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THE IMPACT OF VACCINES AND VACCINATIONS: CHALLENGES AND OPPORTUNITIES FOR MODELERS

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Abstract. This review focuses on how infectious diseases and their prevention and control by development of vaccines and widespread vaccination has shaped evolution of human civilization and of the animals and plants that humans depend on for food, labor and companionship. After describing major infectious diseases and the current status for control by vaccination, the barriers to infection and the attributes of innate and acquired immunity contributing to control are discussed. The evolution in types of vaccines is presented in the context of developing technologies and in improving adjuvants to engender enhanced vaccine efficacy. The special concerns and needs in vaccine design and development are discussed in dealing with epidemics/pandemics with special emphasis on influenza and current global problems in vaccine delivery.

1. **Introduction.** Infectious diseases of animals, plants and humans have shaped the evolution of human civilization ([5, 7, 43, 54, 115, 166, 190]) but the development and use of vaccines and other means to prevent infectious diseases have, in part, begun to partially negate Darwinian evolution of human civilization. It is likely that the first human directed efforts to ameliorate the impact of infectious diseases predated the recognition of the microbial/viral agents of disease and were associated with the domestication of plants and animals during the development of agriculture. In these endeavors during the past 12,000 years or so survivors were always used to generate the next round of progeny. Thus unknowingly, resistance to various infectious diseases, or at least the most severe manifestations of those diseases leading to death, were selectively enhanced over the centuries during which domestication and plant and animal crop improvement occurred. However, with the recognition that specific infectious diseases were caused by bacteria ([68, 97, 98]) and viruses ([12]), it became possible to connect these discoveries with prior successes in developing vaccines to prevent some infectious diseases in animals and humans ([85, 138]). The science of disease prevention in animals and humans took off with the beginning understanding of immunity and our immune system starting with the discoveries of Metchnikoff ([117]) and continuing to the present. Interventions to treat infectious diseases by "magic bullet" drug therapy pioneered by Ehrlich ([47]) led ultimately to discovery of sulfa drugs ([196]) and antibiotics ([52, 87]) as effective therapeutics for control of infectious diseases in animals and humans. Development of anti-fungal drugs ([69]), anti-parasite drugs ([45]) and anti-viral drugs ([152]) and subsequent discoveries and developments of a diversity of anti-microbial/viral therapies have

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contributed greatly to the control of infectious diseases, at least in the developed world. Nevertheless, the increasing global prevalence of infectious disease agents that have acquired resistance and often multiple resistances to these antimicrobial drugs and antibiotics ([63, 65, 88, 175, 195]) has made drug therapy often ineffective with concomitant adverse consequences. These trends in the increasing ineffectiveness of antimicrobial/viral drugs and a decreased rate of discovery and development of new drugs, makes the development and widespread use of vaccines a much more important means to prevent infectious diseases. This topic will thus be the focus of this article. But from a global perspective on infectious disease control, it is important to note that control of infectious diseases of agriculturally important plants, although often controlled by use of fungicides ([49, 172]) and insecticides (to often block transmission of infectious disease agents, ([49, 116]), is and will ultimately be controlled by genetics ([51, 146]). Selection of plant species for resistance to bacterial, fungal and viral pathogens has been impressively successful in the cereal grain crops ([62, 122, 173]) and led to the "green revolution" ([70, 94, 95, 142, 145]) and these approaches are being rapidly augmented by genetic engineering ([119]) to confer pathogen ([30, 38]) and insect ([22, 50]) resistance while improving other agronomically valuable attributes to improve quality and quantity of crop yields ([9]) as well as nutritive value ([11, 159, 162]), to produce natural compounds for medical use ([141]).

2. **Infectious diseases and vaccination.** Based on WHO estimates some 55 million people die each year and some 18 million die as a direct consequence of infection with an infectious disease agent ([193]). However, many cancers are the consequence of infection with bacteria and viruses (Helicobacter pylori, hepatitis B virus, papilloma virus, etc.) and respiratory pathogens undoubtedly contribute to chronic lung diseases ([61]). There is also increased interest in the ability of pathogens such as Chlamydia pneumoniae to contribute to cardiovascular disease ([29]) and other microbes to contribute to autoimmune diseases ([131]). Thus, infectious diseases can potentially be implicated in causing or contributing to up to half of the annual global deaths and to much of the morbidity associated with infectious diseases and other pathogen-induced diseases. These cumulative losses are measured as the number of disability-adjusted life years (DALYs) lost due to premature deaths and disabilities ([126]). Smallpox is one infectious disease that has been eradicated by the availability of the vaccinia anti-smallpox vaccine and a well-coordinated global vaccination effort ([71]). This success came some 182 years after Jenner's discovery and is now estimated to save some five million lives each year had the vaccine not been effectively deployed ([96]). Ten vaccines, against polio, diphtheria, pertussis, measles, mumps, tetanus, hepatitis B, yellow fever, rubella and tuberculosis, are used in much of the world as the targeted diseases of the Children's Vaccine Initiative ([124]) listed in a decreasing scale of use and/or effectiveness and prevent some six million annual deaths. If all these vaccines were maximally effective and universally used to immunize all children, another seven to eight million lives could be saved annually ([160]). However, the BCG vaccine to prevent tuberculosis ([28]) is only effective in preventing miliary tuberculosis in young children and is totally ineffective in preventing the pulmonary form of the disease ([34, 37]) such that we still have some two to three million tuberculosis deaths each year ([154]). We now have

