 per 100 000 and 1-2 per 100 000, respectively. The risk for one or more of these manifestations is much higher in infants and elderly people. Even in economically developed regions, invasive pneumococcal disease carries high mortality; for adults with pneumococcal pneumonia the mortality rate averages 10-20%, whilst it may exceed 50% in the high-risk groups. Pneumonia is by far the most common cause of pneumococcal death worldwide⁵².

Streptococcus pneumoniae is a Gram-positive encapsulated coccus. Based on differences in the composition of the polysaccharide capsule, about 90 serotypes are identified. This capsule is an essential virulence factor. The majority of pneumococcal disease in infants is associated with a small number of these serotypes, which may vary by region. Current data suggest that the 11 most common serotypes cause at least 75% of invasive disease in all regions. Pneumococci are transmitted by direct contact with respiratory secretions from patients and healthy carriers. Although transient nasopharyngeal colonization rather than disease is the normal outcome of exposure to pneumococci, bacterial spread to the sinuses or the middle ear, or bacteremia following penetration of the mucosal layer, may occur in persons susceptible to the involved serotype. Pneumococcal resistance to essential anti-microbials such as penicillins, cephalosporins and macrolides is a serious and rapidly increasing problem worldwide.

Vaccines. Protective immunity is conferred by type-specific, anticapsular antibodies, although the serological correlates of immunity are poorly defined. Antibodies to certain pneumococcal surface proteins have been demonstrated to confer protection in animal models but the role of these antibodies in humans is yet to be determined.

The polysaccharide capsule antigens do not regularly elicit protective levels of antibodies in children aged less than 2 years, and in individuals with advanced immunological impairments (HIV/AIDS). Furthermore, the polysaccharides do not induce immunological memory, which is required for

subsequent booster responses. The spectrum of prevailing capsular types varies with age, time and geographical region, although common serotypes are consistently identified throughout the world. One of the currently licensed vaccine is a polyvalent polysaccharide vaccine containing per dose (0.5 ml) 25 micrograms of purified capsular polysaccharide from each of the 23 capsular types of *S. pneumoniae* that together account for most cases (90%) of serious pneumococcal disease in Western industrialized countries. The marketed versions of this vaccine are almost identical. Relatively good antibody responses (60-70%) are elicited in most healthy adults during the 2-3 weeks following a single intramuscular or subcutaneous dose of this vaccine. The immune response is unreliable in children aged less than 2 years, and in immunocompromised individuals. Following the vaccination of pregnant women, antibodies are transferred both via the placenta and in the breast milk. However, it is not yet documented that maternal vaccination actually protects newborn infants against pneumococcal disease. The polyvalent polysaccharide vaccine is recommended for selected groups aged less than 2 years with increased risk of pneumococcal disease. Such groups include patients suffering from chronic organ failure, diabetes or certain immunodeficiencies. In some countries vaccination is also recommended for healthy elderly people (over 65 years old), particularly those living in institutions. The almost complete lack of information on the burden of pneumococcal disease among adults and the elderly population in developing regions illustrates the urgent need for further epidemiological and disease-burden studies on pneumococcal disease. Properly designed Phase III trials may provide information both on efficacy and disease burden. The polysaccharide vaccine has not been used in developing countries where much of the pneumococcal disease burden is found in the age group under 2 years. Due to poor immune response in children aged less than 2 years, the polysaccharide vaccine is not recommended for routine use in national childhood immunization programmes. The efficacy of this vaccine in protection against pneumococcal pneumonia in adults has been documented in prospective studies in developing country populations. However, randomized controlled trials in healthy elderly people in industrialised countries have failed to show a beneficial effect; the recommendation for its use in this population is based on data from observational studies showing a significant protective against bacteraemic disease but not pneumonia. The possibility that the vaccine may provide some protection to newborn infants through systematic immunization of pregnant women is currently being investigated. Although the polysaccharide vaccine is recommended for HIV infected patients in the USA, a study in Uganda⁵³ has shown that it provides no benefit. On the contrary, vaccinated individuals had higher rates of pneumonia. This has raised questions about the safety of the vaccine in HIV-infected adults in Africa. This could also influence the use of maternal immunization strategies in areas of high HIV prevalence.

Like the polysaccharide vaccines, the conjugate vaccines induce protection only against the serotypes involved, however, higher antibody levels are achieved, and the conjugates elicit an immune response more efficiently in infants and in immunodeficient persons. Moreover, the vaccine induces immunological memory resulting in a booster antibody response on subsequent exposure to the antigen. In analogy with the Hib vaccines, pneumococcal conjugate vaccines have been shown not only to protect against invasive disease, but also to suppress nasopharyngeal carriage of the pathogen. Therefore, these vaccines could reduce bacterial transmission in the community. Such a herd effect would add considerable value to the conjugate vaccines. Conjugate vaccines followed by natural or PS vaccine boosting might provide a foundation for life-long protection against disease due to vaccine serotypes of pneumococcus. This vaccine has been shown to have high efficacy against invasive pneumococcal disease caused by serotypes included in the vaccine. Introduction of the vaccine in the United States resulted in dramatic decline in the rates of invasive disease, with reductions also been seen in unvaccinated individuals as a result of herd immunity. The vaccine also showed moderate protection against otitis caused by vaccine serotypes. However, the decrease in vaccine type otitis media was offset by an increase in disease due to non-vaccine types of *S. pneumoniae* and by *H. influenzae*, a phenomenon referred to as "replacement disease". This phenomenon has not been observed thus far for bacteraemia and meningitis. Although a 7-valent vaccine is licensed ([Wyeth, USA](#)), this product does not protect against a few serotypes of *S. pneumoniae* that cause severe disease in developing countries, notably serotypes 1 and 5.

The development and introduction in developing countries of a conjugate *S. pneumoniae* vaccine is one of the three R&D projects selected by [GAVI](#) as a priority. A plan for the accelerated development and introduction of pneumococcal vaccine has been developed and a team and host institution selected to implement this plan ([ADIP](#)). Several conjugate vaccines that provide more optimal serotype coverage in developing countries are in clinical development including the 9-valent/Mening C DTCRM [Wyeth](#) vaccine (CRM197), and the 11-valent [GlaxoSmithKline](#) vaccine (protein D of *H. influenzae* - OMP). Though preliminary data are available on the effect of vaccination on pneumonia, the major cause of

pneumococcal mortality in developing countries, this is an area that requires further study. Efficacy against pneumonia will be the most important factor in deciding on the use of this vaccine in developing countries. Although far advanced in their clinical development, one cannot foresee these conjugate vaccines before 2006-2008 for EPI programmes in developing countries. However, everything should be done to maintain this objective as a high priority⁵⁴.

New vaccine approaches are developed in order to provide protective immunity against a larger number of pneumococcal types than provided by the current conjugate vaccines. Several pneumococcal proteins, including pneumolysin, PspA, pneumococcal surface adhesin (PsaA), neuraminidase, and autolysin are in early clinical development ([Aventis Pasteur](#), [GSK](#)). In addition, [Shire Biologicals](#), Canada appears to be ready to start clinical testing of its candidate (BVH-3 and BVH-11) vaccines. Another approach is the use of inactivated whole bacteria administered intranasally.

Viral Respiratory Infections

Viruses are a common cause of acute lower respiratory infection in children worldwide. The commonest viral pathogens causing LRI are respiratory syncytial virus (RSV) and the parainfluenza viruses (PIV). In developing countries, available data suggest that dual infection with these viruses and bacterial pathogens are more common as compared to the industrialized countries. In Pakistan, 26% of children infected with RSV also had *S. pneumoniae* or *H. influenzae* bacteremia⁵⁵. In a study in Papua New Guinea, bacteria were isolated from blood culture or lung aspirate in two-thirds of children with viral ARI.⁵⁶ Though the exact relationship between viral and bacterial infection in these cases has not been established, dual infection does seem to increase the severity of the disease and result in higher mortality. Therefore, in these countries viral infections may contribute to the high morbidity and mortality from bacterial LRI.

Development of vaccines to prevent RSV and PIV infection have been complicated by two factors. Firstly, host immune responses appear to play a role in the pathogenesis of disease, especially in the case of RSV. Early studies with a formalin inactivated vaccine showed that vaccine recipients suffered from more severe disease on subsequent exposure to wild virus as compared to unvaccinated controls. Secondly, naturally acquired immunity is neither complete nor durable and recurrent infections occur frequently. Nevertheless, protection against severe disease develops after primary infection.

Respiratory syncytial virus

Disease burden. RSV is the single most important cause of severe lower respiratory tract infections in infants and young children. RSV disease spectrum includes a wide array of respiratory symptoms from rhinitis and otitis media to pneumonia and bronchiolitis; the latter two diseases are associated with substantial morbidity and some mortality. RSV infects nearly all children by 2 years of age. The global annual infection and mortality figures for RSV are estimated to be 64 million and 160 000 respectively.

RSV disease in industrialized countries (see ref. 58). RSV is now well documented as a cause of yearly winter epidemics of acute lower respiratory infection (LRI), including bronchiolitis and pneumonia. Estimates of impact in the USA are that 18 000 to 75 000 hospitalizations and 90 to 1 900 deaths occur annually. Data from Vanderbilt University, (Nashville, Tennessee) shows the rate of culture-proven, RSV-associated LRI in otherwise healthy children to be 37 per 1 000 child-years in the first two years of life and the risk of hospitalization 6 per 1000 child-years. These rates are higher in the first six months of life: 45 per 1 000 child-years for LRI and 11 per 1 000 child-years for hospitalization. Incidence will be higher for children with cardio-pulmonary disease and those with prematurity. Patients with these risk factors constitute almost half of RSV-related hospital admissions in the USA. In eight European countries, 19% of LRI occurring in hospitalized patients under five years of age was attributed to RSV. This represented approximately 80% of proven viral LRI. These studies serve as a basis for anticipating widespread use of RSV vaccines and other interventions in industrialized countries, where the costs of caring for patients with severe lower respiratory tract disease and their sequelae are substantial. There is increasing recognition of the importance of RSV as a cause of substantial morbidity from influenza-like illness in the elderly in industrialized countries.

RSV disease in developing countries (see ref. 58). In developing countries there are few population-based estimates of the incidence of RSV disease, though existing data clearly indicate that this virus accounts for a high proportion of LRI in children. A WHO collaborative study in 10 developing

countries in the early 1960s identified RSV in 19% of children under 6 years of age hospitalized with severe respiratory illness. This proportion was highest in under-6-month olds (37%) but remained at 12-16% of cases in one, two, three, and four-year-old children, falling to 8% in children over 5 years. Similarly, studies in Colombia, Brazil, and Bangkok show that RSV caused 20-30% of LRI cases in children from 1 to 4 years of age. In the Gambia, the peak age has varied in different areas and different years, depending on the density of the population and the frequency of outbreaks. Thus, the proportion of LRI caused by RSV appear to be similar in both developed and developing countries. However, the overall incidence of LRI in children is higher in developing countries. Therefore, one may expect that the incidence of RSV-associated LRI would also be higher in developing countries than in industrialized countries. For example, by applying this proportion of RSV-associated LRI to the incidence of all-cause LRI, the estimated incidence of RSV-associated LRI is 97 to 180 episodes per 1 000 child-years in developing countries. Although these are crude estimates, this developing country rate is 2.6 to 4.8 times the rate of RSV-associated LRI seen in the USA.

In addition to the lack of accurate incidence rates, other important data that are lacking in developing countries are the severity and case-fatality rates for RSV infection at the community level and the median age of first infection. Preliminary data from WHO sponsored community-based studies suggest that the median age of first infection may vary between communities. This information is important for vaccination programme planners, when considering the optimal schedule for vaccination. Maternal immunization against RSV may protect the infant in the first months of life, and would be a desirable strategy to evaluate and adopt if rates of infection during the first two months of life is high.

Another confusing aspect of the epidemiology of RSV infection that may impact vaccine use is the seasonality of the disease. In Europe and North America, RSV disease occurs as well-defined seasonal outbreaks during the winter and spring months. Studies in developing countries with temperate climates, such as Argentina and Pakistan, have shown a similar seasonal pattern. On the other hand, studies in some tropical countries have reported an increase in RSV in the rainy season but this has not been a constant finding. Interestingly, marked differences in the seasonal occurrence of RSV disease have been reported from geographically contiguous regions, e.g. Mozambique and South Africa, and Bangladesh and India. The factors that cause seasonal outbreaks of infection are not completely understood and it is possible that cultural and behavioural patterns in the community may affect the acquisition and spread of RSV infection. Therefore, a clear understanding of the local epidemiology of the disease is critical to the implementation of a successful vaccine development and introduction programme.

Vaccines. RSV belongs to the *Paramyxoviridae* family, genus *Pneumovirus*. The two groups of RSV strains have been described, the A and B groups, based primarily on differences in the antigenicity of the surface glycoprotein G. There is some evidence of cross-reactive immunity between the two. The analogy has been made that the strains belonging to the two RSV groups are as closely related as sequential influenza strains of the same serotype. All current vaccine efforts are directed towards the development of a vaccine that will incorporate strains in both groups, or will be directed against the F protein, which is relatively conserved between the two groups.

Initiatives are now under way for the prevention of RSV disease in industrialized countries. Passive immunization in the form of RSV immune globulin or monoclonal antibodies given prophylactically has been shown to be helpful in prevention of lower respiratory tract disease in those with underlying cardiopulmonary disease, particularly small, premature infants. This demonstrates that humoral antibody does play a role in protection against disease.

The development of an RSV vaccine is difficult but should remain a high priority. The major obstacle to developing a vaccine against RSV is drawn from the experience of earlier clinical trials with formalin-inactivated whole RSV. Therefore, for safety reasons, live attenuated vaccines seem preferable for immunization of naive infants than inactivated or subunit vaccines. The prospect of RSV vaccines is however encouraging. Purified F Protein vaccines (PFP-1 and PFP-2) have been shown to be safe and immunogenic in 12-48 month old RSV-seropositive children. Maternal immunization using a PFP subunit vaccine is a strategy being evaluated to protect infants younger than 6 months of age (who respond poorly to vaccines) from RSV disease. A PFP-2 vaccine was tested in postpartum women and women of childbearing age and shown to be safe and highly immunogenic. A Phase I trial in pregnant women during the third trimester has been carried out recently. [Aventis Pasteur's](#) RSV vaccine is a sub-unit vaccine in Phase II of its clinical programme. Trials to date have been conducted

in Canada and Australia with an excellent safety and immunogenicity profile to date. Launch of the product is anticipated in the 5-10 year time frame. A subunit approach was also investigated using the G protein fragment of RSV-A long strain. A recombinant vaccine candidate, BBG2Na ([Pierre Fabre](#)), developed by fusing the conserved central domain of the G protein (G2Na) of RSV long strain to BB (the albumin-binding region of streptococcal G protein) elicited a protective immune response in animals, but the development of this vaccine was interrupted due to the appearance of unexpected side effects. Another candidate vaccine is a synthetic peptide of the conserved region of the G protein administered intranasally, either alone or in combination with cholera toxin. Protection was conferred to mice even without the cholera toxin. A live attenuated RSV vaccine that could be delivered to the respiratory mucosa is the basis of another approach, already in Phase I clinical trials. The vaccine is based on temperature-sensitive, cold-adapted strains of the virus. Although difficulties for such a vaccine arise from over- or under-attenuation of the virus and its genetic stability, this is probably the most promising approach developed by the pharmaceutical industry and [NIAID \(Jordan Report 2000\)](#).

Parainfluenza virus type 3

Disease burden. Parainfluenza virus type 3 (PIV-3) infections are second only to RSV infections as a viral cause of serious ARI in children. Like RSV, Parainfluenza viruses belong to the *Paramyxoviridae* family. Along with RSV, parainfluenza viruses are also leading causes of hospitalization in adults with community-acquired respiratory disease. The seasonal patterns of parainfluenza virus types 1, 2, and 3 are curiously interactive. Parainfluenza virus type 1 causes the largest, most defined outbreaks, marked by sharp biennial rises in cases of croup in the autumn of odd-numbered years. Outbreaks of infection with parainfluenza virus type 2, though more erratic, usually follow type 1 outbreaks. Outbreaks of PIV-3 infections occur yearly, mainly in spring and summer, and last longer than outbreaks of types 1 and 2. Parainfluenza virus type 4 is infrequently isolated and is therefore relatively unknown and uncharacterized. Associated illness usually is mild, but lower respiratory tract disease has been reported⁵⁷. Though three types of parainfluenza viruses (types 1-3) have been described in developing countries and are a cause of LRI, the disease burden has not been accurately quantified.

The parainfluenza viruses cause a spectrum of respiratory illnesses similar to those caused by RSV, but result in fewer hospitalizations. Most are upper respiratory tract infections, of which 30-50% are complicated by otitis media. About 15% of parainfluenza virus infections involve the lower respiratory tract, and 2.8 of every 1 000 children with such infections require hospitalization. Most children are infected by parainfluenza virus type 3 by the age of two years and by parainfluenza virus types 1 and 2 by the age of five years. Pneumonia and bronchiolitis from parainfluenza virus type 3 infection occur primarily in the first six months of life, as is the case for RSV infection, but with a lower frequency. Croup is the signature clinical manifestation of infection with parainfluenza virus, especially type 1, and is the chief cause of hospitalization from parainfluenza infections in children two to six years of age. However, this syndrome is relatively less frequent in developing countries. PIV-3 is unique among the PIV in its ability to infect young infants less than 6 months of age. In a study performed in Houston, Texas, 69% of children who acquired RSV infection during their first year, 83% were reinfected during their second year, and 46% were reinfected during their third year⁵⁸. At least two thirds of these children were infected by PIV-3 in each of their first two years of life⁵⁹. The proportions of hospitalizations associated with PIV infection for each disease varied widely in the hospital-based studies. Consequently, the annual estimated rates of hospitalization fall within a broad range: PIV-1, 0.32 to 1.59 per 1 000 children; PIV-2, 0.10 to 0.86 per 1 000 children; and PIV-3, 0.48 to 2.6 per 1 000 children. Based on these data PIV-1 may account for 5 800 to 28 900 annual hospitalizations; PIV-2 for 1 800 to 15 600 hospitalizations; and PIV-3 for 8 700 to 52 000 hospitalizations. More precise estimates of PIV-associated hospitalizations would require large prospective studies of PIV-associated diseases by more sensitive viral testing methods, such as polymerase chain reaction techniques⁶⁰. The therapeutic use of ribavirin has been limited because it is expensive and because a beneficial effect on clinical outcome remains unproved.

Vaccines. Integral to immunity and pathogenesis of PIV are the large envelope glycoproteins, which consist of a fusion protein (F) and a second glycoprotein, which in PIV is called hemagglutinin neuraminidase. Antigenic variations in parainfluenza viruses also occur, but they appear to be less important immunologically than the variations in RSV.

Attenuated parainfluenza virus vaccines have been developed from both human and bovine strains. Bovine PIV-3 is closely related antigenically to human PIV-3, protects against challenge with human

PIV-3, and replicates poorly in humans⁶¹. One bovine type 3 vaccine was immunogenic in seronegative but not in seropositive children⁶². However, a human cold-adapted type 3 vaccine appears promising in both seropositive and seronegative children as young as six months. [NIAID](#) has produced an attenuated chimeric PIV-1 that contains type 3 internal proteins with the type 1 surface glycoproteins F and hemagglutinin neuraminidase⁶³. In addition, [Berna Biotech](#) is developing a visoromal formulation of PI-3 vaccine.

Influenza

Disease burden. At unpredictable intervals, and in addition to seasonal mild influenza epidemics caused by antigenic drift or reassortment, antigenic shifts with completely new influenza virus subtypes emerge against which immunity in the human population does not exist. They cause global pandemics that spread rapidly around the world. Three of these pandemics occurred in the last century (1918, 1957, 1968)^{64 65}. The most severe, in 1918, infected approximately 50% of the world's population, of which about 25% suffered clinical disease; the total mortality was estimated between 20-40 million, particularly affecting people in the prime of their lives. This pandemic depressed population growth for the following ten years. The last outbreak with high mortality and pandemic potential occurred in 1997, when a new influenza virus (H5N1) emerged in Hong Kong, killing a third of the affected patients, mainly young adults ([WHO, 2003](#)). Fortunately, the virus was not able to spread from person to person and it was possible to quickly stop the outbreak. A similar virus was isolated in 2003 in Hong Kong ([WHO, 2002](#)). In the USA, the impact of the next pandemic is projected to be 18-42 million outpatient visits, 314-734 000 hospitalizations and 89-207 000 deaths, assuming that the next pandemic will be of a similar magnitude as the impact as the 1957 or the 1968 pandemic, and not like the 1918 pandemic⁶⁶. Extrapolating this projected impact proportionally to the global population, the gross estimate of the global impact of the next pandemic can be estimated at 1-2 billion cases of the flu, 5.3-12.3 million cases of severe illness, and 1.5-3.5 million deaths.

Besides a potential pandemic, annual influenza epidemics caused by drifted variants of influenza A and B viruses infect about 10-20% of the population each season, and cause febrile illness, hospitalizations and deaths. Indirect statistical methods have been used to estimate the total burden of influenza; these include various statistical models that quantify the seasonal increase in morbidity and mortality during influenza epidemic periods⁶⁷. Using this methodology, an average influenza season in the USA is currently associated 25-50 million cases of flu, 150 000 hospitalizations and 20-40 000 deaths. Assuming that the age-specific risk of influenza mortality is similar to that in the USA, the annual average global burden of inter-pandemic influenza may be on the order of ~1 billion cases of flu, ~3-5 million cases of severe illness and 250-500 000 deaths.