effective vaccines against Hemophilus influenzae B ([147, 140]), Streptococcus pneumoniae (the most prevalent forms) ([134]), rotavirus ([112, 130]), Neisseria meningitidis ([21, 64]), Salmonella Typhi ([58, 80]), papilloma viruses ([2]) and influenza virus ([15]). However, most of these vaccines are too expensive for widespread use in the developing world ([90]) and the need for refrigeration and administration by needles can compromise effectiveness and increase costs as well as contribute safety issues, respectively. There are still many infectious diseases for which no effective vaccines exist and these include most of the pathogens that cause respiratory and diarrheal diseases ([155]). These pathogens contribute to high mortality in children under five years of age in the developing world ([155]) and undoubtedly contribute to their malnourishment, which in turn impedes development of the central nervous system ([121]) to lessen development of intellectual skills to recognize and solve problems to improve their economic well-being in adulthood. In this regard, it should be noted that successfully fighting infections requires stimulating the immune system and this requires gene activities and production of protein antibodies and specific classes of lymphocytes ([42]), which diverts nutrients needed for growth and development of tissues and organs. Similarly vaccines against parasitic diseases including malaria, schistosomiasis, sleeping sickness, Chagas disease, etc. do not exist and this is also true for HIV and tuberculosis that account for some four to five million of the annual deaths due to infectious disease agents ([193]).

3. **Barriers to infection and effective immunity.** Animals and humans have numerous natural defense mechanisms to decrease the likelihood of infections. Natural barriers to infection include skin, which can be bridged by insect delivery of infectious disease agents and wounds that expose susceptible tissues, an extensive array of enzymes (lysozyme, proteases, lipases, etc.) ([123]), peptides (defensins, etc.) ([44, 123]) and ion scavengers such as lactoferrin ([184, 188, 189]) present in mucosal secretions of the respiratory, intestinal and genitourinary tracts and harsh environments that have antimicrobial attributes such as stomach acidity and the detergent action of bile secreted into the duodenum. Two types of immunity exist, innate and acquired. Innate immunity is always present and consists of an intricate system by which infection is recognized with production of antimicrobial/viral activities and recruitment of neutrophils and other phagocytic cells to the site of infection to kill or neutralize invading pathogens ([23, 125, 135]). These activities are triggered by the presence of cell-surface and internal Toll-like receptors (TLR) ([125, 135]) and also by internal Nod factors ([125, 135]) that recognize certain pathogen-associated molecular patterns present in or on pathogens. These PAMPs as they are called ([20, 82]) include bacterial cell wall components such as teichoic acid, lipopolysaccharide, flagellin, lipoproteins, peptidoglycan, etc. as well as viral single-stranded and double-stranded RNA and forms of bacterial DNA that differ in methylation at CpG sequences ([82]). The interaction between the PAMPS and the TLRs and Nods elicit production of cytokines and other secretions that result in different degrees of inflammation and call in/recruit different innate defense mechanisms ([82]). Acquired immunity is induced in response to the invading pathogens but is dependent on the innate immune system to facilitate presentation of pathogen antigens to trigger either production of antibodies or stimulate cellular immunity ([1]). Antibody production is dependent on B lymphocytes and for T-cell dependent antigens such as proteins also depends on T cells of the CD4 type ([42]). Most carbohydrates are T-cell independent antigens although CD3-type T

cells are sometimes involved in the stimulation of antibodies by B cells ([192]). This humoral or systemic immunity due to production of antibodies is also divided into two compartments in which antibodies are present in serum or in secretions from mucosal tissues and secretory glands ([177]). The antibodies in serum are mostly IgG but also IgA and IgM ([42]) whereas the mucosal antibodies are mostly IgA that are dimeric and contain a secretory component (S) that permits their transport and secretion from mucosal cell surfaces and secretory glands (mammary, salivary, lacrimal, etc). Serum antibodies are also contained in some mucosal secretions such as in saliva due to leakage of serum IgG from the gingival crevice ([46]) and in the female reproductive tract due to the opening of the oviduct to the peritoneal cavity ([100, 194]). Serum antibodies are effective in neutralizing toxins or viruses to prevent their entry into cells ([109]). Mucosal antibodies can also block attachment/invasion of pathogens entering through a mucosal surface ([78]). Stimulating this line of immune defense is of significant importance since most pathogens use a mucosal portal of entry ([129]). Cellular immunity is dependent on presentation of antigens or parts of antigens termed antigenic determinants or epitopes ([42]) by either of two pathways by antigen presenting cells that are either macrophages or dendritic cells ([42]). In one pathway, antigens are presented in association with the major histocompatibility (MHC) class II antigen to trigger antigen-specific T cell help (via CD4 T cells) for antibody production by B cells ([84, 92]). In the other pathway, antigens are presented in association with the major histocompatibility (MHC) class I antigen to stimulate antigen-dependent CD8 T cells ([92, 101]). These T cells are cytotoxic and can specifically kill host cells in which a pathogen resides ([110]). This type of immunity is very important in preventing infections by pathogens that multiply within host cells as is the case for facultative and obligate intracellular bacterial pathogens, all viruses and many parasites ([25, 77, 89]). T-cell immunity is essential for induction of long-term protective immunity against infectious disease agents ([104, 114, 197]).

4. **Types of vaccines.** Although survival from infection with a pathogen generally results in life-long protective immunity, this is a risky way to acquire immunity, but probably was very important in selecting for survival of those with increased natural abilities to survive infection or more rapidly mount an effective immunity. Use of a pathogen with decreased potential to cause disease due to attenuation or partial inactivation was thus a more acceptable means to induce immunity. This approach, using old stored dried-out pox crusts from individuals with mild non-fatal small pox infections, was successfully used as the first means to prevent smallpox infections during the Qing Dynasty in China ([176]). The one percent mortality from this form of vaccination was preferable to the much higher mortality associated with small pox infection. Because of safety concerns, most current vaccines are killed (heat or chemically inactivated) viruses or bacteria or are purified protein or carbohydrate subunits of the virus or bacteria or are inactivated toxins (toxoids) ([105]). In still other cases, conjugate vaccines have been developed to combine a not very immunogenic protective carbohydrate antigen to a highly immunogenic protein ([4]). These conjugate vaccines become T-cell dependent and importantly induce immunity in infants under the age of two, which is the age at which induction of immunity to carbohydrate antigens becomes possible ([167]). Some vaccines are attenuated derivatives of the pathogen so as to induce a mild non-fatal infection. Some of the means of attenuation derive from Pasteur's pioneering research using

multiple passages of infectious material as a means to attenuate the agent (what ever it was) while retaining its ability to induce protective immunity ([137]). This was used in the development of the live attenuated polio vaccine by Sabin ([156]). Currently, live attenuated vaccines for influenza were developed by selecting for mutant strains of virus that only grow at low temperatures (cold-adapted) and replicate very slowly at our body temperature ([33, 86]). Other live attenuated vaccines have been generated by reassortment of segmented virus genomes so that the recombinant attenuated viruses have an altered host range to reduce replication proficiency in humans ([78]). This has been successfully used in development of the Rota Teq human-bovine reassortment vaccine to prevent diarrheal disease due to rotavirus infections ([35]). Still other live attenuated bacterial vaccines have been generated by introducing attenuating mutations ([58]). These live attenuated viral and bacterial vaccines tend to induce a higher level of immunity of longer duration.