Vaccines. Influenza viruses belong to the *Orthomyxoviridae* family. The currently available influenza vaccines are effective in preventing influenza-related illness and highly effective in terms of preventing hospitalizations and deaths⁶⁸. In all USA-based studies^{69 70}, vaccination of the elderly and those considered at "high risk" for severe outcomes of influenza provided substantial health benefits and cost savings. In spite of these results, even in developed countries that have had influenza vaccination programmes in place for some time, the elderly population has reached high coverage in very few countries while there continue to be even greater difficulties reaching the younger "high risk" populations where vaccine coverage are surprisingly low. In developing countries none of the "high risk" populations have been reached as of yet, due in part to the relatively high price of the vaccine, and the need for annual re-vaccination. In addition, whilst outbreak reports confirm the impact of influenza in developing countries (see Madagascar 2002), medical and economical burden have remained un-assessed in those countries, hampering evidence based prioritization of national communicable disease control action.

Very recently, the use of influenza vaccine has begun to increase worldwide. During the period of 1993-2000, the global use increased from 135 to around 235 million doses, but there is still a sizeable gap in pandemic vaccine demand as the current vaccine production. Indeed, WHO estimates that there are about 1.2 billion people at "high risk" for severe influenza outcomes: 385 million elderly over 65 years of age, 140 million infants, 700 million children and adults over 65 years of age with underlying chronic health problems. In addition, 24 million health care workers should also be immunized to prevent influenza to spread in "high risk" populations. Currently the worlds total vaccine production is limited to about 235 million doses produced by several manufacturers in industrialized countries. Realistically, this current production capacity of influenza vaccine does not even suffice to cover parts

of the global “high risk” population. In reality, it is questionable whether the global infrastructure would be able to handle timely distribution and delivery of pandemic influenza vaccine. In addition, current vaccine technology produces vaccines with a narrow spectrum of protection, and it is therefore most unlikely that vaccines available in stockpiles would protect against a completely new *Influenza* virus pandemic strain. The production of a vaccine tailored to this pandemic strain would take eight months. Therefore, there is a need to improve on the current vaccine production technologies (egg-derived vaccines) and to produce vaccines able to protect against the whole possible spectrum of *Influenza* viruses. Several companies ([Baxter Vaccines](#), [Chiron Vaccines](#), [Aventis Pasteur](#), [Shire](#)) have embarked on projects for the development of cell-culture vaccines; a production technology that would help overcome current vaccine production bottlenecks and time constraints. Furthermore, it would improve possibilities of up-scaling of vaccine production capacities in face of a pandemic (whilst the existing down-stream problems would remain). None of those vaccines is likely to enter the market before 2006. [Solvay Pharmaceuticals](#) is the only company which has already licensed a cell-line (MDCK) for cell-culture vaccine production.

As the above vaccines are based on inactivated Influenza virus preparations, these will not be mentioned further in the analysis below (nor in Annex C), which will only concentrate on new approaches to Influenza vaccines. In collaboration with [MedImmune](#), [Wyeth](#) is currently in Phase III trials with a live cold-adapted influenza vaccine. [Biodiem Limited](#) (Australia) and [Merck](#) will be developing a novel, live attenuated influenza vaccine, delivered by nasal spray. [Berna Biotech](#) is commercializing an influenza vaccine formulated in virosomes, with surface spikes of the three currently circulating influenza strains inserted in the vesicle membrane of three corresponding virosome types. A nasal formulation of this vaccine was recently withdrawn from the market. Yeda R&D Company (Israel) is proposing a synthetic peptide influenza vaccine for nasal administration, [PowderJect](#) (USA) is working on new delivery of influenza vaccine in order to elicit a higher antibody response compared to needle and syringe delivery. Lastly, many groups are concentrating on the evaluation of the potential of the DNA vaccines for influenza (e.g. [CDC](#), [Vical/Merck](#), [Powderject](#)).

Tuberculosis

Disease burden. An estimated third of humanity (approximately 2 billion people) is infected with tuberculosis (TB). Amongst those carrying the pathogen, around 8 million come down with clinical disease every year and out of these, roughly about 1.64 million die, not counting tuberculosis-related deaths in TB-HIV co-infected individuals ([WHO](#), 2002). Over 1.5 million TB cases per year occur in sub-Saharan Africa and nearly 3 million in Southeast Asia. Over a quarter of a million TB cases per year occur in Eastern Europe where TB deaths are increasing after almost 40 years of steady decline⁷¹.

The global epidemic is growing and becoming more dangerous. The breakdown in health services, the spread of HIV/AIDS and the emergence of multidrug-resistant TB are contributing to the worsening impact of this disease. In 1993, WHO took an unprecedented step and declared tuberculosis a global emergency, so great was the concern about the modern TB epidemic. It is estimated that between 2000 and 2020, nearly one billion people will be newly infected, 200 million people will get sick, and 35 million will die from TB - if control is not further strengthened⁷². Left untreated, each person with active TB will infect on average between 10 and 15 people every year. But people infected with TB will not necessarily get sick with the disease. *Mycobacterium tuberculosis*-infected, immunocompetent individuals have an estimated 10% lifetime risk of contracting active TB disease.

Several factors contribute to the rise in TB. HIV is accelerating the spread of TB. HIV and TB form a lethal combination, each speeding the other's progress. HIV weakens the immune system. Someone who is HIV-positive and infected with TB is many times more likely to become sick with TB than someone infected with TB who is HIV-negative. TB is a leading cause of death among people who are HIV-positive, accounting for about 15% of AIDS deaths worldwide. In Africa, HIV is the single most important factor determining the increased incidence of TB in the last ten years (70% of new active TB cases are HIV-infected). In 1996, TB became the leading cause of death in HIV-infected individuals worldwide, responsible for approximately one-third of all AIDS deaths⁷³.

Poorly managed TB programmes are threatening to make TB incurable. Drug-resistant TB is caused by inconsistent or partial treatment, when patients do not take all their drugs regularly for the required period because they start to feel better, doctors and health workers prescribe the wrong treatment

regimens or the drug supply is unreliable. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined as the disease due to TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. MDR-TB is rising at alarming rates in some countries, especially in the Newly Independent States of the former Soviet Union, and threatens global TB control efforts⁷⁴. From a public health perspective, poorly supervised or incomplete treatment of TB is worse than no treatment at all. While drug-resistant TB is treatable, it requires extensive chemotherapy (up to two years of treatment) that is often prohibitively expensive (often more than 100 times more expensive than treatment of drug-susceptible TB), and is also more toxic to patients. Movement of people is helping the spread of TB. Global trade and the number of people traveling in airplanes have increased dramatically over the last forty years. In many industrialized countries, at least one-half of TB cases are among foreign-borne people. In the US, nearly 40% of TB cases are among foreign-born people. The number of refugees and displaced people in the world is also increasing. Untreated TB spreads quickly in crowded refugee camps and shelters. As many as 50% of the world's refugees may be infected with TB.

Vaccines. Although substantial and successful efforts have been carried out worldwide to stop TB, especially through the WHO-recommended treatment DOTS^a strategy for detection and cure of TB, for all the reasons mentioned above plus the relative ineffectiveness of the current BCG vaccines, the development of improved TB vaccines has become a necessity for adequate control and elimination of tuberculosis.

Against miliary TB and TB meningitis in children, BCG's reported efficacy ranges from 46-100%⁷⁵. However, against pulmonary TB, efficacy ranges from 0-80%. Efficacy of BCG vaccination appears to vary with geographic latitude – the farther from the equator, the more efficacious the vaccine (presumably, exposure to nonpathogenic mycobacteria induces a degree of protective immunity in exposed populations, masking any potential protection from BCG)⁷⁶.

Despite some hypothesis-driven approaches towards TB vaccine development, such as the focus on secreted proteins as early targets of the anti-mycobacterial immune response, the search for new TB vaccine candidates has so far largely remained empirical. Consequently, the number of candidates that have been seriously considered for development by now virtually goes into the hundreds, including nearly all approaches in the vaccine developers' armamentarium: BCGs (auxotrophic mutants, added cytokine expression), live-attenuated strains of *M. tuberculosis*, subunit vaccines (culture filtrate proteins combined with new generation adjuvant and Th1 cytokines IL-2 or IL-12, non proteinous antigens such as mycolic acids and carbohydrate moieties), DNA vaccines (major secreted protein, Ag85A, HSP65, 36kD proline-rich mycobacterial antigen, whole genome or expression library immunization approaches), nonpathogenic mycobacteria such as *M. vaccae* or *M. microti*, expression vector systems (Salmonella or Vaccinia virus, expressing Ag85, 19kD or 45kD proteins)⁷⁷. The first of the genuinely new candidates, a recombinant poxvirus construct carrying the secretory antigen 85A has completed phase I safety evaluation in humans in the United Kingdom without major adverse events and is now being evaluated in an endemic country, i.e. The Gambia. At least four other candidates are expected to follow into human clinical trials until early 2004, including two recombinant protein subunit vaccines (fusions of MTB32 plus Mtb39 and ESAT-6 plus Ag85B), a recombinant BCG overexpressing Ag85B and a combination of several peptide epitopes⁷⁸. It is of note, that evaluation of the new subunit candidates is planned, at least initially, in a prime-boost paradigm with BCG as the priming agent. In the absence of a valid surrogate of vaccine-induced protection and in order to avoid long duration and/or enormous cohort sizes, the first phase III efficacy trials of new TB vaccines are likely to be performed in high risk populations such as household contacts of TB patients and health care workers⁷⁹. TB vaccine developers will have to face important ethical issues including withholding BCG vaccine during clinical trials (except for recombinant BCG) and the testing of novel vaccines safely in populations where HIV infection is prevalent. Main players in this area include the Aeras Global TB Vaccine Foundation (supported by the [Bill and Melinda Gates Foundation](#)), a network of European researchers supported by the European Commission, [GlaxoSmithKline Inc.](#) and [IDRI](#)-Corixa.

^a DOTS combines five elements: political commitment, microscopy services, drug supplies, surveillance and monitoring systems and use of highly efficacious regimes with direct observation of treatment. Once patients with infectious TB (bacilli visible in a sputum smear) have been identified using microscopy services, health and community workers and trained volunteers observe and record patients swallowing the full course of the correct dosage of anti-TB medicines (treatment lasts six to eight months).

Buruli Ulcer

Disease burden. Buruli ulcer (BU) is an emerging necrotic skin disease caused by *Mycobacterium ulcerans*. *Mycobacterium ulcerans* is the third most important mycobacterial pathogen of man after *Mycobacterium tuberculosis* and *Mycobacterium leprae*. It is an environmental pathogen, which is transmitted to humans by an unknown mechanism, although some data suggests that insects may play a role. BU has been reported in many tropical and some temperate countries, and is endemic in West Africa at present. There have also been reports in the Americas, Asia, Australia, and Papua New Guinea⁸⁰. The global burden of BU is unknown. Several reports confirm the rise BU in West Africa^{81 82 83 84}, but accurate estimates of incidence by region and country are generally not available. A recent report from Ghana has estimated a national prevalence of 20.7/100 000 in 1999⁸⁵, and in some West African communities, BU has replaced tuberculosis and leprosy as the most prevalent mycobacterial disease, affecting up to 22% of the population. It is generally reported that Buruli ulcer is most common in children (median age: 12 years⁸⁶).

The characteristic pathological lesion—masses of organisms, necrosis of fat, absence of inflammatory cells and edema that extends beyond visible organisms is probably explained by the production of a diffusible toxin (mycolactone⁸⁷), which has both necrotising and immunosuppressive properties. Other toxins and virulence determinants such as phospholipase C may also play a role⁸⁸. BU is associated with severe illness and permanent disabilities in >26% of patients⁸⁹. Currently, surgery involving excision and grafting is recognized to be the treatment of choice for lesion of *M. ulcerans* infection. Unfortunately, many patients do not present until there is extensive and disfiguring ulceration. Depending on extent of lesions, extensive postoperative physiotherapy may be required to achieve a good functional result.

The rising incidence, predilection of the disease for poor rural communities, the cost of complex surgical treatment, lost productivity during illness, and reduced fitness after recovery combine to make BU a major economic burden in West Africa⁹⁰.

Vaccines. Two large randomized controlled trials of BCG vaccination for the prevention of Buruli ulcer were conducted in Uganda during the late 1960s. These field trials have suggested that BCG vaccination may offer a degree of protection against Buruli ulcer^{91 92}. The overall efficacy of BCG was 47%, but the effect was short-lived, ranging from 6 months in the one study to one year in the other. Indeed, *M. tuberculosis* and *M. ulcerans* are closely related and share at least some antigenic determinants⁹³, and BCG has been shown to be effective in a mouse model of *M. ulcerans* using small inoculae, but this protection can be overcome with larger inoculae⁹⁴.

Taken together, the results obtained in the above trials strongly suggested that a new vaccine is needed for the prevention of Buruli ulcer. To our knowledge, no private investment is devoted to the research of Buruli ulcer vaccines at present, and most Buruli ulcer research activities are coordinated by the [Global Buruli Ulcer Initiative](#).

Chlamydia Trachomatis

Disease burden: *Chlamydia trachomatis* is a small bacterium that cannot grow outside a living cell. *Chlamydia trachomatis* is primarily a human pathogen and the causative agent of eye, genital and respiratory diseases. The whole genome of *C. trachomatis* strain D/UW-3/CX (serovar D) was sequenced in collaboration between the University of California, Berkeley ([Chlamydia Genome Project](#)) and the Stanford DNA Sequencing and Technology Development Center ([Stanford Chlamydia Group](#)). *Chlamydia trachomatis* is the most prevalent bacterial pathogen causing sexually transmitted disease (STD) in the western world. *Chlamydia trachomatis* is the most frequent cause of Pelvic Inflammatory Disease (PID) and its long term consequences include chronic pain, ectopic pregnancy and infertility. In both sexes, conjunctivitis (that does *not* progress to blindness) and joint inflammation may occur. In men, *Chlamydia trachomatis* is the commonest cause of non-gonococcal or (less correctly) non-specific urethritis. In both men and women, asymptomatic infection is not uncommon. The organism is transmitted from one partner to another during sexual intercourse. Contamination of

the hands with genital discharge may lead to a conjunctival infection following contact with the eyes. Babies born to mothers with infection of their genital tract frequently present with chlamydial eye infection within a week of birth (chlamydial ophthalmia neonatorum), and may subsequently develop pneumonia. Worldwide, the most important disease caused by *Chlamydia trachomatis* is trachoma, one of the commonest infectious causes of blindness. In some parts of the developing world, over 90% of the population becomes infected. Chlamydia Trachomatis is the second cause of blindness in the world, after cataract, and thus the first cause of preventable blindness despite long-standing control efforts. It is estimated that more than 500 million⁹⁵ people are at risk of contracting the infection, while over 140 million are already infected and about 6 million subjects are already blind⁹⁶ or severely visually impaired as a consequence of the infection. Trachoma thus constitutes a serious public health problem, which affects the poorest of the poor⁹⁷. The disease is prevalent in Africa, Middle East, Central Asia, South East Asia, some countries in Central and South America, India and China (some regions). A review of available data has identified 46 countries as having known areas of blinding trachoma. This disease is particularly prevalent and severe in rural populations living in poor and arid areas of the world where personal hygiene is difficult. Visual loss from trachoma often starts in middle life and is more common in women. It is therefore a major cause of disability in affected communities, attacking the economically important middle-aged female population. Trachoma is a communicable disease of families, with repeated reinfection among family members. The disease starts as an inflammation, and evolves to trachomatous trichiasis (at least one eyelash rubbing on the eyeball, or evidence of recent removal of interned eyelashes) and blindness due to trachomatous corneal opacity.

Clamydiae are sensitive to a number of antibiotics including sulfonamides, erythromycin and tetracyclins. Chemotherapeutic intervention thus consists of topical (tetracyclin) or systemic (azithromycin) treatment with antibiotics. Other interventions consist of surgery of the eyelid⁹⁸. Global elimination of trachoma as a disease of public health importance has been targeted by WHO for 2020.

Vaccines: Antex Biologics has developed a subunit vaccine candidate for *Chlamydia trachomatis*. Phase I studies in the US will begin in the fourth quarter of 2002. This will be the first time that a *Chlamydia trachomatis* vaccine has been in the clinic in 30 years. On 3. February 2003 [Antex Biologics](#) announced that it has filed an initial Investigational New Drug Application for its TRACVAX™ vaccine with the FDA. TRACVAX is a recombinant subunit vaccine designed to prevent and treat infections caused by *Chlamydia trachomatis*. This Phase I clinical trial will be an open label, randomized trial designed to assess the general safety and immunogenicity of the vaccine, along with efficient dosing regimens.

HIV/AIDS

Disease burden. WHO and UNAIDS have estimated that at the end of 2001 the number of adults and children living with HIV/AIDS worldwide will have reached 40 million⁹⁹. It is also estimated that, during 2001, 5 million people (including 800 000 children aged under 15 years) became infected and 2.9 million have died of the disease ([WHO, 2002](#)). Human Immunodeficiency viruses belong to the *Retroviridae* family, lentivirus genus. Two types have been described: HIV-1 and HIV-2. HIV infections are now almost equally distributed between men and women, with an estimated 17.6 million women aged 15-49 living with HIV/AIDS. The vast majority of people living with HIV/AIDS are not aware that they are carrying the virus. The HIV/AIDS epidemic continues to claim a large number of lives, with an estimated 3 million deaths during 2001. Deaths in women also continue to increase, accounting for an estimated 46% of adult deaths due to HIV in 2001. HIV/AIDS is the leading cause of death in sub-Saharan Africa and the fourth biggest killer worldwide. As of 25 November 2001, a total of 2 784 317 AIDS cases had been estimated. This is an increase of 471 457 cases.

Some regional trends can be observed. During 2001, in sub-Saharan Africa AIDS killed 2.3 million people while an estimated 3.4 million people have been newly infected with HIV, bringing the number of Africans living with HIV/AIDS to 28.1 million. Sub-Saharan Africa remains the hardest-hit region, accounting for 68% of the 5 million people infected worldwide with HIV during 2001, 71% of the people living with HIV/AIDS and 77% of AIDS deaths¹⁰⁰. An estimated 7.1 million adults and children are living today with HIV in Asia and the Pacific. In 2001, AIDS has claimed the lives of 435,000 people. Most infections continue to be concentrated in a few large countries and selected population groups. While prevalence in the adult population continues to be relatively low in most Asian countries, increasing sex trade, use of illicit drugs, rates of sexually transmitted infections and large population movements contribute to an increased vulnerability in this region. The estimated number of

adults and children living with HIV in Latin America and the Caribbean at the end of 2001 is 1.8 million. While in some countries HIV infections remain concentrated mainly in men who have unprotected sex with other men and injecting drug users, others are experiencing increasing rates of heterosexual transmission. AIDS mortality has been reduced in some countries thanks to antiretroviral therapy.

Eastern Europe and Central Asia continue to experience some of the sharpest increases in HIV infections. During 2001, there were an estimated 250 000 new infections, bringing the number of people living with HIV/AIDS to 1 million. Most of the infections continue to occur among injecting drug users. In North Africa and the Middle East, the number of people living with HIV/AIDS now totals 440 000. While HIV prevalence continues to be low in most countries in the region, an increasing number of HIV infections have been detected in several countries, particularly in countries experiencing complex emergencies. During 2001, highly active antiretroviral therapy has continued to reduce progression to AIDS, deaths and HIV transmission from mother to child in the industrialized countries of North America, Western Europe and the Pacific. However, these successes in treatment and care are not being matched by progress in prevention. During 2001, 75 000 individuals became infected with HIV in industrialized countries, where an estimated 1.5 million people are living with HIV. New evidence of rising HIV infection rates is emerging, particularly in marginalized communities.

Vaccines. While HIV/AIDS continues to spread in all regions of the world, there are positive signs. In both industrialized and developing countries, an increasing number of HIV-positive people can live longer and healthier lives thanks to antiretroviral therapies. Large-scale prevention programmes have reversed epidemic trends in some Asian countries. The estimated number of new infections in most Central/East/West African countries seem to decline, and there are some initial indications that the epidemics might have peaked in southern Africa. But above all, a new determination to fight the epidemic has emerged following the United Nations General Assembly Special Session on HIV/AIDS in July 2001. However, despite these encouraging trends, a preventive vaccine is more than ever needed, in particular for developing countries. The development of a safe and effective vaccine is hampered by the high genetic variability of HIV¹⁰¹, the paucity of knowledge on the immune mechanisms of protection, the absence of relevant and predictive animal models, and the complexity of the implementation of efficacy trials, especially in developing countries^{102 103}. Several vaccine candidates have been tested over the past 15 years and are in the pipeline for further human testing^{104 105}. The first Phase I trial of an HIV vaccine was conducted in the US in 1987. Since then, over 30 candidate vaccines have been tested in over 80 Phase I/II clinical trials, involving over 10 000 healthy human volunteers (adults and infants)¹⁰⁶. The majority of these trials have been conducted in the US and Europe, however, trials have also been conducted in developing countries (Brazil, China, Cuba, Haiti, Kenya, Thailand, Trinidad and Uganda). The effort to develop and evaluate HIV vaccines must increase, especially in Africa¹⁰⁷. This effort will be strengthened by the [African Aids Vaccine Programme \(AAVP\)](#), which has been recently established following an initiative in WHO and UNAIDS. Only two efficacy trials have been started so far, both using the same approach of a monomeric gp120, one in the United-States (with sites in Canada and in the Netherlands), the other in Thailand ([Vaxgen/CDC](#)). Definite results from the USA trial have been reported in March 2003. The study did not show a statistically significant reduction of HIV infection within the study population as a whole, which was the primary endpoint of the trial. However, the study did show a statistically significant reduction of HIV infection in certain vaccinated groups. Protection appeared to correlate with the higher level of vaccine-induced neutralizing antibodies observed in these groups, according to [Vaxgen](#). A third efficacy trial of a recombinant canarypox HIV vector prime-gp120 boost vaccine in heterosexual volunteers in Thailand is expected to start late in 2002 or early in 2003 ([WRAIR/NIH/Vaxgen/Aventis Pasteur](#)). Other interesting approaches based on DNA prime and recombinant poxviruses (MVA, fowlpox) boost are being tested in humans. Recombinant adenoviruses represent another promising approach, already tested in Phase I in humans. Other candidate vaccines include recombinant Salmonella ([IAVI/Institute for Human Virology, University of Maryland](#), USA), VEE ([Alphavax](#), USA), subunit HIV proteins ([GSK](#), Center for Genetic Engineering and Biotechnology ([CIGB](#)) in Cuba, among others), DNA vaccines (Wyeth/University of Pennsylvania, among others) or peptides (Wyeth/Duke University). The development of a safe, effective, and affordable HIV vaccine remains the scientific and public health challenge of this new century. However, it is urgent that the availability and affordability of a safe and efficient HIV vaccine should by any means strongly encourage, strengthen, and expand the efforts of traditional prevention against HIV infection proven to be efficient in some countries.

Herpes simplex virus type 2

Disease burden. HSV-2 prevalence is increasing worldwide¹⁰⁸. There is now ample evidence that *Herpes simplex virus type 2* (HSV-2) infection, the most common cause of genital ulcers worldwide, is a major cofactor favoring HIV infection. HSV-2 prevalence is generally higher in developing than in developed countries and in urban than rural areas. The HSV-2 prevalence in developing countries, although very high, varies widely according to the countries, the gender, urban versus rural areas, ranging from 2-74%, very few being available from Asian and South American countries¹⁰⁹. HSV-2 incidence data are scarce. In the community-based survey, HSV-2 prevalence increased with age until 25 years, leveled off at 50% in both genders. The same independent predictors of HSV-2 infection were identified in both genders: older age, higher lifetime number of sexual partners, positive HIV serology, and positive *Treponema pallidum* hemagglutination serology¹¹⁰. In Bangladesh a study conducted in truck drivers showed a high HSV-2 prevalence of disease were HSV-2 (25.8%), compared to serological syphilis (5.7%), gonorrhoea (2.1%), chlamydia (0.8%). In New Zealand, increased rates of HSV-2 acquisition after age 21 may be due to a higher prevalence of infection in the pool of potential partners encountered during the third decade of life. Factors related to partner choice may have more influence on the risk of HSV-2 infection than the number of sexual partners alone¹¹¹. Overall prevalence is higher in women compared with men, especially among the young^{112 113}, and rates up to 40 % have been reported among women aged 15-19 in Kisumu (Kenya)¹¹⁴. Prevalence is higher in the USA (22% in adults)¹¹⁵ compared to Europe (generally lower than 15%). It is now estimated that in the USA alone, 40 to 60 million people are HSV-2-infected, with 2 million incident cases per year and 600 000 clinical cases. The clinical spectrum of HSV-2 includes primary infection, a first episode of genital herpes and recurrent episodes of clinical disease (4-5 per year). In addition, subclinical infection may be associated with infectious viral shedding. The proportion of infections which are symptomatic is estimated to be between 13 and 37%, although this is higher in HIV positive individuals¹¹⁶. The risk of neonatal herpes is very low (less than 3%) among HIV-negative pregnant women living in developed countries, but few data are available on neonatal herpes in developing countries. A recent meta-analysis of thirty-one studies addressing the risk of HIV infection in HSV-2-seropositive persons was performed. For nine cohort and nested case-control studies that documented HSV-2 infection before HIV acquisition, the risk estimate was 2.1 (95% confidence interval, 1.4-3.2). Thus, the attributable risk percentage of HIV to HSV-2 was 52%, and the population attributable risk percentage was 19% in populations with 22% HSV-2 prevalence but increased to 47% in populations with 80% HSV-2 prevalence. For 22 case-control and cross-sectional studies, the risk estimate was 3.9 (95% confidence interval, 3.1-5.1), but the temporal sequence of the two infections cannot be documented¹¹⁷. Control strategies for HSV-2 need to be incorporated into control of sexually transmitted infections as a strategy for HIV prevention.

In developed countries, acquisition of HSV-1 in childhood has decreased as HSV-2 seroprevalence has increased, suggesting a possible protective effect of HSV-1 against HSV-2 acquisition¹¹⁸. However, studies have shown discrepant results in this respect. Although HSV-1 does not seem to modify the risk of HSV-2 acquisition^{119 120}, it seems to increase the proportion of asymptomatic seroconversions¹²¹ and, in one study, to increase the rate of HSV-2 shedding¹²². Infection with HSV-1 in childhood is almost universal in developing countries, where HSV-2 prevalence is very high, and this confirms that HSV-1 provides limited protection against HSV-2 infection.

Vaccines. Herpesvirus 2 belongs to the enveloped *Herpesviridae* family. The first generation of vaccines was recombinant subunit viral glycoproteins. [Chiron](#) developed a two-component (30 µg of both gB2 and gD2 glycoproteins) subunit vaccine formulated in MF59 adjuvant and SmithKline Beecham a monovalent vaccine (gD2) formulated in alum + monophosphoryl lipid A (Corixa). The [Chiron vaccine](#) induced very high antibody titres, and efficacy in women for the first 5 months was 26%, but this protection against infection was not sustained. Chiron doesn't have an active HSV-2 vaccine at present. The Phase III trials of the vaccine developed by [GlaxoSmithKline](#) (glycoprotein gD2 formulated with adjuvant) showed limited efficacy, depending on gender and previous exposure to HSV-1. Indeed, these trials showed a 73% and 74% efficacy (P=0.01 and 0.02, respectively) against genital herpes disease in HSV-1- and HSV-2-negative women. Trends towards protection in women against HSV infection were also seen in both studies (39-48% efficacy), although not statistically significant. The main disadvantages of this vaccine are the apparent failure to improve on protection provided by HSV-1 infection and the need for frequent administration to boost host immunity. Further

efficacy trials of this vaccine, which has already been administered in about 7 500 individuals, are pending in collaboration with [NIAID](#). A novel HSV-2 candidate vaccine has been developed by Cantab Pharmaceuticals (now [Xenova](#))/[GlaxoSmithKline](#) based on a genetically Disabled Infectious Single Cycle (DISC, glycoprotein H-deleted, ICP8 gene mutation) replicative vaccine, which is believed could have higher efficacy than previous vaccines. This new candidate vaccine has been tested in Phase II trials in the US and UK, showing good tolerance and inducing neutralizing antibodies and CTL in 83% of the vaccine recipients. Nevertheless, no difference in time to recurrence was observed in this therapeutic candidate vaccine in HSV-2 seropositive symptomatic, and no difference was recorded in shedding. Therefore, Xenova (without GSK) is refocusing their programme on prophylactic applications for their DISC vaccine. One complexity of evaluating protection against infection induced by the DISC vaccine is that, because of the similarity of the disabled virus and wild HSV-2, it will not be possible to distinguish natural infection from vaccine-induced immunity. Another live, replication-impaired vaccine is currently under development by [Avant Immunotherapeutics](#). [AuRx, Inc](#) concentrate on live genetically-attenuated replication-competent vaccines and [PowderJect](#) and [Merck](#) on DNA vaccine formulations.

A vaccine which protects only women would be expected to reduce HSV infection and disease in vaccinated women, decrease the rate of neonatal HSV infection, have an impact on the epidemic spread of genital herpes in men and women, and finally possibly reduce acquisition and transmission of HIV infection. Failure to protect HSV-1 seropositive women may result if vaccination does not add to the natural protection provided by HSV-1. In this case administration of vaccine to young children, before HSV1 occurs, would not be particularly helpful. Lack of efficacy of vaccines in HSV1-infected individuals would render the vaccine useless in developing countries, where HSV-1 infection is almost universal.

Malaria

Disease burden. Malaria is by far the world's most important tropical parasitic disease, and kills more people than any other communicable disease except tuberculosis. Malaria is a public health problem today in more than 90 countries, inhabited by a total of some 2 400 million people - 40% of the world's population. Malaria is endemic in a total of 101 countries and territories: 45 countries in WHO's African Region, 21 in its Americas Region, 4 in its European region, 14 in its Eastern Mediterranean Region, 8 in its South-East Asia Region, and 9 in WHO's Western Pacific Region. Worldwide prevalence of the disease is in the order of 300-500 million clinical cases each year. More than 90% of all malaria cases are in sub-Saharan Africa.

Mortality due to malaria is estimated to be over 1.1 million deaths each year ([WHO](#), 2002). The vast majority of deaths occur among young children in Africa, especially in remote rural areas with poor access to health services. Malaria kills one child every 30 seconds. This preventable disease has reached epidemic proportions in many regions of the world, and continues to spread unchecked. In absolute numbers, malaria kills 3 000 children under five years of age per day. It is a death toll that far exceeds the mortality rate from AIDS. African children under five years of age are chronic victims of malaria, suffering an average of six bouts a year. Fatally afflicted children often die less than 72 hours after developing symptoms. In those children who survive, malaria also drains vital nutrients, impairing their physical and intellectual development. Malarial sickness is also one of the principal reasons for poor school attendance. Other high-risk groups are women during pregnancy, and non-immune travelers, refugees, displaced persons and laborers entering endemic areas. During pregnancy, malaria causes severe anemia, and is a major factor contributing to maternal deaths in malaria endemic regions. Pregnant mothers who have malaria and are HIV-positive are more likely to transmit HIV to their newborn. In many developing countries and in Africa especially, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost. The causative agents in humans are four species of *Plasmodium* protozoa (single-celled parasites) - *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae* transmitted by Anopheline mosquitoes, the number and type *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae* transmitted by Anopheline mosquitoes, the number and type of which determine the extent of transmission in a given area. Transmission of malaria is affected by climate and geography, and often coincides with the rainy season. Of these, *P.falciparum* accounts for the majority of infections and is the most lethal. Malaria is a curable disease if promptly diagnosed and adequately treated.

The geographical area affected by malaria has shrunk considerably over the past 50 years, but control is becoming more difficult and gains are being eroded. Malaria's reach is spreading. In malaria endemic parts of the world, a change in risk of malaria can be the unintended result of economic activity or

agricultural policy that changes the use of land (e.g. creation of dams, irrigation schemes, commercial tree cropping and deforestation). "Global warming" and other climatic events such as "El Niño" also play their role in increasing risk of disease. The disease has now spread to highland areas of Africa, for example, while El Niño events have an impact on malaria because the associated weather disturbances influence vector breeding sites, and hence transmission of the disease. Many areas have experienced dramatic increases in the incidence of malaria during extreme weather events correlated to El Niño. Moreover, outbreaks may not only be larger, but more severe, as populations affected may not have high levels of immunity. Quantitative leaps in malaria incidence coincident with ENSO (El Niño/Southern Oscillation) events have been recorded around the world: in Bolivia, Columbia, Ecuador, Peru and Venezuela in South America, in Rwanda in Africa, and in Pakistan and Sri Lanka in Asia.

More than any other disease, malaria hits the poor. Malaria endemic countries are some of the world's poorest. Costs to countries include costs for control and lost workdays - estimated to be 1-5% of GDP in Africa. For the individual, costs include the price of treatment and prevention, and lost income. Rural communities are particularly affected. In rural areas, the rainy season is often a time of intense agricultural activity, when poor families earn most of their annual income. Malaria can make these families even poorer. In children, malaria leads to chronic school absenteeism and there can be impairment of learning ability. Urban malaria is increasing due to unplanned development around large cities, particularly in Africa and South Asia.

The estimated costs of malaria, in terms of strains on the health systems and economic activity lost, are enormous. In affected countries, as many as 3 in 10 hospital beds are occupied by victims of malaria. In Africa, where malaria reaches a peak at harvest time and hits young adults especially hard, a single bout of the disease costs an estimated equivalent of 10 working days. Research indicates that affected families clear only 40% of land for crops compared with healthy families. Knowledge about malaria is markedly low among affected populations. In one recent survey in Ghana, for example, half the respondents did not know that mosquitoes transmit malaria. The direct and indirect costs of malaria in sub-Saharan Africa exceed US\$ 2 billion, according to 1997 estimates. According to [UNICEF](#), the average cost for each nation in Africa to implement malaria control programmes is estimated to be at least US\$ 300 000 a year. This amounts to about six US cents (US\$ 0.06) per person for a country of 5 million people.

A limited number of drugs for treatment of malaria are available today. Due to worsening problems of drug resistance in many parts of the world, adequate treatment of malaria is becoming increasingly difficult. Although some new drugs have appeared in the last 20 years (e.g., mefloquine, halofantrine, artemisinin derivatives, malarone, atovaquone + proguanil, co-artemether), new (especially inexpensive and affordable) drugs and more practical formulations of existing drugs/compounds are badly needed.

Vaccines. International efforts to combat malaria are unprecedented (see [WHO website](#)) through a [Global Malaria Control Strategy](#) whose activities are coordinated by WHO's [Programme on Communicable Diseases \(CDS\)](#), [Roll Back Malaria](#) coordinating the efforts of four UN-System agencies ([UNDP](#), [UNICEF](#), [WHO](#) and the [World Bank](#)) launched on 30 October 1998, [Multilateral Initiative on Malaria \(MIM\)](#) launched in Dakar in January 1997 when a number of institutions (from both public and private sectors) joined forces to promote malaria research in Africa. The UNDP/World Bank/WHO [Special Programme on Tropical Diseases](#) (WHO/TDR) has joined the initiative, establishing a Task Force to address the needs of endemic countries and to fund activities related to strengthening research capacities in malaria. The Task Force has mobilized around 40 countries and 161 partners for submitting proposals for review. Fifteen partnership projects involving 20 African and 5 European countries and the USA have been funded. The main malaria vaccine funding agencies are the USA [NIH](#), the [European Union](#), either directly or through the [European Malaria Vaccine Initiative \(EMVI\)](#), the [USAID](#), the [Malaria Vaccine Initiative \(MVI\)](#) and [Rockefeller Foundation](#). There are four general categories of malaria vaccine candidates, each representing a different stage of intervention. Virtually all the malaria vaccine candidates (with the exception of GPI anchor antigen described below) are cell surface antigens present during one of the three developmental stages of the *Plasmodium* parasite. Pre-erythrocytic (sporozoite) vaccines are those directed against the sporozoite and liver stages of the malaria parasite. The sporozoite is the form of the parasite introduced into the human host by the bite of an infected mosquito and that invades liver cells. A sporozoite vaccine could prevent infection either by blocking invasion of liver cells (antibody response) or destroying infected liver cells (cell-mediated response) by preventing release of parasites into the bloodstream. The asexual blood-stage (erythrocytic) vaccines are directed against the merozoite stage of the parasite, which invades and replicates in the red blood cells. A blood-stage vaccine would be expected to reduce both the severity

and duration of the disease by decreasing the blood parasite density, which correlates with reduced disease symptoms and risk of death¹²³. The transmission-blocking vaccines are designed to raise antibodies (in humans) against the gamete stage of the parasite present in the mosquito gut¹²⁴. Such antibodies taken up by a mosquito during a blood meal should block further parasite development in the mosquito, becoming a non-infectious vector. Blocking transmission of the parasite could reduce infectivity of the mosquitoes (carrying fewer parasites) and extend the useful life of a pre-erythrocytic or blood-stage vaccine by preventing transmission of antibody-resistant mutants. A fourth type of potential malaria vaccine is an anti-disease vaccine. This approach to a vaccine involves the identification of parasite toxins that contribute to disease. The glycosylphosphatidyl inositol (GPI) anchor, which tethers several of the parasite antigens to the membrane, has been shown to be highly toxic in mouse models¹²⁵. GPI as a vaccine would have to be detoxified enough to be safe but the potential for disease attenuation with this approach is real. Most investigators now acknowledge that a combination vaccine (multi-antigen, multi-stage) will probably be the best approach to effective vaccination. The various malaria vaccine candidate antigens are expressed and manufactured in a number of different ways including recombinant protein, DNA vaccine constructs, viral-vectored constructs, synthetic peptides, and chimeric proteins. The *Plasmodium* genome is very A-T rich, unlike most of the microbial organisms (bacteria, yeast or virus) or animals used to express recombinant parasite antigens, and the organism has quite different codon usage. Enhanced expression of recombinant *Plasmodium* antigens may be obtained by creating synthetic genes using optimized codons according to the organism used for expression.

Pre-erythrocytic vaccine candidates

The most advanced and well-documented pre-erythrocytic (liver-stage) vaccine candidates are derived from the circumsporozoite (CS) antigen present on the sporozoite. Such a vaccine candidate developed by [GlaxoSmithKline](#) and the [Walter Reed Army Institute of Research \(WRAIR\)](#), is referred to as RTS,S/ASO2. This vaccine is comprised of the antigenic C-terminus (amino acids 207-395) of the CS gene from *P. falciparum* fused to the hepatitis B surface antigen. This chimeric polypeptide containing the hepatitis B surface antigen is co-expressed with hepatitis B surface antigen in *Saccharomyces cerevisiae*. Initial Phase I clinical trials of RTS,S formulated with the ASO2 adjuvant (containing MPL, QS21 and an oil-in-water emulsion) showed protection against sporozoite challenge in 6 out of 7 volunteers¹²⁶. More recently, a dose-range Phase I/II study showed levels of efficacy from 30% (single dose) to 55% (3 doses). Overall protective efficacy was 41% among 41 vaccinees. Further trials in a pediatric population in The Gambia are now in progress (www.malariavaccine.org). Current studies are aimed at combining RTS,S with the blood-stage antigen MSP1 (see below).

Another CS-based vaccine candidate includes a 102-amino acid synthetic peptide representing the antigenic C-terminus of the circumsporozoite antigen^{127 128}. The peptide was safe and immunogenic in humans (no challenge). This vaccine candidate will soon enter Phase I/II trials for safety and efficacy in Europe. In addition, [Apovia \(USA\)](#) is developing with MVI funding a vaccine based on CS determinants expressed on Hepatitis B core particles¹²⁹. This vaccine should soon enter clinical development.

The Department of Defense (USA) in collaboration with [Vical, Inc.](#) is developing DNA vaccines for malaria (the Multi-Stage DNA Operation, MuStDO) that includes a liver-stage DNA vaccine candidate encoding the CS protein of *P. falciparum*¹³⁰. This DNA vaccine tested in Phase I showed no serious adverse events and no detectable DNA autoantibodies after a one-year follow-up but the vaccination failed to induce antigen-specific antibodies.

A multiple-antigen version of the DNA vaccine, MuStDO 5¹³¹, encodes five different liver-stage antigens including CS and the additional antigens: liver stage antigens 1 and 3 (LSA 1 and 3), exported protein 1 (EXP1), all having shown to be protective in mouse models¹³², and sporozoite surface protein 2 (SSP2, previously tested in animals as part of a DNA vaccine mix¹³³ and also known as thrombospondin-related adhesive protein, TRAP). MuStDO 5 is manufactured as a combination of five separate plasmids. The DNA vaccine administered with GM-CSF DNA as adjuvant was safe and well tolerated in mice and rabbits. The LSA 3 protein has been demonstrated to induce protective immunity against *P. falciparum* infection in the chimpanzee model. Various formulations of this antigen (peptides, lipopeptides, and DNA vaccine) are currently targeted for clinical development.

Several groups are using a prime-boost approach by priming with a DNA vaccine and boosting with either recombinant antigen or viral vectors, shown to be more immunogenic than either vaccine alone¹³⁴.

Another vaccine development approach targeting the pre-erythrocytic parasite is focused on the intracellular liver stage parasite. Several known antigens expressed by sporozoites or merozoites can also be expressed by liver stage parasites. Various studies conducted in endemic areas have linked liver stage antigen 1 (LSA-1) with protective immunity. B-cell and T-cell epitopes in LSA-1 and LSA-3 have been associated with protective immune responses in these studies¹³⁵.

Additional antigens that have been targeted for vaccine development because of identification of epitopes associated with protective immunity include the sporozoite and liver stage antigen (SALSA)¹³⁶, sporozoite threonine and asparagine rich protein (STARP – also expressed in sporozoites)¹³⁷, and the glutamate-rich protein (GLURP)¹³⁸. The progress in the field of peptide synthesis now permits the synthesis of long chains of synthetic peptides (LSP) which allows for the inclusion of multiple protective epitopes. This long synthetic peptide approach to malaria vaccine design is being pursued by several collaborative groups in Europe and Africa.

Asexual blood-stage vaccine candidates

The most advanced asexual blood stage vaccine is merozoite surface protein 1 (MSP1). MSP1 forms part of a complex that is thought to be involved in red blood-cell invasion and antibodies to MSP1 have been shown to block parasite invasion of red blood cells *in-vitro*. MSP1 contains the presence of two connected cysteine-containing epidermal growth factor (EGF)-like modules required to maintain the conformation-dependent epitopes needed to generate protective antibodies^{139 140}. The EGF-like domains is conserved across all species of *Plasmodium*, thus this region is a prime focus of current MSP1 vaccine candidates. Several groups work on MSP1 either as the entire molecule, the 42kDa C-terminal moiety, the further-processed 19kDa fragment, or as part of a hybrid molecule. Baculovirus, *E. coli*, and yeast (*Saccharomyces* or *Pichia*) are the expression systems used for recombinant antigen production. Recombinant MSP1 (42kDa or 19kDa), produced in each of these different systems, has been shown to protect both mice and monkeys against lethal parasite challenge¹⁴¹. Several DNA vaccine constructs are also being tested. Most of these vaccines are in pre-clinical stage of optimization prior GMP manufacturing for Phase I/II trials.

A number of additional merozoite surface protein (MSP) antigens are under development as vaccine candidates (MSP2, 3, 4, 5, 8 and 9)^{142 143 144}. These related molecules contain one or more of the hallmark EGF-like domains present in MSP1. MSP5 is of particular interest because it lacks the sequence variation between different isolates of *P. falciparum* from different geographical locations (typically seen with most of the merozoite surface proteins), which may simplify vaccine formulation.

Currently most advanced along the vaccine development pathway of blood-stage malaria vaccine candidates is the 'Combination B' vaccine candidate. This vaccine combines MSP-1 and MSP-2 with *Plasmodium falciparum* ring-infected erythrocyte (RESA). Recently, phase I/IIb trials of this vaccine in Papua New Guinea children aged 5-9 showed a 62 % reduction in parasite density in participants who were not pre-treated with sulfadoxine-pyrimethamine before vaccination¹⁴⁵. This trial highlighted the issue of clearing parasitemia before vaccination with treatment as the efficacy of the vaccine was only significant in the group who were not pre-treated. It also demonstrated possible vaccine-induced selective pressure on the MSP-2 component of the vaccine underlining the importance of development strategies that focus incorporating all significant genotype or highly conserved antigens in vaccine design.

Two other promising *P. falciparum* asexual blood-stage candidate antigens are the apical membrane antigen-1 (AMA-1)^{146 147} and erythrocyte binding antigen-175 (EBA-175)^{148 149}. There are currently 4-5 different laboratories developing these antigens as vaccine candidates expressed either in *E. coli*, *Pichia pastoris* or as a DNA prime-boost vaccine.

Transmission-blocking vaccine candidates

The leading candidate vaccines contain the *P. falciparum* surface protein antigens Pfs 25 and Pfs 28 or the *P. vivax* homologues referred to as Pvs25 and 28. Currently, transmission-blocking antigens Pvs28, Pfs28, and Pfs25 are being developed at the [NIH](#) (USA)^{150 151} as recombinant yeast-secreted proteins (*S. cerevisiae*). Initial human Phase I safety, immunogenicity and *in vitro* efficacy trials have been done

for Pfs25 and might be following soon for Pvs25. Other sexual stage-specific antigens that are being developed as transmission-blocking vaccines are Pfs48/45¹⁵² and Pfs230¹⁵³.

Anti-toxic candidate vaccine – the anti-GPI vaccine

Anti-disease vaccines are also being developed that involve direct immunization against parasite toxins that are identified as the cause of disease pathology. The identification of these toxins must be followed by characterization of the immune response to the toxin, focusing on its subsequent neutralization by antibodies and most importantly, prevention of disease pathology, in order to be considered as a potential anti-disease malaria vaccine candidate.

The glycosylphosphatidylinositol (GPI) anchor, which tethers several of the parasite antigens to the membrane, has been shown to be highly toxic in mouse models, and is currently being developed as a carbohydrate anti-toxic vaccine. A study testing synthetic GPI in rodent models of malaria appeared to show that the candidate anti-toxic vaccine was immunogenic and protected the rodent model from significant malaria pathologies and mortalities¹⁵⁴. Following this initial proof-of-principle in an animal model, further development of the malaria toxin neutralization as a vaccine strategy continues.

Leishmaniasis

Disease burden. Leishmaniasis is caused by several species of protozoan parasites (*Leishmania donovani*, *L. tropica*, *L. infantum* (*L. chagasi*), *L. major*, *L. amazonensis*, *L. aethiopic*a, *L. mexicana*, *L. guyanensis*, *L. peruviana*, *L. venezuelensis* and others) transmitted by the bite of the female phlebotomine sand fly. Leishmaniasis is currently prevalent in the four continents, being endemic in 88 countries, 72 of which are developing countries, threatening 350 million men, women and children with a worldwide prevalence of 12 million cases. Annual number of deaths in 2001 are estimated around 59,000 (WHO, 2002): 90% of all visceral leishmaniasis cases occur in Bangladesh, Brazil, India, Nepal and Sudan; 90% of mucocutaneous cases occur in Bolivia, Brazil and Peru, and 80% of cutaneous forms occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. For many years, the public health impact of the leishmaniasis has been grossly underestimated, mainly due to lack of awareness of its serious health impact. As declaration is obligatory in only 32 of the 88 countries affected, a substantial number of cases are never recorded. Of the 1.5-2 million new cases estimated to occur annually, only 600 000 are officially declared. In addition deadly epidemics of visceral leishmaniasis periodically flare up. In Varanasi district, Bihar, India, 12.9% of a village population suffered kala-azar, with a case-fatality rate of 10.5%¹⁵⁵. In the 1990s Sudan suffered a crisis with an excess mortality of 100 000 deaths among people at risk. An epidemic of cutaneous leishmaniasis is ongoing in Kabul, Afghanistan with an estimated 200 000 cases. The precise disease burden of leishmaniasis is not known but its economic and social impact is tangible. The expansion of leishmaniasis is related to environmental changes such as deforestation, building of dams, new irrigation schemes and migration of non-immune people to endemic areas. It seriously hampers productivity and vitally needed socioeconomic progress. Epidemics have significantly delayed the implementation of development programmes in Saudi Arabia, Morocco, the Amazon and the tropical regions of the Andean countries. More recently, as a result of epidemiological changes a sharp increase in the overlapping of HIV infection and visceral leishmaniasis has been observed, especially in southwestern Europe and intravenous drug users. This situation may soon worsen in Africa and Asia where the prevalence and detections of co-infections are probably largely underestimated. In response to this situation, WHO and UNAIDS have set up a surveillance system in 28 institutions worldwide¹⁵⁶.

Vaccines. The first line drugs for treatment of leishmaniasis (antimonials) are over a half-century old remain expensive, require repeated injections for one month (for visceral disease), are associated with side effects and are not always available where needed. Drug resistance is also becoming common in certain areas (i.e. Bihar, India) making their use ineffective. The recent drug developed against visceral leishmaniasis (miltefosine) is a break through as it is active orally but has not been widely used yet to determine its possible side effects and is not affordable for those who need it most. Vector and reservoir control may be useful under certain conditions but are not applicable in every epidemiological setting and require infrastructure and vigilance beyond the capability of many endemic countries. Vaccination remains the best option for control of all forms of the disease.

There is no effective vaccine for any form of leishmaniasis as yet. The first generation vaccines (whole killed parasites with or without BCG as adjuvant) have been tested in humans. A single injection of a combination of autoclaved promastigote form of *L. major* with low dose of BCG was tested in Iran

against two different forms of cutaneous leishmaniasis without showing efficacy, except maybe in those who converted their skin reaction¹⁵⁷ to leishmanial antigens (leishmanin) and unexpectedly in boys¹⁵⁸. Clinical trials of the same vaccine against visceral leishmaniasis in Sudan did not confer significant protection against visceral leishmaniasis¹⁵⁹. However, again in the vaccine group, a lower incidence of disease was observed in the converters to positive leishmanin skin test (43% lower incidence rate as compared to non-responders: 7.2% vs. 12.7%). Similar observations were made earlier in Brazil¹⁶⁰. New trials are on-going using the addition of alum as adjuvant.

New approaches based on surface antigens (gp63, lipophosphoglycan), promastigote antigen derived from *L. amazonensis* (46kD protein), enzyme receptor (LACK), have shown some protection in mice. Th1-driving adjuvant such as IL-12 or oligodeoxynucleotides with leishmanial antigens, or a recombinant leishmanial antigen (TSA, LmSTI1) have conferred protection in mice. DNA constructs encoding gp63 and LACK conferred protection against *L. major* in mice. The [Bill and Melinda Gates Foundation](#) funded the development of a chimeric vaccine made as a fusion of three recombinant leishmanial antigens (LeIF, LmSTI-1 and TSA) in monophosphoryl lipid A adjuvant ([IDRI-Corixa](#)) to be tested in Phase 1 in the United States as well as efficacy trials in several countries around the world^{161 162}. Lastly, attenuated vaccines by gene deletion have shown some promise in mice.

Schistosomiasis

Disease burden. Schistosomiasis, also known as bilharziasis is second only to malaria in public health importance (1.8 million DALYs ([WHO, 2002](#)) and 15,000 deaths ([WHO, 2002](#)) in 2001). *Schistosoma haematobium* is found in 53 countries in the Middle East and Africa, including the islands of Madagascar and Mauritius. There is also an ill-defined focus of *S. haematobium* in India. With the recent introduction of *S. mansoni* to Mauritania, Senegal and Somalia, intestinal schistosomiasis is now found in 54 countries, including the Arabian peninsula, Egypt, Libya, Sudan, sub-Saharan Africa, Brazil, some Caribbean islands, Suriname and Venezuela. *S. intercalatum* has been reported from 10 countries within the rain forest belt of central Africa. *S. japonicum* is endemic in China where bovines are the main reservoir, Indonesia and the Philippines (with dogs and pigs as reservoir) and has been reported from Thailand. Another oriental schistosome, *S. mekongi* is found in Cambodia and Laos, along the Mekong River. Environmental changes linked to water resources development, population movements and population growth have led to the spread of the disease to previously low or non-endemic areas, particularly in sub-Saharan Africa. A few striking examples can be cited in this respect. The building of the Diama dam on the Senegal River has introduced intestinal schistosomiasis to both Mauritania and Senegal. Refugee movements and population displacements in the Horn of Africa have also introduced intestinal schistosomiasis to Somalia, and more recently to Djibouti.

Globally, about 120 million of the 200 million infected people are estimated to be symptomatic and 20 million are thought to suffer severe consequences of the infection. Annually an estimated 20 000 deaths are associated with the severe consequences of infection, including bladder cancer or renal failure (*S. haematobium*) and liver fibrosis and portal hypertension (*S. mansoni*). Much of the available data on schistosomiasis is however limited to numbers of individuals harboring the infection (prevalence). The related mortality are highly likely vastly under-estimated. In sub-Saharan Africa where schistosomiasis constitutes an important public health problem, a recent survey of disease-specific mortality indicates that 70 million individuals out of 682 million (year 2000 figures) had experienced haematuria and 32 million dysuria associated with *S. haematobium* infection. It was estimated that 18 million suffer bladder wall pathology and 10 million hydronephrosis. Infection with *S. mansoni* was estimated to cause diarrhoea in 0.78 million individuals, blood in stool in 4.4 million and hepatomegaly in 8.5 million. As the associations between prevalence of *S. mansoni* infection and prevalence of diarrhoea and blood in stool were not very clear, the resulting estimates might be underestimations. Using the very limited data available, it is estimated the mortality rates due to non-functioning kidney (from *S. haematobium*) and haematemesis (from *S. mansoni*) at 150 and 130 thousand per year¹⁶³. Despite substantial progresses mentioned above the search for a schistosomiasis vaccine for sub-Saharan Africa and the Mekong region and the Philippines.

Although these are global estimates of the schistosomiasis disease burden, the public health impact of schistosomiasis in the field has been poorly evaluated and is still subject to controversy. Apart from a few situations where schistosomiasis is or was recognized as an obvious public health problem as in China, the Philippines, Egypt, Brazil, northern Senegal and Uganda, the disease is often not a priority for health authorities and lack of a simple clinical case definition that would enable rapid identification by health personnel. Due to its non-specific signs and symptoms and its insidious nature, affected

persons often do not perceive schistosomiasis as a serious health problem, thus favoring the development of late, irreversible sequelae. In many endemic areas, the public health importance of these late sequelae is poorly documented because of the lack of adequate diagnostic facilities. Although its existence is generally acknowledged, the impact of "subtle" morbidity, such as anemia, impaired growth, development and cognition, and poor school performance, caused by schistosomiasis in school age children, has not been documented as well as for the soil-transmitted helminth infections¹⁶⁴. Also precise data on the socio-economic burden of schistosomiasis is lacking.

The global distribution of schistosomiasis has changed in the past 50 years, with control successes achieved in Asia, the Americas, North Africa and Middle East. Schistosomiasis has been eradicated from Japan and some of the islands in the Lesser Antilles. Transmission has been stopped in Tunisia, and is low in Morocco, the Philippines, Saudi Arabia, and Venezuela. There is now ample evidence from countries where schistosomiasis control was implemented, that the WHO-recommended strategy for morbidity control is effective. Four national control programmes (Brazil, China, Egypt, and the Philippines) demonstrate that concerted control efforts together with economic development can decrease morbidity to low levels. Chemotherapy was central to these successes. The current drug of choice for schistosomiasis, praziquantel, reverses pathology – in as little as six months after treatment in *S. haematobium* infections, significant changes in the urogenital tract can be reversed, particularly rapidly in children. Therefore, when chemotherapy is used widely, morbidity can be substantially reduced, as has been the case in Brazil, China, Egypt, and the Philippines. In Brazil, the mortality due to schistosomiasis decreased by 56% between 1979 and 1997. In the age group 0-14 years old, the decrease was as much as 87%. Between 1984 and 1997, hospitalizations due to schistosomiasis in all age groups decreased also by 43%. These encouraging results have to be balanced against the cost of chemotherapy. The cost of praziquantel has decreased significantly over the past 20 years and it is now possible to purchase the drug as low as US\$ 0.08 a tablet. Nonetheless large scale use of the praziquantel can impose a heavy burden on health systems. In addition, there remain concerns over the potential threat of the emergence of praziquantel resistant parasites.

Vaccines. As large-scale population-based chemotherapy programmes can be difficult to implement and costly to sustain, despite the now lower cost of praziquantel, there would be considerable advantages to have a vaccine available for long-term prevention. Schistosomula appear to be a major source of the antigens that are currently being considered as vaccine candidates¹⁶⁵. Significant levels of protection against challenge *S. mansoni* infection have been obtained when irradiated cercariae have been used to immunize a variety of species. In tests of vaccine candidate antigens in mice, both type 1 (Th1) and type 2 (Th2) helper-T-cell responses may be involved in protection. In recent Phase 1 and 2 clinical trials involving human volunteers, a schistosome-derived molecule, the *S. haematobium* glutathione *S*-transferase (Sh28GST) ([Bilhvax](#), Institute Pasteur de Lille, France), was safe and demonstrated good immunogenicity in France, Niger and Senegal¹⁶⁶.

The Schistosomiasis Vaccine Development Programme ([SVDP](#)), based in Egypt and supported by USAID¹⁶⁷, has focused on two *S. mansoni* antigens: paramyosin and a synthetic peptide construct containing multiple antigen epitopes (MAP) of the schistosome triose phosphatase isomerase (TPI) (Bachem Company, Los Angeles, USA). A third approach ([FIOCRUZ](#), Rio de Janeiro, Brazil) is currently engaged in the scaling up of Sm14, a fatty acid-binding *S. mansoni* antigen.

Developing a vaccine for *Schistosoma japonicum* is the focus of a number of Asian countries. The profile of a candidate vaccine for *S. japonicum* has been debated there was diversity of opinion over what is expected from the vaccine. In China, the situation seems to call for a vaccine for animal use that would reduce transmission of infection to humans; but in The Philippines, a vaccine is required for human use with the aim of reducing morbidity. Key indicators for any potential vaccine would include an impact on acquisition of infection, worm burden, fecundity, and egg output in the vaccinated groups. In addition, a reduction in transmission would be desirable. Recent studies in water buffaloes of the protection afforded by the *S. japonicum* antigens paramyosin, an invertebrate muscular protein, (Sj-97) and GST-26 (Sj-GST26) have yielded some encouraging results, leading to the suggestion that vaccine that blocks transmission could be produced for use in reservoir hosts¹⁶⁸. However, it is clear that there is a need for the development of standardized protocols and standard operating procedures (SOPs) to allow more direct comparisons of trial results.

Dengue

Disease burden. Dengue viruses are the most widespread arthropod-borne viruses (arboviruses). They are members of the *Flaviviridae* family, which includes more than 70 related but distinct viruses, most of which are urban-dwelling mosquito-borne (*Aedes aegypti*, *Aedes albopictus*). During the 20th century, the distribution and density of the mosquito vector, *Aedes aegypti*, expanded dramatically in tropical areas, beginning in large cities then spreading to the countryside. This was followed by global circulation of the four dengue viruses, a virus group closely related to yellow fever. Due to complex phenomena related to the circulation of multiple serotypes, a new yellow fever-like viral hemorrhagic fever emerged dengue haemorrhagic fever (DHF). An unprecedented 1.3 million cases of dengue fever and dengue hemorrhagic fever were reported to WHO in 1998, including over 3500 deaths. The pandemic largely affected the WHO Regions of the Americas (AMR/PAHO), South-east Asia (SEAR) and the Western Pacific (WPR). More than 55% of the cases, mostly of dengue fever, and only 2% of the deaths, were reported from AMR. However, in this region, dengue fever and dengue hemorrhagic fever are reported separately, whereas in SEARO and WPRO the data are aggregated and the great majority of reported cases are hospitalized cases of dengue hemorrhagic fever¹⁶⁹. The burden of severe disease remains proportionately much greater in the affected Asian and Pacific countries. In 1999, dengue was present on most continents, and more than one-half of all United Nations member-states were threatened by dengue.

It is estimated that 2.5 billion people (including 1 billion children) are exposed to infection, 100 millions of dengue clinical cases and 500 000 cases of hemorrhagic fever (DHF) and dengue shock syndrome occur each year with a case fatality rate of over 1%. Overall, it is estimated that in 2001 dengue was responsible for 656,000 deaths ([WHO, 2002](#)) and 653,000 DALYs ([WHO, 2002](#)). Epidemics continue to emerge in areas previously untouched. The westward expansion of dengue in Asia was first documented in the late 1980s by the increased epidemics in India and Sri Lanka. Dengue emerged more preeminently in Africa in the late 1990s (Somalia, Saudi Arabia). The disease will continue to spread as newly urbanized areas become infested with mosquito vectors. Typical of post-epidemic periods, dengue activity was much lower in the year after the pandemic, but the number of reported cases increased to over 0.5 million in 2000. Preliminary data for the year 2001, up to September, for two of the three regions (AMRO and SEARO), show a further, large increase of reported cases (over 525 913 cases) with nearly 500 deaths. These data suggest a level of activity comparable in magnitude with that of 1998¹⁷⁰.

In recognition of the magnitude of this global public health problem, an international conference with over 700 public health specialists from 41 countries was held in Chiang Mai, Thailand, on 20-24 November 2000. The delegates recommended that all countries at risk for dengue transmission develop and implement sustainable prevention and control programmes¹⁷¹.

Vaccines. There are four closely related, but serologically distinct dengue viruses (1 to 4). As there is no cross-protection between the four types, a population could experience several type-specific epidemics in a row. To date antiviral drug chemotherapy has not been successful; most forms of therapy are supportive in nature. As attempts to eradicate mosquito vectors have not been successful in developing countries, the control of dengue will be possible only after an efficient vaccine has been developed. Clearly, the phenomenon of immune enhancement may be a major problem in developing an effective dengue vaccine. In order to avoid production of monotypic-enhancing antibodies that might lead to DHF associated with subsequent natural infections, the development of a multivalent vaccine against all four serotypes seems necessary. Progress in vaccine development has been slowed, mainly because these viruses grow poorly in cell culture and there is no acceptable animal model for DHF.

Two live attenuated tetravalent dengue vaccines^{172 173} were developed by passages of wild type strains of dengue viruses in cell culture. The first was developed at Mahidol University, Thailand and [Aventis Pasteur](#). Study with adult volunteers in USA demonstrated promising results. Individuals immunized with tetravalent vaccine gave multivalent antibody responses, the highest antibody titers being against dengue virus type 3. A Phase I/II study was also performed in children in Bangkok. The second live attenuated tetravalent vaccine is being developed at the [Walter Reed Army Institute of Research \(WRAIR\)](#), USA. In a pilot studies in human, three doses of this vaccine induced 50% and higher seroconversion to all four dengue serotypes. Many formulations of this vaccine were tested and two of them have been selected for further evaluation. The dissemination rates of live attenuated tetravalent vaccine viruses in mosquitoes were low and it is therefore unlikely that these viruses would be transmitted under natural conditions.

Several research groups are successfully exploring an infectious clone technology for the development of a dengue vaccine. The ChimeriVax™ system, originally developed to construct JE vaccine, has now been applied to dengue viruses by [Acambis](#) in the USA. A chimeric YF-dengue viruses were prepared using a recombinant cDNA infectious clone of a YF vaccine strain as a backbone, into which the pre-membrane and envelope genes of dengue viruses were inserted. (see ref. 151, 152). This vaccine was shown to be safe and immunogenic in a monkey study. A monovalent formulation is currently submitted for evaluation in clinical trials. Another approach is based on the use of a dengue type 4 mutant containing a deletion in a non-coding region as a genetic background for the construction of a dengue chimeric vaccine. Viruses with deletion mutations are genetically stable and are less likely to revert to the genotype of the parent virus than the mutants with point mutations when propagated in vaccinated individuals. Phase I clinical trials of a deletion mutant were carried out in adult human showing good safety and immunogenicity profile. This attenuated virus will be used as the backbone for the construction of chimeric dengue viruses of serotypes 1, 2 and 3. Work at the [US CDC](#) is based on the use of non-structural genes of dengue-2 vaccine strain and structural genes of the dengue 1, 3 and 4 serotypes. Other approaches for the development of dengue vaccines include DNA technology, inactivated and subunit vaccines, or recombinant vaccinia virus vectors^{174 175}.

The establishment of a Regional Regulatory Authority that would harmonize guidelines and define clear manufacturing, quality control, preclinical and clinical testing criteria for vaccine acceptability would facilitate dengue vaccine development for developing countries. In addition, the threshold of vaccine efficacy acceptable for public health use is not defined. As it was done for HIV vaccines, a modeling of the epidemic with the introduction of a dengue vaccine (with various efficacy rates and vaccine coverage strategies) would be helpful in this regard.

Japanese Encephalitis

Disease burden. Japanese encephalitis (JE) is a mosquito-borne arbovirus infection, where pigs play the role of amplifying host in rural areas, with seasonal distribution, is endemic in parts of China, India, Korea, Japan, South East Russian Federation, Islands in the Torres Strait Australia, Nepal, Thailand, Vietnam, Cambodia, Lao PDR, the Philippines, Taiwan, Indonesia, Malaysia, and Sri Lanka. JEV belongs to the *Flaviviridae* family, genus flavivirus. Large outbreaks of JE in India and Nepal have highlighted the continuing expansion of the geographic range of JE in recent years^{176 177 178 179}. The epidemic situation in Bangladesh and Bhutan needs to be clarified. It is the leading cause of virus encephalitis and neurological infection in Asia. Although severely under-reported, 50 000 cases are annually reported throughout Asia, with 15,000 deaths annually ([WHO](#), 5-35% case fatality rate) and a 75% JE-related disability rate (767,000 DALYs, [WHO, 2002](#)). An annual incidence ranges from 10 to 100 cases per 100 000 inhabitants has been reported in heavily endemic areas. It is estimated that on average 1 in 300 infections results in symptomatic illness. The majority of people living in JE-endemic areas are infected with the virus before the age of 15. A recent study of cost-effectiveness of routine immunization to control JE in Shanghai, China showed that compared with no immunization, a programme using the inactivated JE vaccine would prevent 451 JE cases, 113 JE deaths, and the loss of 6 888 DALYs per 100 000 persons. A live-attenuated JE vaccine could prevent 461 cases, 115 deaths, and the loss of 7 035 DALYs. Both immunizations are cost saving, but the live-attenuated vaccine strategy resulted in more than a 40% greater savings (US\$ 579 210) compared with the inactivated vaccine strategy (US\$ 408 272)¹⁸⁰. However, additional data on JE disease burden, JE-associated disability, cost-effectiveness and JE control programme analysis, as well as demand for vaccine, remain to be determined and should deserve further research.

Vaccines. There is no JE-specific therapy other than supportive care. JE control programmes include mosquito control (spraying, impregnated bed nets), pig control (segregation, slaughtering, and vaccination) and human vaccination. Several vaccines are now available and others under development¹⁸¹. A formalin-inactivated [JE vaccine](#) propagated in mouse brain tissue has been used successfully to reduce the incidence of JE in Japan, Taiwan, Korea, Thailand, and Vietnam. The vaccine is available internationally, but remains expensive. Since 1988 it is included in EPI programme in Thailand. However, it has been hampered by high demand, a limited production capacity far below the global requirement, the short-term protection provided by the vaccine, and reports of neurological reactions after vaccination.

A live-attenuated vaccine (SA 14-14-2) has been developed and tested in China. The vaccine is produced on primary hamster kidney cells. This attenuated vaccine appears to be safe and effective in annual Chinese immunization programmes involving millions of children. A recent review of 13 000 vaccinated and control children in Chengdu Province, China, indicated low rates of acute systemic and local side effects, and no central nervous system infection were reported¹⁸². In collaboration with Chengdu, South Korea (Glovanx is developing this vaccine under improved GMP conditions. Another inactivated JE vaccine based on the same SA 14-14-2 strain is currently being developed by a US company (VaccGen) and tested in Phase II by [WRAIR](#) in Thailand. Vero cell-derived inactivated JE vaccines have been developed in China, where the vaccine is now licensed and 2 millions doses are produced annually, as well as in Japan, where Biken and Chemo-Sero Therapeutic Research Institute are testing in Phase I a Vero cell-derived JE vaccine.

Recombinant JE vaccines using pox vectors expressing the premembrane, envelope, NS1 and NS2A protein genes have been tested in monkeys¹⁸³ and in humans¹⁸⁴. This vaccine approach was stopped. A single intramuscular immunization of DNA vaccine of Japanese encephalitis and West Nile viruses protected mice and horses from virus challenge. The use of DNA vaccines in multivalent and/or combination vaccines designed to immunize against multiple flaviviruses is thus a promising area of development¹⁸⁵, although the immunogenicity of DNA vaccines in humans has yet to be improved.

Recently, a very attractive chimeric vaccine concept of live-attenuated vaccines using the 17D yellow fever strain cultivated on Vero cells has been developed by [Acambis](#)^{186 187}. The prototype vaccine has been tested successfully in US adults, showing good safety and immunogenicity. A trial in Thai adults and children is planned end of 2002.

Tick-Borne Encephalitis

Disease burden. Tick-Borne Encephalitis (TBE) virus is a flavivirus responsible for endemic infection mostly in Russia and Eastern and Central Europe. The endemic ar4a spreads from the Rhine to the Urals, from Scandinavia to Italy and Greece¹⁸⁸. In addition, it was conclusively demonstrated that the TBE virus is endemic in Japan, where TBE virus was isolated from the blood samples of sentinel dogs, tick pools, and rodent spleens since 1995¹⁸⁹.

TBE is transmitted to humans usually by the bite of a tick (either *Ixodus persulcatus* or *Ixodus ricinus*). Occasionally, cases occur following consumption of infected unpasteurized milk. Transmission is seasonal and occurs in spring and summer, particularly in rural areas. Outbreaks of disease in urban populations have been described when groups of susceptible individuals occasionally enter forests or work on farms. In recent years, such intermittent travel of city dwellers to rural areas has accounted for many new cases of TBE. A rise in incidence of TBE has been observed in recent decades in some regions. This is probably related to global warming, because it is believed that warmer weather leads to more active ticks and to larger rodent populations which are the main hosts and reservoirs of the virus. There are two subtypes of TBE virus: Eastern and Western, and they differ slightly in the structure of viral proteins. In some publications, diseases caused by them are referred to as Russian spring/summer encephalitis and Central European encephalitis, respectively. TBE is a serious case of acute central nervous system disease, which may result in death or long-term neurological sequelae in between 35-58% of patients. The TBE morbidity rate in Russia recently increased dramatically from 6-10 000 persons per year. In Europe, 11 356 cases were reported in 1999, including 9427 cases in Russia¹⁹⁰. The proportion of cases involving subclinical infection varies between 70% and 98%. Symptomatic infection occurs in all age groups. According to a study conducted in 1958, the proportion of TBE in the total number of CNS viral diseases in Austria was 56%¹⁹¹. The virus subtype largely determines the clinical course of the disease. Compared with the virus prevalent in central Europe, the eastern variant has proven to be more virulent and to lead to severe diseases far more often. The fatality rate associated with clinically manifested infection is 0.5-20%.

Vaccines. There is no specific treatment for TBE. Prevention consists of individual prophylactic measures (self-examination and systematic extraction of ticks after exposure, use of repellents), and immunization. At least four inactivated cell-derived TBE vaccines are available. They are produced in Austria ([Baxter Vaccine](#), previously Immuno AG), Germany ([Chiron](#), previously Beringwerke AG) and Russia (Institute of Poliomyelitis and Viral Encephalitis and Virion Company). Active surveillance in Austria has demonstrated a dramatic decline in the incidence of TBE in vaccinated groups. On the basis of this observation, a vaccine efficacy of 95% has been reported. The main reported side effect is

postvaccination fever with currently available vaccines. In Germany, the vaccine is widely used to immunize children in high-risk areas. To reduce side effects in infants and children, smaller doses of vaccine are being used. The Russian vaccine induces high seroconversion rates and is believed to be highly effective.

A project is now in progress in Russia ([Virion](#)) to develop a new inactivated vaccine with improved protective efficacy and to arrange its large-scale production to meet the country needs for TBE vaccines. So far, attempts to develop live attenuated vaccine have been unsuccessful, although promising preliminary results were reported¹⁹². Other approaches to develop TBE vaccines are based on the DNA vaccines or infectious clone technologies.

West Nile encephalitis

Disease burden. West Nile virus (WNV) belongs to the family Flaviviridae and is a member of the Japanese encephalitis virus complex of arthropode-borne flaviviruses¹⁹³. The virus can infect humans, birds, mosquitoes, horses and some other mammals¹⁹⁴. Over 110 species of birds can be infected with WNV. WNV is one of the most widely distributed flaviviruses with a geographic range including Africa, Australia, Europe, the Middle East and West Asia¹⁹⁵. During 1999, WNV also established itself in the USA. In endemic regions, most human WNV infections are asymptomatic or cause mild illness with symptoms of low-grade fever, headache, body aches, rash, myalgia and polyarthropathy. However, severe disease has been reported in epidemics in Israel, France, Romania, and Russia. In acute severe illness, the virus can cause hepatitis, meningitis, and encephalitis, leading to paralysis and coma, resulting in death. The neuropathologic lesions are similar to those of Japanese encephalitis, with diffused central nervous system inflammation and neuronal degeneration. The virus is also found in the spleen, liver, lymph nodes and lungs of infected individuals. During 1999-2000 the virus extended its range throughout much of the eastern parts of the USA, and its range within the Western hemisphere is expected to continue expanding. During 1999-2001, 142 cases of neuroinvasive WNV viral diseases of the central nervous system (including 18 fatalities), and seven cases of uncomplicated WN fever were reported in the USA. An investigation conducted by the [CDC](#), the Food and Drug Administration (FDA), the American Red Cross, and state health departments in Georgia and Florida has confirmed transmissions of West Nile Virus from a single organ donor to four organ recipients¹⁹⁶.

Vaccines. Protective neutralizing antibody titers to WNV in recipients of a licensed inactivated Japanese encephalitis (JE) vaccine were not detected in any volunteer despite successful immunization to related flaviviruses. Vaccination against JE or dengue is therefore unlikely to prevent WNV infection but may still protect against disease. Indeed, in animal models, immunization with heterologous flavivirus vaccines reduces the severity of subsequent WNV infection¹⁹⁷.

A candidate live attenuated vaccine strain¹⁹⁸ based on chimeric dengue virus type 4 (DEN4) strain was also constructed, the genes for the structural pre-membrane and envelope proteins of DEN4 present in an infectious cDNA clone were replaced by the corresponding genes of WNV strain NY99. The WN/DEN4 chimera was highly attenuated in mice compared with its WNV parent. Nonetheless, the WNV/DEN4 chimera was immunogenic and provided complete protection against lethal WNV challenge. A novel technology platform for live attenuated recombinant vaccines (ChimeriVax) represents a promising approach for rapid development of a West Nile vaccine¹⁹⁹. This technology ([Acambis Inc](#)) uses yellow fever 17D as a live vector for envelope genes of the WNV. Infectious clone technology is used to replace the genes encoding the prM and E structural proteins of yellow fever 17D vaccine virus with the corresponding genes of WNV. The resulting virion has the protein coat of West Nile, containing all antigenic determinants for neutralization and one or more epitopes for cytotoxic T lymphocytes.

West Nile encephalitis has also emerged as a significant problem for the equine industry. One major veterinary manufacturer (Ft. Dodge) is developing formalin-inactivated vaccine. This product has been given conditional approval for use in horses²⁰⁰. Other technologies include notably naked DNA vaccines²⁰¹ and attenuated WNV variants²⁰². These variants have lost the neuroinvasion trait of the parental virus through serial passages in mosquito cells and neutralization escapes from WNV-specific monoclonal antibody.

Meningitis *N. meningitidis*

Disease burden. Bacterial meningitis remains a serious threat to global health, accounting for an estimated 171 000 deaths worldwide per year²⁰³. Even with antimicrobial therapy and the availability of sophisticated intensive care, case fatality rates from bacterial meningitis remain at 5-10% in industrialized countries, and are even higher in the developing world. Between 10-20% of survivors develop permanent sequelae such as epilepsy, mental retardation or sensorineural deafness. Three species, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*, are responsible for most cases of bacterial meningitis occurring beyond the neonatal period. Since the introduction of *H. influenzae* type b conjugate vaccines, *N. meningitidis* and *S. pneumoniae* have become the commonest causes of bacterial meningitis in the world. *N. meningitidis* moreover is the only bacterium capable of generating epidemics of meningitis.

N. meningitidis is spread by person-to-person contact through respiratory droplets of infected people. It is a common inhabitant of the mucosal membranes of the nose and throat, where it usually causes no harm. Up to 5-10% of a population may be asymptomatic carriers. These carriers are crucial to the spread of the disease as most cases are acquired through exposure to asymptomatic carriers. A small minority of the persons who contract the disease will develop an acute inflammation of the meninges, the membranes covering the brain and the spinal cord. The disease is mainly affecting young children, but is also common in older children and young adults. Groups, A, B, C, and recently Y and W135 account for up to 90% of all disease. All five groups may cause epidemics, although their nature differs from one group to another.

Group A meningococcus has historically been the main cause of epidemic meningococcal disease and still predominates in Africa during both endemic and epidemic periods. The highest number of cases and the highest burden of disease occur in sub-Saharan Africa in an area that is referred to as the meningitis belt. This is the area between Senegal and Ethiopia. Epidemics occur in irregular cycles every 5 to 12 years, last for two to three dry seasons, dying out during the intervening rainy seasons. The size of these epidemics can be enormous with attack rates as high as 400-800/100 000. During the 1996 group A epidemic in sub-Saharan Africa, around 200 000 cases were reported with 20 000 deaths²⁰⁴. Few studies have examined the rates of group A meningococcal disease in Africa in non epidemic years where rates are typically 5-10 times those of western European countries. In Niger, the average annual incidence of meningitis due to *N. meningitidis* in 11 inter-epidemic years between 1981 and 1994 was 30/100 000²⁰⁵. This contrasts with an incidence in the United Kingdom of 2-3/100 000²⁰⁶. Three-quarters of cases occur in people less than 15 years of age. Disease peaks in March-April towards the end of the dry season and is more common in males. In the last two years, the potential emergence of group W135 as the cause of epidemics has added complexity to the epidemiological situation in the region. In 2000 and 2001, W135 has been associated with outbreaks in Saudi Arabia²⁰⁷ and this year a W135 epidemic with around 14 000 cases has been reported from Burkina Faso²⁰⁸.

Although the highest burden of disease is currently in Africa, group A epidemics can occur in any part of the world. Asia has had some major epidemics of group A meningococcus in the last 30 years (China 1979 and 1980, Mongolia 1994-1995)²⁰⁹.

This serogroup accounts for 50-85% of meningococcal cases in North America and Europe, with most of the remaining cases caused by group C strains. The proportion of group B strains is especially high in Norway, The Netherlands, Germany and Denmark, while high or increasing proportions of group C strains are reported from Slovak and Czech Republics, Greece, Republic of Ireland, Spain and the United Kingdom. In all countries, the incidence of group B disease is highest in infants less than one year old. In the United States, in addition to groups B and C, meningococcal disease is also caused by Y strains, with each capsular group accounting for approximately a third of cases. In North America, meningococcal disease occurs at a rate of 1 case per 100 000 population per year, producing 2 725 cases notified in the US in 1998 and 155 laboratory confirmed cases in Canada in the same year²¹⁰. A majority of these cases occur in the winter season and in early childhood, with a case fatality rate of approximately 10%. 33% of cases were due to serogroup B in the USA, making for around 1 000 meningitis B confirmed cases and of 100 deaths per year. In Canada in 1995-96, 47% of cases were due to *Neisseria meningitidis* serogroup B²¹¹, which allows for a figure close to 80 cases meningitis B per year. Group B epidemics have also occurred in the USA, and in some middle-income countries of the Americas (Cuba, Colombia, Brazil and Chile).

For reasons that are unknown, epidemics caused by group B strains are rare in Africa or in other areas of the developing world. Since 1991, New Zealand has experienced an epidemic of group B

meningococcal disease with incidence rates of up to 10 times the background incidence, and with much higher age-, area- and ethnic-specific rates. In this country, the annual incidence of meningococcal disease increased from 53 cases (1.6 per 100 000 population) in the pre-epidemic year of 1990 to a peak of 613 (16.9 per 100 000) in 1997, followed by consistently raised rates. Over the 1996-2000 period, there was an average of 502 cases per year (13.9 per 100 000). The epidemic has resulted in 3 547 cases since. Of the total cases, 158 (4.5%) were fatal²¹². As of 2001, the epidemic shows no signs of abating, with some of the highest rates recorded in the industrialized countries. Serogroup B meningococci differ from serogroups A and C in disease epidemiology. In contrast to serogroup A and C epidemics, which usually resolve in 1 to 3 years, serogroup B outbreaks begin slowly, usually reach country-wide rates of 5 to 20 cases per 100 000 population per year and may persist for 5 to 10 years or longer, as seen in Cuba, Norway, areas of Chile and currently in New Zealand.

From data available on meningitidis serogroup B infection, and in the absence of an official WHO reported figure, meningitidis B incidence was estimated between 20 000 and 80 000 cases per year, with 2 000-8 000 deaths annually.

Vaccines against meningitidis serogroups A, C, Y and W135. Polysaccharide vaccines against groups A and C, or A, C, Y and W-135, are licensed ([GlaxoSmithKline](#) and [Aventis Pasteur](#)) and available worldwide. The first successful capsular polysaccharide vaccines against groups A and C were developed 30 years ago in response to meningitis epidemics among military recruits in the USA and were widely tested in Europe, Latin America and Africa. They proved to be safe and effective in preventing group C disease in U.S. military recruits, and in controlling group A epidemics during mass campaigns in Africa. However, most polysaccharide vaccines are poor immunogens in young infants and fail to induce immunological memory at all ages, and therefore they have not been routinely used²¹³. During the epidemic season in the African meningitis belt, vaccine from an international stockpile is made available to countries through the International Coordinating Group on Vaccine Provision for Epidemic Meningitis ([ICG](#)) in WHO, set up in 1997. Since 2002, the W-135 serogroup of *N. meningitidis* has emerged as a major epidemic strain in some countries of Africa. This has prompted the development of a trivalent PS A C W135 vaccine by [GSK](#).

Experience with *Hib* and pneumococcal conjugate vaccines has shown that the immunogenicity of polysaccharides can be improved by chemical conjugation to a protein carrier, thereby eliciting a T-cell dependent anti-saccharide antibody response. The resulting polysaccharide-protein conjugate vaccines are safe, immunogenic in young infants and induce long-term protection. Immunization also decreases nasopharyngeal carriage and transmission of the pathogen. In November 1999, meningococcal group C conjugate vaccine was introduced into the routine UK immunization programme. Infants receive three doses of vaccine at the same time as their routine primary immunizations given at 2, 3 and 4 months of age. Currently, no booster is given²¹⁴.

Bivalent A plus C polysaccharide conjugate vaccines have been evaluated in clinical trials and found to be well tolerated and immunogenic in infants, toddlers and adults^{215 216}. Vaccine manufacturers are currently developing conjugate vaccine combinations incorporating groups A, C, Y and W-135 polysaccharides. Wyeth is currently testing a conjugate 9 valent *S. Pneumonia*/mening C vaccine in Phase III trials as well as a tetravalent A, C, Y, W135 conjugate vaccine. [Aventis Pasteur](#)'s meningitis tetravalent conjugate vaccine is in Phase II/III trials and [GSK](#)'s *N. meningitidis* A/C conjugate vaccine is in Phase II evaluation. No major technical problems are anticipated with these vaccines. Thus, it seems highly likely that multivalent meningococcal polysaccharide-protein conjugate vaccines will be available in the USA and Europe within a few years, and will be highly effective in controlling disease caused by groups A, C, Y and W135 strains.

Vaccines against meningitidis serogroup B: The serogroup B capsular polysaccharide is a poor immunogen, probably because it is structurally identical to carbohydrate antigens (glycoproteins) expressed by a variety of host tissues, and attempts to improve its immunogenicity raise critical safety issues. Consequently, vaccine research against serogroup B meningococcus has focused largely on cell-surface protein antigens contained in outer-membrane vesicles (OMV). Of these outer membrane proteins (OMP), the PorA is the most important. This protein is expressed by almost all meningococci and is the major inducer of and target for serum bactericidal antibodies. However there are numerous PorA proteins and mounting an immune response against one does not confer protection against strains of meningococci type B with alternative antigens. Thus, OMV vaccines are more appropriate for use as strain-specific vaccines against clonal outbreaks than for routine infant immunization aimed at the

prevention of endemic disease caused by diverse strains. The two most studied outer membrane protein vaccines, called outer membrane vesicle (OMV) vaccines, are those produced in response to national outbreaks in Norway, and Cuba. Both of these vaccines have been used for epidemic control in their respective countries and, in the case of the Cuban vaccine, in other Latin American countries. They were found to be 50-80 % effective, but not effective to protect the very young, and immunity wanes over time. Neither of those vaccines contain the appropriate PorA protein to mount an immune response against the NZ strain which expresses a different PorA protein. The [RIVM](#) in The Netherlands have used recombinant technology to produce both a monovalent PorA vaccine and a hexavalent vaccine containing 6 PorA proteins, including this of the New Zealand strain. The performance of the New Zealand PorA antigen in the hexavalent formulation was poor, but it was shown to stimulate a satisfactory immune response in toddlers in the monovalent format. The [Finlay Institute](#) is producing the vaccine for New Zealand in collaboration with [GSK](#) (Phase II trials), while NIPH have formed an alliance with [Chiron](#). [Wyeth](#) is also currently involved in the development of a Meningitis B vaccine in collaboration with RIVM. The successful sequencing of the meningococcal genome has allowed discovery of several new proteins and raised potential for the development of new candidate vaccines. Among other, [Microscience](#) (USA) has identified novel surface-located vaccine candidates which are currently in preclinical evaluation.

Hepatitis C

Disease burden. Hepatitis C is comparable to a 'viral time bomb'. WHO estimates that about 200 million people, 3% of the world's population, are infected with hepatitis C virus (HCV) and 3 to 4 million persons are newly infected each year with a global 170 million chronic carriers at risk of developing liver cirrhosis and/or liver cancer (at least 85% of infected persons become chronically infected and about 70% develop chronic hepatitis)²¹⁷. Around 844,000 DALYs ([WHO, 2002](#)) and 46,000 deaths ([WHO, 2002](#)) have been attributed to HCV in 2001. HCV belongs to the *Flaviviridae* family, and has been classified in the separate genus hepaciviruses. Research indicates that HCV is responsible for 50-76% of all liver cancer cases, and two thirds of all liver transplants in the developed world. Despite the risk of developing life-threatening chronic liver diseases, 90% of HCV patients who are in need of treatment against HCV today cannot afford it. Updated hepatitis C prevalence data based on published studies and/or data submitted to WHO by 131 countries/areas as of June 1999 show that hepatitis C is clearly an epidemic disease, likely to be a serious problem in newly independent states of Eastern Europe, and which affects also millions of poor people in developing countries. For instance, 8 countries - Bolivia, Burundi, Cameroon, Egypt, Guinea, Mongolia, Rwanda, and Tanzania - have reported HCV prevalence above 10% in certain population. Seven countries/areas - Gabon, Libya, Papua New Guinea, Suriname, Vietnam, Zaire, and the United Nations Relief and Works Agency for Palestine Refugees in the Near East - have reported HCV prevalence between 5 and 10%. Due to differences in the population groups studied, methods of data collection and of interpretation between countries, and since data from several countries are limited, the prevalence shown does not necessarily represent the true prevalence in a country²¹⁸. The seroprevalence of HCV in the Nile delta of Egypt increases with age from 19% in 10-19 year olds to about 60% in 30-year-old persons and is thought to be the major cause of high prevalence of liver cirrhosis²¹⁹. In terms of annual mortality, [CDC](#) mentions traditionally 8 000/10 000 deaths in the US, expected to triple within the next five years. However, it must be stressed that numbers of these deaths occur in HIV-infected individuals who present hepatic diseases (80% of HIV-infected IVUs, 25% of HIV-infected high risk sexual behavior subjects). Such data are unknown in developing countries. However, if in the US, an up-to-date country for therapeutic management of hepatitis C, 10 000 deaths occurred in 2001, representing 0.25% of the 4 millions HCV-infected American patients, plus the 10% HIV-infected deaths with liver viral infection, a total of 12 000 deaths is reached in the US for 2001. This figure can be compared to the 18 000 deaths due to HIV the same year. One can easily imagine that the situation is worse in developing countries. Better and more extensive disease burden data are urgently needed in developing countries, including those with high HIV prevalence.

Hepatitis C prevalence in Europe is not clearly established. It could be at least 1.03% of the general population, which represents 8.9 million infected people. Patient communities like hemophiliacs, drug addicts or people transfused with blood before 1990 are particularly affected by the disease. Furthermore, the situation has not been assessed in many countries and varies according to the area and population sample. Thus, there is a pressing need to support multicentre studies conducted in larger samples to obtain more accurate and reliable data on the prevalence, mortality and morbidity related to hepatitis C in the European general population.

Mother-to-child HCV transmission has been widely documented. The risk of perinatal infection ranges from 3-15% in different populations. However, correlates of transmission remain to be defined and targeted studies are urgently needed to provide adequate counseling to HCV infected pregnant women and to identify possible preventive measures.

Vaccines. To minimize the health and economic impact of the disease, more efficient, better-tolerated, cost-effective therapies are needed, especially for non-responders to the current treatments. Up to 60% of all HCV-infected patients do not experience significant long-term benefits from the current interferon- and ribavirin-based combination and/or polyclonal HCV antibody therapies. No vaccine is yet available. Several vaccine approaches, essentially therapeutic, are currently in development, mostly in developed countries. In Europe, Innogenetics is a Phase IIa of an E1-based therapeutic vaccine. [Berna/Pevion](#) are testing HLA-A2- restricted core epitope peptides formulated with influenza virosomes as carriers for both therapeutic and prophylactic vaccine strategies. Several other projects are EU-funded including HCVACC, Memovax, Protarvac, Theravacc, Euroamp, and in the US, GenPhar, and [Epimmune](#), [Merix](#), and mostly [Chiron](#). Two Chinese teams are also making significant progress in HCV vaccine research: [Fudan University](#), and the [National Taiwan University, Taipei](#).

Hepatitis E

Disease burden. HEV is a non-enveloped, polydenylated, single-stranded, positive-sense RNA virus, remaining unclassified. The pace of hepatitis E research is increasing as global interest in emerging infectious diseases grows. An important proportion of HEV research is taking place in South Asia, particularly in the Indian Sub-continent, where epidemic infection was first recognized. Data on the endemicity of HEV infection have predominantly been collected in areas where outbreaks have been reported (see exhaustive review²²⁰). HEV was first identified in India in 1955, and has since been recognized in the Middle and Far East, South Asian countries, in northern and western Africa, the central Asian Republics of the former Soviet Union, in China and Hong Kong. Outbreaks (see details below) have been reported from Algeria, Bangladesh, Borneo, China, Côte d'Ivoire, Egypt, Ethiopia, Greece, India, Indonesia, Iran, Jordan, Kazakstan, Libya, Mexico, Myanmar, Nepal, Nigeria, Pakistan, southern Russia, Somalia, eastern Sudan, Tajikistan, The Gambia, Thailand, Turkmenistan, Uzbekistan, and Vietnam. In the Mekong delta river region of Viet Nam, a relatively low seroprevalence of hepatitis E indicates considerable outbreak potential given the favorable background of poor, water-related hygiene and sanitation, dependence on a likely human and animal waste-contaminated Mekong riverine system and periodic river flooding²²¹. During the last two decades, the following figures were reported: 119 000 cases in China between 1986 and 1988, 11 000 cases in Somalia, and about 4 000 cases in Mexico between 1988 and 1989, 79 000 cases in Kanpur, India, in 1991.

Recent evidence suggests that there is a low prevalence of HEV in some developed countries, indicating that HEV may cause sporadic illness or unapparent infections. Low incidence is reported in Italy and Spain (1995). Most cases of acute hepatitis E in the US, central and Western Europe have been reported among travelers and soldiers (Soviet Army in Afghanistan) returning from high HEV-endemic areas. Most outbreaks occurred following monsoon rains, heavy flooding, fecal contamination of well water, or massive uptake of untreated sewage into city water treatment plants. There is no evidence for sexual transmission or for transmission by transfusion. Vertical transmission of HEV from mothers to their infants has been reported. The risk of infection through breastfeeding is also not known. Regardless of whether HEV is endemic in the respective human population, hepatitis E is enzootic in pigs, probably worldwide²²². A zoonotic spread of HEV is not excluded, since monkeys, pigs, cows, rodents, sheep and goats are susceptible to infection with HEV (possible non-human reservoir of virus)²²³. One of the peculiar characteristics of HEV infection is that, in epidemic situations, the highest incidence of infection occurs in subjects between 15 and 40 years of age with a higher incidence in males than females²²⁴. In Nepal 75% of the infections occurred in people aged 15-34 years. Less than 10% of children under age 10 have antibodies to HEV. The low amount of intact HEV particles present in patient stools accounts for the generally lower secondary attack rate of person-to-person transmission of hepatitis E (2%) when compared with that of hepatitis A (10-20%). In most cases, HEV infection remains asymptomatic (75% of the cases during the outbreak in India in 55-56).

Clinically, HEV and HAV infections are virtually undistinguishable with pre-icteric and icteric Phases. HEV is the first cause of hospitalization for jaundice in Nepal. The disease is self-limited, and most

patients recover completely without complications or sequelae. Viremia is thought to last between 14 and 28 days in most patients with clinical disease. Both IgG and IgM antibody responses are detected soon after infection, with peak antibody titers at 2-4 weeks. No chronic or carrier state has been demonstrated after HEV infection. A low mortality rate (0.5-4%) is associated with HEV infection with the dramatic exception of pregnant women during the third trimester who can develop fulminant hepatitis with a case fatality rate of 10-42% (during outbreaks or in clinical series of sporadic infections)^{225 226 227}. An Ethiopian study found that 35% of HEV-infected hospitalized pregnant women had premature deliveries.

Vaccines. To date, four different strains of HEV have been completely sequenced (Myanmar, Mexico, Pakistan, and China). A recent phylogenetic analysis of nucleotide sequences from 26 HEV strains suggests that there are three principal genotypes: Asia-Africa (I), United-States (II), and Mexico (III). The Asian subgenotype is divided into China/Pakistan and India/Myanmar. Although HEV does not replicate well in cell culture, animal models have been developed (cynomolgus, macaques, chimpanzee, rat, tamarin). At present, no commercially available vaccines exist for the prevention of hepatitis E. However, several studies for the development of an effective vaccine against hepatitis E are in progress.

A 56kDa recombinant HEV-derived ORF2 protein produced in insect cells infected with recombinant baculoviruses has been used to vaccinate rhesus monkeys against different strains of hepatitis E. Although primates could still be infected, the vaccine protected them from the symptoms of disease²²⁸²²⁹. In collaboration with [WRAIR](#), GSK has conducted several clinical trials and is now in Phase III efficacy trial in Nepal. Results are expected in 2003. Moreover, the direct intramuscular injection of purified plasmid DNA containing the full-length ORF2 of HEV has induced a prolonged humoral immune response (less than 12 months) to the expressed structural protein ORF2 in 80% and 100% of two separate groups of challenged mice, respectively.²³⁰ Lastly, as swine HEV is immunologically cross-reactive with human HEV and their capsid genes are much conserved, swine HEV may prove useful as an attenuated vaccine for immunization against human hepatitis E through the "Jennerian" approach.²³¹

Helicobacter pylori

Disease burden. In 1982 the isolation of *Helicobacter pylori* (HP) radically changes the conceptualization of several chronic gastrointestinal illnesses including gastritis, peptic ulcer, gastric lymphoma and gastric cancer. HP is a Gram-negative, spiral, microaerophilic, and flagellated bacterium, with a unique ecological niche, the stomach, almost exclusively extracellular, and whose unique host is human being (very ancient adaptation and co-evolution). HP may be now considered as one of the most widespread infection worldwide, approaching a 50-60% prevalence rate of the population. Initially thought to be restricted to developed countries, evidence came that developing countries not only were affected but even more heavily: seroprevalences were 4-6 times higher in subjects from developing countries at early as 10 years of age and remained constantly and significantly higher in older persons²³². Its transmission is from person to person, oral-oral and fecal-oral²³³. It affects high-density population with lower socio-economic status including in developed countries. A natural reservoir in an animal species has not been identified to date²³⁴. Most newly acquired HP infections happen before the age of 10 years²³⁵ or even below the age of 2 years (Turkish in Germany and developing countries)²³⁶. The acquisition is mainly intrafamilial, mother-to-child²³⁷, or also child-to-child²³⁸. Its persistence is life-long.

HP is responsible for several pathological features: chronic (antral) gastritis, atrophic gastritis, chronic gastritis, up to 15% of them evolving into duodenal ulcer²³⁹, gastric ulcer, gastric B-cell lymphoma, gastric adenocarcinoma (defined "carcinogen" by WHO in 1994): more than 1 million new cases each year ([IARC](#) 1997). In Japan, more than 90% of cases are associated with HP and 5% of HP positive will develop gastric cancer over 10 years, i.e. 300 000 new cases per year²⁴⁰. The HP disease burden is unknown with precision. In developing countries, the HP prevalence is very high (over 80%) starting from very young ages (under 2 years old). It is responsible for hypochloridria, diarrhoea, growth retardation; favouring cholera, salmonellosis, and malnutrition^{241 242 243}. It had been postulated that in Africa high HP (sero) prevalence was associated with low gastric pathology. However, the active invasive search of accurate gastric pathology shows evidence of the contrary.^{244 245}

The treatment is based on the inhibition of the proton pump and antibiotherapy using two to three of the following antibiotics for one to two weeks: amoxicillin, clarithromycin, metronidazole, tetracycline, bismuth salts. Although in controlled trials, the treatment is more than 90% efficient, the efficacy at the level of general practitioners is likely lower. In addition, the treatment cost is high and the compliance poor (several tablets / day depending on the therapeutic scheme), accompanied by side effects (abdominal pain, nausea, diarrhoea, etc), and increased resistance (50% or more to metronidazole; 20% or more to macrolides; 20-40% to ampicillin). Moreover, reinfections are possible (from 1% to 50% reported). There is a high proportion of severe gastric pathology without previous history of symptoms.

Vaccines. The organism has been shown to be heterogeneous at a genetic level ([Jordan Report 2000](#)). Micro heterogeneity can indeed be very high at the level of single genes or at the level of some specific areas in these genes; however, at the genomic level, the variability is no higher than about 7%. Several vaccine approaches are being pursued and prophylactic vaccination has been proven feasible in animal models (mice, dogs, etc). Whole-cell vaccines and selected antigens (urease, VacA, CagA, NAP, hsp, catalase, etc) are efficacious by mucosal (oral, nasal) and parenteral (intramuscular) immunisations, but there is no known correlate of protection²⁴⁶. It is of note that the above antigens are relatively well conserved among various bacterial strains. In humans, several Phase I studies have been conducted: (i) oral live-attenuated *Salmonella* expressing urease showing no to poor immunogenicity²⁴⁷ (Oravax, now [Acambis](#)), project discontinued), (ii) oral whole-cell plus LT mutant as adjuvant, immunogenic but eliciting diarrhoea²⁴⁸ ([Antex](#)), (iii) purified urease co-administered with wild type LT ([Acambis](#), on hold), (iv) intramuscular delivery of recombinant VacA, CagA & NAP in alum, safe and strongly immunogenic ([Chiron](#)). Other companies involve Commonwealth Serum Labs (Australia). A prophylactic vaccine would be cost-effective in preventing gastric cancer²⁴⁹ and in preventing peptic ulcer (Institute of Medicine, Washington DC, 2001). An ideal vaccine against HP infection would be administered orally or parentally early in infancy with the EPI vaccines.

Human Papilloma Virus

Disease burden. Human Papillomavirus (HPV) causes cervical cancer²⁵⁰ and is the second biggest cause of female cancer mortality worldwide with 288 000 deaths yearly²⁵¹. About 510 000 cases of cervical cancer are reported each year with nearly 80% in developing countries²⁵²: 68 000 in Africa, 77 000 in Latin America, and 245 000 in Asia.

In the absence of screening programmes (routine Pap smear), cervical cancer is detected too late and leads to death in almost all cases. The highest yearly incidences are found in some countries of Central and South America (93.8 per 100 000 women in Haiti, [IARC 1996](#)), highest national incidence in the world), in Southern Africa (61.4 per 100 000 women in Tanzania), and in Asia (30 per 100 000 in India)²⁵³. The prevalence of HPV infection in the world is around 630 million, and that of clinical infections about 190 million. Worldwide prevalence of cervical cancer is 2 274 000: 1 300 000 in Asia, 409 000 in Europe, 218 000 in Africa, 172 000 in Latin America, 167 000 in Northern America, and 8 000 in Australia²⁵⁴. Epidemiological studies have reported that in the US, 75% of the 15-50 year old population is infected, 60% with transient infection (antibodies), 10% with persistent infection (detection of DNA), 4% with cytological signs, and 1% with clinical lesions²⁵⁵. The prevalence of HPV infection among sexually active women may range from 18 to 25%, especially in some populations of sexually active teenagers. In each case, these women may transmit HPV to their partners or to their infants²⁵⁶. In neonates, HPV infection may lead to papillomas in the oral cavity and in the upper respiratory tract. In HIV-infected individuals, HPV infection appears to cause extensive warts and severe and rapidly progressing disease.

Vaccines. HPV belongs to the *Papovaviridae* family. Of the many types of HPV molecularly identified to date (over 100), more than 30 types have been shown to infect the genital mucosa. It has been established that over 95% of cervical cancer cases contain HPV DNA, and five specific oncogenic HPV types (HPV-16, 18, 33 and 45) cause more than 80% of the cervical cancers diagnosed worldwide. The fact that over 99% of cervical cancer cases are associated with the presence of sexually transmitted HPV virus DNA has substantiated the basis for vaccine development. Viral recombinant proteins are being studied as antigenic components of prophylactic and therapeutic vaccine candidates. Prophylactic vaccine candidates are based on recombinant capsid proteins (L1 and L2), that self assemble into virus like particles (VLPs), which can induce antibodies that neutralize the infectious virus, while therapeutic vaccine candidates are based on the well characterized viral

oncogenic proteins E6 and E7, and are designed to induce cell mediated immune responses to eliminate infected cells.

Three prophylactic vaccine candidates are at the level of Phase III clinical evaluation. The [US National Cancer Institute](#) is developing a monovalent HPV-16 VLP vaccine produced in insect cells using the recombinant baculovirus technology. [GlaxoSmithKline](#) has licensed the product development programme from [MedImmune](#) and is now pursuing the Phase II clinical trials focusing on the bivalent HPV-16/18 VLPs vaccine candidate, also based on baculovirus technology. [Merck](#) is developing a quadrivalent vaccine using yeast-recombinant technology and based on VLPs from HPV-6, 11, 16, and 18, which is currently well into Phase III in the USA, Europe, Southeast Asia and South America. The results of a controlled efficacy trial of HPV-16 VLP became available recently²⁵⁷ and showed that the incidence of persistent HPV-16 infection and HPV-16-related cervical intraepithelial neoplasia was reduced in vaccinated women with a 100% efficacy rate over a 1.7 year follow-up period. These results suggest that immunizing HPV-16-negative women may eventually reduce incidence of cervical cancer.

Three therapeutic vaccine candidates are in Phase II clinical evaluation. Live recombinant vaccinia virus expressing the E6 and E7 genes from HPV-16 and 18 (TA-HPV) has been tested in Phase I/II trial by [Xenova](#) as therapeutic vaccine to be used in conjunction with post-surgical treatments of cervical lesions. TA-CIN, a recombinant fusion protein made up of the L2, E6 and E7 proteins of HPV-16, produced in *E. coli*, has also been tested in Phase I trial. The present development programme is aimed at developing both products in a prime and boost strategy, but still targeting only therapeutic use in cervical lesions. A similar recombinant bacterial fusion protein of HPV-16 E6 and E7 has been made by CSL and preliminary results of a Phase I study showed good immune responses. [Transgene S.A.](#) has also developed a vaccinia virus based product, carrying modified E6 and E7 proteins of HPV-16, aimed to treat cervical as well as ano-genital dysplasias, currently in Phase II clinical evaluation. [Stressgen](#) has initiated Phase II clinical trials with a fusion protein of E7 and heat shock protein (Hsp-E7) to stimulate immune system to remove diseased cells. Stressgen reported data on the efficacy of HspE7, demonstrating that HspE7 is likely active in anal intraepithelial neoplasia in converting most patients from high grade to low grade squamous intraepithelial lesions within 3-6 months of starting therapy. Elucidation of the full extent and duration of the clinical benefit requires additional long-term follow-up. In addition, [Zycos Inc.](#) is developing a DNA plasmid based therapeutic vaccine, and a Phase 2b study demonstrated resolution of 43% pre-cancerous lesions caused by HPV in vaccinated women as compared to 23% of patients treated with placebo.

The greatest applicability would be obtained from a vaccine that possesses both prophylactic and therapeutic properties. In this respect, the [US NCI](#) and [Medigene AG](#) in Collaboration with Schering A.G. have developed the so-called "chimeric" VLP (CVLP) technology, which allows combining both prophylactic and therapeutic components. These vaccine candidates are based on L1 or L2 recombinant proteins fused to the E7 or E2 modified oncogenic antigens. The safety of such vaccine candidates has been successfully tested by Medigene their but immunogenicity and efficacy remains to be established.

Epstein Barr Virus

Disease burden. In developed countries 25-50% of adolescents are seronegative to Epstein Barr Virus (EBV). In 25-70% of these seronegative subjects, EBV infection results in infectious mononucleosis. In limited geographical areas, EBV is associated with Burkitt's lymphoma and nasopharyngeal carcinoma. In immunocompromized individuals, including AIDS, EBV is associated with lymphoproliferative diseases and lymphomas²⁵⁸. Recent evidence suggests a possible association with Hodgkin's lymphoma, T-cell lymphomas, and about 10% of gastric carcinomas.

Burkitt's lymphoma (BL) is a malignant form of tumor associated with EBV and endemic to central parts of Africa and New Guinea with an annual incidence of 6-7 cases per 100 000 with a peak incidence at 6 or 7 years of age. It is associated with holoendemic malaria and occurs sporadically within these regions. The transformation of B-lymphocytes by EBV occurs within the first few years of life. The epidemiological involvement of EBV in Burkitt's lymphoma is based on the recognition of the EBV viral genome in tumor cells, based on DNA fingerprinting, using techniques such as Southern blotting and PCR. An elevated antibody titer against EBV (VCA) is also observed in BL patients. The distribution of BL in Africa, the "lymphoma belt," a region of high incidence in Africa, extending from West Africa to East Africa between 10th degree north and south of the equator and continuing down the

eastern coast south. Temperature and humidity were associated with the belt, which was later considered to be the reason for an association of malaria with BL. In areas where EBV infection occurs at a very early age and malaria is holoendemic, the incidence of association with BL is highest. In African countries in the lymphoma belt, such as Uganda, there is a very high association between BL and EBV (97%). In Northern Africa, the association drops to 85% in Algeria. However, in France and the United States, the rare cases of Burkitt's lymphoma are only associated with EBV in 10-15% of all reported cases²⁵⁹.

Nasopharyngeal cancer (NPC) incidence rates are less than 1 per 100 000 in most populations. In persons of Chinese descent, these rates are much higher, with elevated cases in southern China, with an annual incidence more than 20 case per 100 000. Isolated northern populations such as Eskimos and Greenlanders also show higher incidence. Southeast Asian populations show lower incidences, Japan and Northern China show almost none. In Tunisia, Morocco, and Algeria there is moderate incidence. There is also moderate incidence in Kuwait, the Sudan, parts of Kenya, Uganda, and in Israel. Men are twice as likely to develop nasopharyngeal carcinoma as women. The rates general increase from ages 20 to around 50. In the United States, African-Americans develop NPC at a higher rate than Caucasians until middle age, with a sevenfold increase in the under 20 age group. Chinese-Americans comprise the majority of NPC patients, with a peak similar to the general rates. Mortality rates in Taiwan have shown an association between NPC and salt workers and miners. Studies related to nutrition and diet seemed to confirm an association between eating highly salted foods and NPC. Vitamin C deficiency at a young age may also be a contributing factor. In the United States, those workers who were exposed to fumes, smoke, and chemicals were at highest risk, implying a role for chemical carcinogenesis. A study of MHC haplotypes revealed a genetically distinct subpopulation in Southern China, which may account for the highest incident areas. Chinese NPC patients were shown to have an increased frequency of HLA-A2 and a decreased frequency of antigens detected at the second locus. This blank was named Sin-2 (or B46). This haplotype, A-2-B-Sin-2 was shown to have an elevated relative risk of developing NPC. Study of MHC haplotypes in other populations has indicated a possible genetic predisposition as well²⁶⁰.

Vaccines. The principal target of EBV neutralizing antibodies is the major virus surface glycoprotein gp220/350. A range of cell-mediated responses to EBV infection has also been described and is likely to be important in controlling persistent infection. CTL specific for the latent EBV nuclear antigens EBNA-3A, -3B, and -3C are predominant in a large portion of seropositive adults and children. Several vaccine candidates based on the gp220/350 have been developed. The soluble recombinant subunit is safe in humans but needs strong adjuvants to elicit acceptable efficacy (co-development by [MedImmune](#), GlaxoSmithKline (GSK) and Henogen, a GSK joint venture managing clinical trials up to Phase 3). Live recombinant vaccinia vectors have been used to express the gp220/350 and confer protection in primates and in EBV-negative Chinese infants. Clinical trials of an EBNA-3A peptide have been conducted in Australia ([Jordan Report 2000](#)).

Poliomyelitis

Disease Burden. The number of estimated annual poliomyelitis cases for the year 2000 was 3 500 with 350 deaths. The World Health Report 2002 mentions 1 000 deaths ([WHO, 2002](#)). Poliovirus belongs to the *Picornaviridae* family. Wild poliovirus remains endemic in 8 countries. The eradication of wild poliovirus, a long-sought public health goal is possible and close. However, a recent outbreak of paralytic polio in Hispaniola (Haiti and the Dominican Republic) has important implications for current global efforts²⁶¹. Rapid progress in poliovirus eradication owes its success largely to the widespread of the oral poliovirus vaccine (OPV). After ingestion, OPV replicates in the human intestine, with the generation of revertant phenotypes, which may resemble the neurovirulence of wild poliovirus. A high proportion of immunized subjects, perhaps 30% or more, excrete revertant OPV strains or vaccine-derived polioviruses (VDPV). These characteristics have important implications. If a population is immunized close to 100%, the vaccines are exposed to the attenuated vaccine virus and develop immunity before the VDPV can cause paralysis. However, if immunization coverage is incomplete, then a VDPV may spread sequentially through several non-immunized persons, accumulating mutations and reversions, increasing the likelihood of paralytic poliomyelitis in this population. Such conditions were those described in Hispaniola. The "endgame" in polio eradication poses a new challenge then. Once complete eradication of wild poliovirus has been achieved and certified, there are several options including discontinuation of OPV and replacement by IPV that may also be discontinued provided the assurance that OPV and VDPV have disappeared from the world, final round of mass OPV and /or IPV with careful monitoring and surveillance of wild poliovirus and VDPV. The

ultimate goal of the eradication programme is the discontinuation of all polio immunization. Inevitably, an increasing number of people would become susceptible to these viruses²⁶². The destruction of virus stocks and the possible threat of biological terrorism may be new challenges to face.

Measles

Disease burden. Measles, in spite of available vaccination, remains a heavy public health burden worldwide especially in developing countries with 30-40 million cases, 26 million DALYs ([WHO, 2002](#)) and 745 000 deaths ([WHO, 2002](#)) for the year 2001. This represents 50-60% of the estimated million deaths attributable to vaccine-preventable diseases of childhood. Measles may be ultimately responsible for more child deaths than any other single agent because of complications from pneumonia, diarrhoea and malnutrition. Measles is also the major cause of preventable blindness in the world, affecting the same disadvantaged populations.

Of the deaths attributable to measles, 98% occur in developing countries, where vitamin A deficiency is common. Case-fatality rates in these countries are usually estimated to be in the range 1-5% but may reach 10-30% in some situations. Specific goals for reduction in measles mortality and morbidity were set by the World Health Assembly in 1989 and the World Summit for Children in 1990, as major steps towards the eventual eradication of the disease. Subsequently, target dates of 2000, 2007 and 2010 for its elimination were established for the Region of the Americas, the European Region and the Eastern Mediterranean Region respectively. The aim in the African Region, the South-East Asia Region and Western Pacific Region is to reduce measles mortality.

Several strategies are now developed to increase coverage of immunization including a two-dose schedule, mopping up strategies, supplementary immunization strategies such as vitamin A supplementation, one-round national and regional mass immunizations, and development of high-quality case-based measles surveillance supported by regional measles laboratory.

Vaccines. Measles vaccination is one of the most cost effective health interventions available and one of the most powerful tools for providing health equity to poor children. It is cost-effective to improve routine measles vaccination, as preliminary estimates suggest that the cost per life-year gained for expanding measles coverage from 50% to 80% is US\$ 2.53 in areas with high disease incidence and high measles case-fatality ratios. Measles vaccine is highly effective, safe and inexpensive. With US\$ 0.15 for one measles vaccine dose, children in developing countries can survive exposure to measles without sequelae. However, coverage with measles vaccine is low in many countries due to limited resources. Coverage could be greatly enhanced if the method of administration could be simplified. Current measles vaccination requires injection with a needle and syringe. The drawbacks of the needle and syringe technology are as follows: 1) it requires highly skilled personnel to administer the vaccine; 2) it is associated with a risk of transmitting blood-borne diseases such as hepatitis and HIV if syringes and needles are re-used. This risk can be minimized if auto-disable syringes are used and 3) injection may be painful and present a risk of infection if a proper technique is not used.

As the natural route of infection for measles virus is the respiratory tract, administration of live attenuated measles vaccine through the respiratory tract has been investigated as an alternative to injection. Early studies have shown fewer acute adverse events following aerosol vaccination, as compared to conventional parenteral vaccine. Aerosolized vaccine is immunogenic and effective in seronegative and seropositive children. More than 4 million children were vaccinated with aerosolized measles vaccine in mass immunization campaigns in Mexico with good public acceptance. Aerosol vaccination can be performed by non-medical staff with some training. As aerosol vaccination uses the same vaccine formulation as parenteral vaccination, most cold chain procedures are identical. Now that the development of a respiratory route of administration is so promising for measles vaccine, WHO has convened a Product Development Group (PDG) to identify critical licensure steps, define clinical trial strategies and assist in protocol design, identify sites for clinical trials and ensure adequate implementation, monitoring and documentation of good practice. Following the current work plan aerosolized measles vaccine could be licensed in 2007 and introduced in practical use in 2009. This project is managed as a partnership between WHO, [CDC](#) and the [American Red Cross](#), with funding from the [Bill & Melinda Gates Foundation](#).

In addition, studies are in progress to develop new measles vaccine effective for immunization of infants before 6-months of age. Indeed, infants are refractory to conventional measles vaccines in the

presence of maternal anti-measles antibodies. To reach this objective several technologies are currently being tested including DNA vaccines, bacterial vectors, viral vectors (e.g. adenoviruses, poxviruses, alpha viruses) or ISCOMS.

Rabies

Disease burden. More than a century after the first successful prevention of rabies by vaccination in 1885, this zoonotic viral disease continues to plague humankind, especially in developing countries, in South and South East Asia, Africa, and to a lesser extent South America. According to WHO data²⁶³, more than 2.5 billion people are at risk in over 100 countries reporting the disease. According to a preliminary estimate there are still about 50-60 000 human deaths annually although effective vaccines for post-exposure prophylaxis (PEP) are available. More than half of the deaths occurs in India and Bangladesh. The true disease burden of rabies is thought to be largely under-estimated especially in Africa. For example, the annual death rate per 100 000 persons is 18 in Ethiopia, whereas 2-4 and 1.75 in India and Bangladesh, respectively. In Tanzania, predicted human rabies mortality was estimated to be 1 499 deaths per year equivalent to an annual incidence of 4.9 deaths/100 000 when active surveillance data on bite incidence were used, and 193 deaths per year, corresponding to an annual incidence of 0.62 deaths/10 000 when national statistics were used²⁶⁴. The vast majority (95-98%) of these deaths worldwide occur in canine (dog rabies) endemic regions with large stray dog population²⁶⁵. Control of disease is hampered by cultural, social and economic realities (Buddhist and Hindu ethics restrain culling of the canine population. India has prohibited the killing of stray dogs by individuals or municipalities). The ineffective control of the stray dog population in countries such as India and the too often unavailable and inadequate human PEP are responsible for this high mortality rates.

Vaccines. Rabies virus belongs to the *Rhabdoviridae* family of negative and enveloped viruses. Several effective vaccines have been developed and are currently available worldwide^{266 267}. The nerve tissue-derived vaccines prepared from sheep, goat, or rabbit brains (Fermi, Semple vaccine in India) and from suckling mouse brains (Fuenzalida vaccine in South America) are phenol-inactivated, not very immunogenic requiring numerous injections, may not be safe (possibility of sheep contamination with prions) and highly reactogenic (contamination with brain proteins). This type of vaccine is unfortunately still manufactured and widely used in South and South East Asia. However, very recently India took the decision to ban the use of brain-derived rabies vaccines, and to replace them by cell line-cultured vaccines like the PCECV and will attempt to develop the use of intradermal schedules (Thai Red Cross). The avian-derived vaccine (duck embryo), purified by ultra centrifugation (PDEV) is no longer manufactured in Europe (Berna Biotech). The new generation of rabies vaccines is derived from cell cultures: human fibroblasts (HDCV, [Chiron Behring](#), [Aventis Pasteur](#)), fetal rhesus cells (RVA, Bioport), primary Syrian hamster kidney cells (PHKCV, local manufacturers), chick embryo (PCECV, Chiron Behring, 10% of the Indian market) and Vero cells (PVRV, [Aventis Pasteur](#)). They are inactivated by β -propiolactone or formalin. Manufactured mainly in the developed world but distributed worldwide, they are safe and immunogenic. A promising technology developed within the framework of the WHO/[Rockefeller](#) technology transfer programme for rabies vaccine production consists in production on Vero cell using high density microcarriers in a continuous extraction small (20 liters) bio-reactor and chromatography purification. The technology and final product would need to be further tested for GMP batch consistency and in human trials prior to implementation in the field. Of importance for the supply of rabies vaccine is the use of the Thai Red Cross intradermal route schedule, which reduces the number of vaccine vials and the cost of PEP by 60% to 80%²⁶⁸.

Rabies immunoglobulins. However, it has been for long well established that PEP with vaccine alone is not sufficient and its use as only therapeutic agent is responsible for heavy death toll, especially in case of severe head and face bites, in particular in countries still poorly immunogenic using nerve-derived vaccines. The analysis of failure of PEP is not the failure of cell culture vaccines but inadequate management of PEP particularly in regions where short incubation periods are frequently observed and where vaccines and more often rabies immunoglobulins (RIG) are in short supply and very expensive. The worldwide shortage of RIG represents a real public health threat and new challenge^{269 270}. According to the WHO World Survey of Rabies 2000 (see [WHO website](#)), more than 7.6 millions PEP worldwide are offered, the vast majority without RIG because of their unavailability and unaffordability. Some Asian countries (notably China, Myanmar, Nepal, some Indian states, and former Soviet Central Asian republics) have made the calculated decision to accept treatment failures

because of other priorities and the unavailability of RIG. Two types of RIG Human (HRIG in developed countries and Thailand) and equine (ERIG) are currently manufactured. ERIG are either pepsin-digested (Thai Red Cross, India, and formerly Berna, Chiron, Aventis Pasteur) or highly purified (heat treatment and chromatography purification steps). This latter product was developed and is manufactured by Aventis Pasteur in order to meet the highly stringent regulatory environment in Europe and North America. The supply of affordable RIG for developing countries can be seen from two different points of view: either reinforce and expand the local manufacturing of ERIG or to develop new technologies thought to be more appropriate for large scale manufacturing and hopefully more affordable in the future such as rabies monoclonal antibodies²⁷¹. It is to be noted that, in view of the current cost of HRIG (over US\$ 100) and ERIG (over US\$ 40), the price of a cocktail of 2-3 mouse monoclonal antibodies (MRIG) might be highly competitive (expected price over US\$ 10). Interestingly, whereas ERIG can in some experimental conditions suppress the vaccine activity, MRIG were not shown to be suppressive (Charles Rupprecht, personal communication). Rather than being a PEP adjuvant, MRIG could be used for curative treatment, but this would need extensive clinical trials to validate the threshold of MRIG potency for their use as adjuvant of vaccines. Such “high tech” products may appeal some countries and foster the use of RIG. India, The Philippines and China have already expressed their strong interest in developing such technology.

Group A *Streptococcus*

Disease burden. The spectrum of diseases caused by group A β -hemolytic *Streptococcus pyogenes* (GAS) is broad, ranging from simple and uncomplicated pharyngitis and pyoderma to severe invasive infections and the post streptococcal nonsuppurative sequelae of acute rheumatic fever and acute glomerulonephritis. The incidence of rheumatic fever has declined in industrialized countries since the 1950s and now has an annual incidence of around 0.5 cases per 100 000 children of school age. GAS is highly transmissible, and their pattern of spread in families and communities is dynamic; predominant serotypes are constantly being replaced by others. “Crowding” is important when considering the epidemiology of GAS respiratory tract infections in socially and economically disadvantaged populations. Current understanding of the epidemiology of GAS infections comes largely from studies in industrialized countries, where pharyngitis and tonsillitis are very common in children aged 5–15 years, whereas streptococcal pyoderma is less common and mostly seen in children aged under 5 years. Although penicillin has been the antibiotic of choice to treat GAS infections for persons over 50 years, to date there has never been a clinical isolate of GAS that is resistant to penicillin. Despite this fact, bacterial failure of treatment with penicillin has been reported in up to 25% cases of GAS pharyngitis. Half of these are also associated with clinical failure. This is attributed to protection of GAS from penicillin by other beta lactamase-producing organisms in the pharynx. Resistance to the sulfa drugs and tetracyclines quickly became a problem following their introduction into clinical practice. The only other group of antibiotics to which resistance has developed to any important level has been the macrolides.

In developing countries, rheumatic fever remains an endemic disease with annual incidences ranging from 100 to 200 per 100 000 school-aged children and is a major cause of cardiovascular mortality²⁷². Australia's Aboriginal population suffers the highest incidence worldwide. GAS, *S. pneumoniae* and *Staphylococcus aureus* are important causes of severe infection in young children in the Papua New Guinea highlands²⁷³. The importance of GAS in severe infections in Gambian infants is relatively low compared to other bacterial pathogens²⁷⁴. However, the disease burden attributable to GAS is far from being clear. Observations raise the intriguing question of how this apparently low prevalence of group A streptococci in the upper respiratory tract (over 5%) and a high incidence of pyoderma can be correlated with high rates of acute rheumatic fever in the same communities, in light of the generally accepted dogma that acute rheumatic fever follows only group A streptococcal infection of the throat, not of the skin. Several explanations have been proposed. One is that in tropical regions, throat-isolation rates of groups C and G are higher than those for group A. In Indian school children, the prevalences of group G and GAS were 43.2% and 28.8%, respectively²⁷⁵. Groups C and G streptococci can cause invasive disease and pharyngitis. Group C *Streptococcus* can cause acute poststreptococcal glomerulonephritis. Both may express M protein, and there is evidence of horizontal M-protein gene transfer between group A and group G *Streptococcus*²⁷⁶. It is unclear whether these other serogroups of β -hemolytic streptococci may alter the epidemiology and recovery of group A streptococci or to what extent they may have the potential to cause suppurative or nonsuppurative complications.

The lack of implementation of primary prevention of rheumatic fever in the developing world has occurred for a number of reasons. These include the lack of financial and medical resources, the scarcity of laboratory facilities necessary to reduce the overuse of penicillin and other antibiotics (in the absence of proper diagnosis), and difficulties with providing adequate professional education for health care workers.

There is a clear need for well-planned, prospective, longitudinal studies to understand more completely the epidemiology of GAS in developing countries and to implement more effective public health prevention programmes. There is also a need for continuing laboratory research to better understand the pathogenesis of acute rheumatic fever and acute post streptococcal glomerulonephritis. This is essential to the development of a cost-effective group A streptococcal vaccine(s)²⁷⁷.

Vaccines. Immunity to group A streptococci is mediated by antibodies against the M protein, a coiled-coil alpha helical surface protein of the bacterium. Vaccine development faces two substantial obstacles. Although opsonic antibodies directed against the N terminus of the protein are mostly responsible for serotypic immunity, more than 100 serotypes exist. Furthermore, whereas the pathogenesis of rheumatic fever is not well understood, increasing evidence indicates an autoimmune process. To develop a suitable vaccine candidate, researchers have first identified a minimum, helical, non-host-cross-reactive peptide from the conserved C-terminal half of the protein and displayed this within a non-M-protein peptide sequence designed to maintain helical folding and antigenicity, J14. As this region of the M protein is identical in only 70% of GAS isolates, the optimal candidate might consist of the conserved determinant with common N-terminal sequences found in communities with endemic GAS. Seven serotypic peptides were linked with J14 using a new chemistry technique that enables the immunogen to display all individual peptides pendant from an alkane backbone. This construct demonstrated excellent immunogenicity and protection in mice²⁷⁸. Moreover, a 24-valent M protein based vaccine is currently undergoing evaluation. In addition, there are a few candidate vaccines based on non-M protein antigens not detailed in this analysis. Lastly, [ID Biomedical](#)²⁷⁹ is currently evaluating their peptide vaccine in Phase I/II.

The duration of protection would be a critical parameter to consider for the introduction of this vaccine in EPI, since mainly targeting school-aged children. Efficacy trials may be difficult and long in their implementation, requiring large sample sizes, especially if efficacy endpoints are clinical endpoints.

Group B *Streptococcus*

Disease burden. Women colonized with Group B streptococcus (GBS) during pregnancy are at increased risk of premature delivery and perinatal transmission of the organism. Pregnancy-associated GBS disease is most often manifest during labor or within the first few days of an infant's life. It can result in maternal sepsis, and very rarely, meningitis. GBS is also the leading cause of chorioamnionitis and is one of several bacteria now thought to enhance the risk of preterm premature rupture of membranes. Newborns can acquire GBS by aspiration of infected amniotic fluid or during passage through the birth canal. Vaginal colonization with GBS occurs in about 20% of American women, 6% in Peru, 6.2% in Thailand, 12% in India and Pakistan to 22% in North Africa and the Middle East, and 27.6% in Saudi Arabia^{280 281 282 283}. However, GBS is not a very common cause of invasive disease in newborn infants in both developed and developing countries. In the USA, GBS is the most common cause of neonatal infection²⁸⁴. The incidence of neonatal sepsis and meningitis due to GBS is 0.5-3 cases per 1 000 live births with substantial geographical and racial differences. In the USA, the case-fatality ratios are now much lower than in the 1970's (over 50%) and the 1980's (15-25%)^{285 286}. The apparent lack of significant clinical disease due to GBS in less developed countries is puzzling. In a WHO collaborative study on serious infections in young infants conducted in 4 developing countries, GBS comprised only 2 of 167 blood culture isolates and 1 of 40 CSF isolates. In contrast, GAS comprised 29/167 blood isolates and 3/40 CSF isolates²⁸⁷. In India, the incidence of GBS bacteraemia was 0.17 per 1 000 live births²⁸⁸. However, such data may suffer of some bias. Detection of early-onset infections may be obscured by the large proportion of deliveries that take place outside health centers and the probability that infants who develop GBS sepsis on the day of birth will not survive. Other clinical presentations of GBS morbidity may predominate such as miscarriages. Since exposure to the organism seems to be similar in pregnant women in developing and industrialized countries, the failure to recognize GBS as an important cause of neonatal sepsis in developing countries could reflect the insufficient surveillance or true population differences in clinical diseases. One hypothesis is that GBS-related morbidity among women in developing countries may more often manifest through preterm delivery, in which case

infants may not survive to develop confirmed sepsis. Further community-based studies are warranted to determine with more accuracy the disease burden of GBS in developing countries.

Vaccines. Early-onset of GBS disease in neonates can be prevented by the use of intrapartum chemoprophylaxis, based on risk factors on the mother. The implementation of such a strategy, suggested in South Africa for example²⁸⁹, depends however largely on the availability, accessibility and quality of health care in developing countries. These factors vary greatly among countries. It is therefore unlikely that such strategies will significantly reduce the GBS disease burden. Immunization of mothers represents a very attractive alternate strategy²⁹⁰. GBS vaccine development is quite advanced. Capsular polysaccharide conjugate vaccines (conjugated to either recombinant cholera toxin B subunit or tetanus toxoid - TT) have been tested in mice^{291 292}, including by intranasal immunization,²⁹³ and in nonpregnant women²⁹⁴. In this latter group, the bivalent Ia- and Ib-TT conjugate vaccine was well-tolerated and the immune responses to the conjugates were dose-dependent and correlated with *in vitro* opsonophagocytosis. [Microscience](#) (USA) is developing a vaccine based on novel protein vaccine candidates. A first difficulty in developing GBS vaccines is the existence of several serotypes with different geographical distribution and the very heterogeneous cross-reactivity between serotypes. A vaccine suitable for Asian or European populations may not be suitable for African populations^{295 296}. Continuous monitoring of GBS serotypes seems necessary for the design of a GBS vaccine²⁹⁷. Another major difficulty similar to that of GAS vaccines is the implementation of efficacy trials. The evaluation of investigational GBS vaccines would require large sample sizes and would take a long time to evaluate efficacy if the vaccines were administered to women before pregnancy. Administering the vaccine to pregnant women may be difficult because of the incidence of birth defects: their occurrence with vaccination would be difficult to evaluate. To overcome these obstacles, licensure of such vaccines may be based on the induction of GBS type-specific antibody without efficacy trial. It was recently shown that a vaccine that induces IgG GBS Ia antibody levels $\geq 5\mu\text{g/mL}$ in mothers can be predicted to confer a high degree of type-specific immunity to EOD to their infants²⁹⁸.

Leptospirosis

Disease burden. Leptospirosis is an acute bacterial infection which affects humans and a wide range of animals. It is probably the world's most widespread zoonosis (over 100 000 human cases and over 1 000 deaths worldwide annually²⁹⁹), due to infection by *Leptospira* (23 serogroups and more than 200 serovars). It remains underdiagnosed largely to the broad spectrum of signs and symptoms attributable to this spirochetal pathogen. At the first meeting of the [International Leptospirosis Society](#) (ILS) held in Nantes (France) in 1996, a proposal was endorsed to collect worldwide figures on the incidence of leptospirosis. The ILS expressed concern that reliable figures on morbidity and mortality were generally lacking and that the disease was often overlooked and underreported in tropical countries. The ILS (through the WHO/FAO Collaborating Centre for Reference and Research on Leptospirosis, Western Pacific Region) issued surveillance questionnaires to members from representative countries. The data returned were to be collated and reported in collaboration with the World Health Organization. This is the first-ever attempt to collect such data. The website holds all previously published quarterly and annual reports from the surveillance programme (<http://www.health.qld.gov.au/qpsb/sciencsr/who/home.htm>). The worldwide survey of leptospirosis over the years 1998, 1999 and 2000 undertaken by [the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis](#), Brisbane, Australia, has revealed an average of over 15 000 severe leptospirosis cases annually with a mortality ranging (on the average) between 5 and 20%. The survey covered 8% of the world population is therefore an underestimation of the disease burden. More precise estimates, based on a previous worldwide survey, is currently about 5 million cases and 500 000 severe cases worldwide, with a mortality ranging from 5-0%.

The disease occurs more commonly in tropical countries. Leptospirosis is highly endemic in warm humid areas, especially in areas of rice culture, with typical incidence numbers for severe (hospitalized) cases of at least 10 per 100 000 annually. Poor and slum areas are high-risk areas for leptospirosis. Urban disease may be considered as mainly a poverty disease.

In SEAR countries, leptospirosis is endemo-epidemic and documented in India, Indonesia, and Thailand. The National Institute of Communicable Diseases in India ([NICD](#)) reviewed the published literature in 1998³⁰⁰. In Gujarat and Kerala, 112 cases (12 deaths) and 211 cases (11 deaths) were reported in 2000, respectively. Animals reported as hosts were rats, cattle, sheep, rodents, mongoose, donkeys, horses, camels, bandicoots, pigs, dogs, and cats. In the wake of massive floodings in

Indonesia in January 2002, a leptospirosis outbreak occurred, notably in the capital Jakarta in Java. In Thailand, the annual number of cases reported between 1982 and 1995 ranged from 55 to 272 (incidence 0.3/100 000). Severe disease is mainly predominant in Northern Thailand in farmers working bare foot in rice paddy fields during the rainy season from June to October. Thailand spends every year between US\$ 6-7 million for the treatment of severe leptospirosis.

Man becomes infected through direct or indirect contact with infected animal urine or, less frequently, from animal bites, handling infected animal tissues or swallowing contaminated food or water. Person to person spread is exceptionally rare. The bacteria enter through skin abrasions or through the eyes, nose and mouth. The primary reservoir is rodents because, once infected, they shed the organisms for life. Dogs are also a major source for human infections.

Most cases present with an influenza-like illness, sometimes associated with diarrhoea and vomiting or meningitis. In a few cases a generalized infection with bacteremia is observed. Multiplication in the kidneys leads to shedding in the urine, which may persist for years. Damage to the endothelial lining of capillaries and renal failure are the most common reasons for death. Occasionally the central nervous system may become involved.

Vaccines. Immunity against *Leptospira* is primarily humoral. Some inactivated vaccines are currently available in very limited quantity in countries that vaccinate workers highly exposed to infection in China, Japan, Italy, France and Spain. These vaccines are however not very immunogenic. Thailand has expressed the interest to develop such a vaccine for their country and possibly for the region, assuming that sero-epidemiological data would be available for the Mekong Region. Publication of the whole *Leptospira* genome is eagerly awaited. Several other vaccine approaches are pursued: in Russia inactivated vaccine (tested in humans)³⁰¹, in China DNA expressing p68 tested in guinea-pigs³⁰² and recombinant BCG expressing the outer envelop antigen gene OmpL1³⁰³, and in the USA OmpL1 and LipL41 outer membrane proteins³⁰⁴. An inactivated vaccine formulation has also been tested in Cuba. New efforts to develop leptospirosis vaccines should be strongly encouraged³⁰⁵.

Section II - Current research and development advancement status for new vaccines and biologicals

Disease or syndrome	DALYs (000) in 2001 (WHR 2002)	Estimate of annual cases (000) for 2001	WHO estimate of annual deaths (000) for 2001 (WHR 2002)	R&D advancement status	Pharmaceutical company and/or institution
Diarrhoeal Diseases	62 451	> 900,000	2,001		
Rotavirus		125 000	600	<ul style="list-style-type: none"> - Lamb-derive live attenuated (LA), licensed in China but controlled Phase III pending, China - Human neonatal-derived strain Phase II Europe, US and Latin America, Banglades, South Africa - Bovine reassortant quadrivalent LA, Phase III - Neonatal-derived LA, Phase I - Neonatal-derived LA 	<i>Lanzhou Institute Biomed. Products, China</i> GSK Merck Australia <i>Bahrat, India</i>
ETEC		400 000	700	<ul style="list-style-type: none"> - Inactivated, with cholera TB, oral, Phase I/II, Sweden, Egypt, Bangladesh, Israel, Nicaragua - Live attenuated CVD, shigella/ETEC, oral, preclinical - Live attenuated, Phase I - Live attenuated S.typhi strain expression ETEC gene, preclinical - Encapsulated colonization factor, Phase I - Transcutaneous injection CS6-LT 	U. Göteborg, Sweden U. Maryland <u>Acambis, Berna</u> <u>Microscience</u> USA US Navy MRI
Shigella		200 000	1 100	<ul style="list-style-type: none"> - Live attenuated strain SC 602, Phase I, USA, Bangladesh - O-rEPA, Phase III - Live attenuated CVD 1207 - Whole cell inactivated, preclinical - Ribosomal preparation, preclinical 	Institut Pasteur (Paris), WRAIR, <u>IVI</u> NIH CVD, U. Maryland <u>Antex</u> <u>IVI/WRAIR</u>
Cholera		137	5	<ul style="list-style-type: none"> - Killed O1 and O139, oral, Phase II, Viet Nam - Live attenuated strain, oral, Phase II - Live attenuated strain, oral, Phase I, Cuba - O Ag-conjugated, Phase I (USA), preclin. (France) 	<i>Nha-Trang, Viet Nam</i> <u>Avant Ther./BioSidus</u> <i>Finlay I.</i> NIH, Institut Pasteur

				- Killed O1	<u>Powderject, Sweden</u>
Typhoid		16 000	600	- Vi-EPA conjugated, Phase III, Mekong Delta - Ty800 live attenuated, oral, Phase I, USA - live attenuated strain, oral, Phase I/II - ACAM 948-CVD live attenuated, oral, Phase I, USA, Swiss	NIH <u>Avant</u> <u>Microscience</u> <u>Acambis, Berna</u>
Caliciviruses		1-10 000	100-500	- rNVL VLP, Phase I	CVD, U. Maryland
Acute Respiratory Infections	94,037	> 70 000	3 947		
<i>Streptococcus pneumoniae</i>		10-100 000	1 000	- Conjugate 9-valent/Mening C DTCRM vaccine, Phase III - 11-valent conjugate vaccine, Phase III - Pneumolysin, PsPA, PsaA, neuraminidase, autolysin, Phase I - BVH-3 and BVH-11 protein vaccines	Wyeth GSK Aventis Pasteur, GSK <u>Shire Biologicals</u>
Respiratory Syncytial Virus		64 000	160	- PFP-1 and PFP-2, Phase I/II in children and pregnant women - Pre-clinical - Subunit vaccine, Phase II - Synthetic peptide based on G2 protein, preclinical - Live attenuated, nasal, Phase II - virosomes, preclinical	NIH, Wyeth <u>MedImmune</u> Aventis Pasteur Wyeth <u>Berna Biotech</u>
Para Influenza Virus type 3		> 10 000	10-100	- Bovine PIV-3, Phase I/II - Human cold-adapted PIV-3, Phase I - Chimeric PIV-1/3, preclinical - Phase I - Phase I/II - virosomes, preclinical	NIH NIH <u>MedImmune</u> Wyeth <u>Berna</u>
Influenza Virus		1 000 000 (pandemic: <2,000,000)	200-500 (1.5 000-3.5 000)	- Live attenuated cold adapted strain, Phase III - Virosomes - Live attenuated, nasal spray - peptides - DNA vaccines	<u>MedImmune/Wyeth</u> <u>Berna Biotech</u> <u>Biodem Ltd/Merck</u> <u>Yeda R&D</u> <u>CDC, Vical, PowderJect</u>
Tuberculosis	36 040	8 000	1 644	- Modified BCG, preclinical	UCLA, NIH, <u>Sequella</u>

				<ul style="list-style-type: none"> - Live-attenuated <i>M. tuberculosis</i>, preclinical - DNA Ag85A, HSP65, 36kD, preclinical - Subunits, peptides, nonprotein antigens, vector expression, preclinical - BCG prime/poxvirus recombinant boost, Phase I 	I. Pasteur, A. Einstein I. <u>Sequella Intercell, Vienna</u> ; GSK-Corixa ; SSI, Copenhagen; UCLA; <u>MPI, Berlin</u> Oxford University
Buruli Ulcer		<200 (underreported)		- BCG	Global Buruli Ulcer Initiative (GBUI)
HIV/AIDS	88 429	5 000	2 866	<ul style="list-style-type: none"> - gp120 B/B, Phase III US, B/E Phase III Thailand - ALVAC+gp120 B/B', Phase II Caribbean, Brazil, performed - ALVAC+gp120 B/E, Phase III pending in Thailand 2002 - Recombinant adenovirus, Phase I, US - DNA, Sindbis virus, gp120 - VEE gag, Phase I US, planned in South Africa 2002 - DNA+MVA clade A, Phase I/II, UK, Kenya; Phase I/II planned in Uganda, South Africa - MVA clade C, preclinical; Phase I planned in India - DNA+fowlpox, clade E, Phase I planned in Australia and Thailand 2003 - Recombinant Salmonella, Phase I, US - Subunit with adjuvants, Phase I - DNA vaccine, Phase I - peptides, Phase I - For other approaches, see ref. 80-83 	<u>VaxGen/CDC</u> <u>VaxGen/Aventis/NIH</u> <u>VaxGen/Aventis/WRAIR/NIH</u> Merck/NIH, Chiron <u>NIH/AlphaVax</u> IAVI/BMRC IAVI/ <u>Therion/ICMR</u> Australia, <i>HIVNAT</i> IAVI/U. Maryland GSK/NIH Wyeth/U. Pennsylvania Wyeth/Duke U.
HSV-2		600 a	10-100 b	<ul style="list-style-type: none"> - Live attenuated DISC, Phase II, UK - Live genetically-attenuated replication-competent vaccines - Subunit with adjuvant, Phase III - DNA vaccine formulations, preclinical 	<u>Xenova</u> <u>AuRx Inc.</u> GSK/NIH <u>PowderJect, Merck</u>
Malaria	42 280	300-500 000	1 124	- Various recombinant blood stage antigens (MSP1 to 5, RAP2, preclinical	<u>Biotech/La Trobe U,</u> <u>Progen/Monash U,</u>

				<ul style="list-style-type: none"> - MSP1-42 - EBA175 - CS peptide in HBcAg particles- - DNA+MVA polyepitope, Phase I/II, UK, Gambia - 25kD yeast-produced protein, Phase I (transmission blocking vaccine) - Hybrid particles with HB surface antigen, Phase II, US, Belgium, Gambia, Kenya - DNA Phase I - peptides, mimetics, virosomes 	<p>Queensland IMR, <u>E MVI</u>, U. Hawai, I. Pasteur, WRAIR, <i>ICGEB India</i>, <u>EntreMed</u>, <i>Wanxing Biopharm, Shanghai</i></p> <p>WRAIR/GSK</p> <p><i>Barhat India</i></p> <p><u>Apovia/MVI</u></p> <p>Oxford U, <u>Oxxon</u>, BMRC NIH</p> <p>GSK/MVI</p> <p>US Navy MRI</p> <p><u>Pevion Biotech</u></p>
Leishmaniasis	2 357	1.5-2 000	59	<ul style="list-style-type: none"> - Killed promastigote <i>L. major</i>, Phase III, Colombia, Brazil, Iran - Killed promastigote <i>L. major</i> with BCG and alum, Phase I/II, Iran, Sudan - DNA (gp63, LACK), preclinical - Trifusion of leishmania antigens LeIF, LmSTI-1 and TSA, subunits, preclinical 	<p><i>Biobras, Brazil</i></p> <p><i>Razi Institute, Teheran</i></p> <p>Various institutions</p> <p>IDRI/<u>Corixa</u></p>
Schistosomiasis	1 760	120 000	15 f	<ul style="list-style-type: none"> - <i>S. haematobium</i> Sh28GST, Phase I, II, France, Niger, Senegal - <i>S. japonicum</i> paramyosin and Sj-GST26, preclinical - <i>S. mansoni</i> paramyosin and peptide TPI, preclinical, Egypt - <i>S. mansoni</i> Sm14 	<p>Institut Pasteur, Lille</p> <p><i>China, Australia</i></p> <p><u>Bachem</u>, USAID</p> <p><i>FIOCRUZ, Brazil</i></p>
Dengue	653	100 000	21	<ul style="list-style-type: none"> - Live attenuated tetravalent, Phase II - Live attenuated tetravalent, Phase I - Live attenuated, YF-derived, preclinical - Recombinant live attenuated dengue 2 	<p>Aventis</p> <p>WRAIR/GSK</p> <p><u>Acambis/Aventis</u></p> <p>NIH</p>

				- DNA, subunits, recombinant vaccinia, preclinical	
Japanese Encephalitis	767	50	15	- Inactivated mouse brain derived vaccine (licensed) - Attenuated SA 14-14-2, primary hamster kidney cells (licensed) - Inactivated JEV Vero cell-derived, licensed - Inactivated SA 14-14-2, Phase II, Thailand - Inactivated JEV Vero cell-derived, Phase I, Japan - Live YFV Chimera, Phase I, Phase II in Thai children pending - Several recombinant approaches, preclinical	<u>Biken</u> , <i>China, Thailand, Vietnam, Taiwan</i> <i>Glovax, Chengdu, China</i> <i>China</i> <u>VaccGen/WRAIR</u> <u>Biken</u> , Chemo-Sero Therapeutic Research Institute, <u>Acambis</u>
Meningitis	6 420 e		172		
Meningitis A, C, Y, W135		300	25-30	- A & C conjugate vaccine, Phase II/III, Africa - conjugate 9 valent pneumo/mening C, Phase III - conjugate A, C, Y W135 - A & C conjugate vaccine	Chiron Wyeth Wyeth, Aventis Pasteur GSK
Meningitis B		20-80	2-8	- OMV (Outer-membrane vesicles) containing the PorA surface protein - Subunit vaccine, Phase II - Subunit	<u>RIVM</u> , <u>Wyeth</u> , <u>NIPH</u> , Chiron <i>Finlay Institute/GSK</i> , <u>Microscience</u>
Hepatitis C		3-4 000	46	- E1-based vaccine, Phase II, therapeutic, Europe - Core epitope peptides, Phase I, prophylactic and therapeutic - Other approaches, preclinical	<u>Innogenetics</u> Chiron <u>Berna/Pevion</u> , EU, USA, <i>China, and Taiwan</i> <i>biotechs</i>
Hepatitis E		100-1 000	<10	- Subunit vaccine, Phase II, Nepal - DNA (ORF-2), preclinical - Live attenuated swine vaccine, preclinical	GSK/WRAIR US Navy MRI NIH
Helicobacter pylori	8 149 d		850 d	- Oral whole-cell with LT mutant, Phase I - Recombinant intramuscular VacA, CagA, NAP,	<u>Antex</u> Chiron

				Phase I - Recombinant oral urease - Other antigens	<u>Acambis</u> , Aventis CSL
Human Papilloma Virus	3 827		258	- VLP L1 quadrivalent, yeast, Phase III, USA - VLP L1 16, 18, baculovirus, Phase II - VLP L1, 16, baculovirus, Phase III, Costa Rica - TA-HPV 16, 18, therapeutic, Phase I/II - rVV E6, E7 HPV16, therapeutic, Phase II - Fusion protein Hsp-E7, therapeutic, Phase II - CVLP 16, baculovirus, Phase I, USA - VLP L1, 16, nasal/aerosol, Phase I, Swiss	Merck GSK NCI <u>Xenova</u> <u>Transgene</u> <u>Stressgen</u> NCI, <u>Medigene</u> U. Lausanne
Epstein Barr Virus		<100	<10	- Recombinant subunit, Phase II - Live rVV expressing gp220/350, Phase I, China - EBNA-3A peptide, Phase I, Australia	<u>MedImmune</u> / GSK <i>China</i> Australia
Measles	26 495	30-40 000	745	- Mucosal delivery systems (aerosol), Phase II - Recombinant Salmonella	<i>Mexico</i> U. Maryland
Rabies		> 7 000 c	50-60	- RMAB, preclinical	US CDC, I. Pasteur
Group A Streptococcus		100-200/100 000 RF in school- aged children		- common N-terminus of M protein (preclinical) - peptide vaccine, Phase I/II - Non-M protein (J14) fusions - 24-valent non-M protein	NIH <u>ID Biomedical</u>
Group B Streptococcus		0.5-3/1 000 LB neonatal sepsis, meningitis		- Capsular polysaccharide TT conjugate, Phase I in non-pregnant women - recombinant protein antigens, preclinical	NIH <u>Microscience</u>
Leptospirosis		100 000	>1000	- recombinant BCG, DNA vaccine, preclinical - recombinant proteins, preclinical - inactivated formulation, phase I	China USA Cuba

Raw estimates of disease burden are highlighted in yellow and do not represent WHO estimates for the year 2002.

Large Pharmaceutical Industries in the developed world are mentioned in bold characters, smaller industries or biotechs in developed countries are underlined, vaccine manufacturers in developing countries are in italics, and academic institutions are in normal characters.

a - clinical cases, but 2 million annual infections, b - confounded with HIV-related deaths, c - post-exposure prophylaxis in Asia,

WHO/IVR

d - all stomach cancers, e - all etiologies confounded (Hib, pneumo, meningo), f - probably largely underestimated
i - refers to carriers. The diseases for which a vaccine already exists but needing improvement are highlighted in gray, while those with an existing efficient and widely used vaccine are highlighted in blue. Those for which assumptions were made to calculate disease burden scores are highlighted in yellow.

Acknowledgements

This document was prepared by Dr. Jean-Louis Excler, IAVI, New Delhi, India, with the help from:

Teresa Aguado, Coordinator, Research on Bacterial Vaccines, IVR, WHO, Geneva
Bruce Aylward, Programme Manager, Polio Eradication, WHO, Geneva
Julian Bilous, Coordinator, Expanded Programme on Immunization, WHO, Geneva
Ladaporn Bodhidatta, Department of Enteric Diseases, AFRIMS, Bangkok, Thailand
Brenton Burkholder, Vaccines and Biologicals, WHO-SEARO, Delhi, India
Guy Blaudin de The, Department of Epidemiology, Institut Pasteur, Paris
Thomas Cherian, Research on Bacterial Vaccines, IVR, WHO, Geneva
Giuseppe Del Giudice, Senior Research Director, Chiron SpA
Anil Dutta, Medical Director, International, Aventis Pasteur, Lyon, France
Jose Esparza, Coordinator, Research on Viral Vaccines (including HVI) WHO/UNAIDS, Geneva
Uli Fruth, Research on Bacterial Vaccines, IVR, WHO, Geneva
Roger Glass, Centers for Disease Control, Atlanta, USA
Elwyne Griffiths, Coordinator, Quality Assurance and Safety of Biologicals, WHO, Geneva
Rudy Hartskeerl, Leptosiris Reference Centre (WHO/FAO/OIE/RIVM)
Bernard Ivanoff, Consultant to IVR, WHO, Geneva
Julie Jacobson, Programme for Appropriate Technology in Health (PATH), Seattle, USA
Luis Jodar, Research on Bacterial Vaccines, IVR, WHO, Geneva
Paul Johnson and the Ulcer Initiative, WHO/CDS
Marie-Paule Kieny, Director, Initiative for Vaccine Research (IVR), WHO, Geneva
Jean Lang, Chief of vector-born disease vaccines, R&D, Aventis Pasteur, Marcy l'Etoile, France
Ying-Ru Lo, HIV/AIDS and TB/HIV, WHO-SEARO, Delhi, India
Mammen P. Mammen, Jr, Chief, Department of Virology, USAMC-AFRIMS, Bangkok, Thailand
Bjorn Melgaard, WHO Representative, Bangkok, Thailand
François-Xavier Meslin, Department of Communicable Disease, WHO, Geneva
Philippe Moingeon, R&D, Aventis Pasteur, Marcy l'Etoile, France
Khin Saw Aye Myint, Chief, Hepatitis Section, USAMC-AFRIMS, Bangkok, Thailand
Isabelle Parent du Chatelet, Association pour l'Aide a la Medecine Preventive, Paris
Stanley Plotkin, Aventis Pasteur, USA
Sonia Pagliusi, Research on Bacterial Vaccines, IVR, WHO, Geneva
Basil Rodrigues, Expanded Programme of Immunizations, UNICEF-EAPRO, Bangkok, Thailand
Rozita Rosli, Faculty of Medicine & Health Sciences, University Putra Malaysia, Kuala Lumpur, Malaysia
Charles Rupprecht, Centers for Disease Controls, Atlanta, Georgia, USA
Dennis Shanks, Director, USAMC-AFRIMS, Bangkok, Thailand

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Philippe Stoeckel, President, Association pour l'Aide a la Medecine Preventive, Paris

Klaus Stohr, Department of Communicable Diseases, WHO, Geneva

Daniel Tarantola, Director, Vaccines and Biologicals, WHO, Geneva

Jay Wenger, Accelerated Vaccine Introduction, WHO, Geneva

Marc-Alain Widdowson, Centers for Disease Control, Atlanta, USA

Henry Wilde, Chulalongkorn University, WHO Rabies Reference Center, Bangkok, Thailand

David Wood, Quality Assurance and Safety of Biologicals, WHO, Geneva

Michel Zaffran, Programme Manager, Vaccines and Biologicals, WHO, Geneva

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