5. **Innate immunity and recruitment by adjuvants.** As stated above, all animals have evolved to display a multitude of barriers as means to confer natural resistance to infection by infectious disease agents. In addition, host tissues, especially mucosal tissues recognize and respond to both commensal harmless as well as infecting harmful microbes and elicit responses accordingly, which constitute the innate immune system ([42]). As stated above, innate immunity is dependent on a large number of cell surface and internal toll-like receptors (TLRs) that recognize various conserved constituents of microbial pathogens such as fimbrial and flagellar appendages, lipoteichoic acids, lipopolysaccharide and lipo-protein constituents of the cell walls of bacteria, single- and double-stranded RNA from viruses, and CpG dinucleotides from bacterial nucleic acids and other internal molecules termed Nod factors that recognize peptidoglycan components of bacteria. These PAMPs and the interaction between PAMPS and TLR and Nod ligands leads to induction to a specific array of inflammatory or non-inflammatory cytokines and these in turn lead to recruitment of neutrophils, macrophages and dendritic cells to the site of infection to non-specifically defend against infecting pathogens or to result in tolerance to benign commensal invaders ([179]). Many of these signal transduction pathways can lead to the development of acquired immunity to infecting pathogens due to the ability of recruited macrophages and dendritic cells to destroy invading pathogens and present pathogen antigens to MHC class I and class I antigens to commence to stimulate acquired immune responses ([179]). However, many injectable subunit vaccines do not possess PAMPs or have PAMPs but not in a form to trigger TLR and Nod factors to recruit innate immunity and are therefore not very effective in inducing protective immunity. We are currently learning the consequence of this problem with the recent recurrence of whooping cough caused by Bordetella pertussis with increased infections and more mortality than observed in prior years ([120]). This is presumably due to the shorter-term immunity induced by the safer new acellular pertussis vaccine, which lacks PAMPs, compared to the old whole cell killed pertussis vaccine that contained PAMPs but was more reactogenic. These lower immunogenicity problems with some subunit vaccines have been partially overcome by the addition of adjuvants to subunit vaccines ([39]), which have the ability to elicit production of inflammatory cytokines that in turn recruit macrophages and dendritic cells ([42]) capable of presenting the pathogen protective antigens in the subunit vaccine to the immune system to initiate induction of acquired immunity. For many years, the only approved adjuvant for use in human vaccines was alum

that acts, in part, by prolonging the presence of the subunit antigen at the site of injection ([60, 66]). More recently, mono-phosphoryl lipid A ([144]) has been approved as a very effective adjuvant. It is a non-toxic derivative on the lipid A endotoxin from gram-negative bacteria and is very efficient at recruiting innate immunity by a specific interaction with TLR4 ([41]). Other adjuvants that are quite effective are used in veterinary vaccines ([174]) and some of these will undoubtedly eventually be approved for use in human vaccines. Still other immuno-enhancer attributes are being designed into improved vaccines ([106, 148]) to increase the level and duration of induced protective immunity.

6. **Vaccination and induction of acquired immunity.** Most of the successful vaccines are against infections by pathogens in which serum antibodies are able to confer complete protection ([147]). Most of these vaccines are administered by injection and therefore do not induce mucosal immunity. Since most of these successful vaccines do not induce very good cellular immunity, repeat booster immunizations are needed throughout life. Some of the live attenuated vaccines against polio ([157]), rotaviruses ([112, 130]) and typhoid ([80]) are administered orally whereas others against influenza ([14, 15, 16]) are administered intranasally. In these cases, mucosal immunity is induced to reduce the likelihood of infection ([185]). The mucosal immunity barrier is never absolute but it is postulated that a higher dose of a pathogen would be needed to result in successful transit of a sufficient pathogen dose to result in infection. However, these vaccines also stimulate significant serum antibody titers to preclude onset of disease ([76]). Another advantage of vaccines that induce mucosal immunity is that they should reduce transmissibility of the infectious agent in populations of immune and non-immune individuals. It should be obvious that administering vaccines at mucosal sites, especially orally, eliminates the use of needles with their associated costs and risks of transmission of disease agents by their reuse. However, oral immunization is not equally effective in all populations and young children in many developing countries develop a lower level of immunity after oral immunization than children of the same age from developed countries ([83, 139]). This is thought to be due to repeat inflammatory trauma of intestinal mucosal tissues due to recurrent infections with diarrheal pathogens and parasites ([18, 111, 139]) to impair invasion and/or uptake of orally administered vaccines ([24]). Such problems can be overcome by increasing the oral dose of vaccine administered ([165]) or by oral immunization at an earlier age such as in newborns or neonates ([6, 168]). Another highly beneficial attribute of live attenuated vaccines, including recombinant attenuated viral and bacterial vaccines still under development, is that they multiply and/or persist in the immunized host to stimulate humoral and cellular immunities ([3]). This is not always the case, however, since some live attenuated vaccines such as some derivatives of vaccinia ([133, 143, 171]) and assortment vaccines of rotavirus with different host specificities ([9, 161]) are defective/impaired in replication and are therefore not as effective in stimulating long-lived protective immunity as live vaccines that are attenuated due to other attributes but are more replication proficient ([91, 107, 183]). Since the immunized host serves as the factory to manufacture the protective antigens, the vaccine dose needed to successfully immunize an individual is likely reduced to further reduce costs of vaccine manufacture. This benefit is correlated, however, with the degree of replication proficiency of the live vaccine. Manufacturing costs are also reduced by the fact that these live attenuated vaccines are replicative (under

some defined conditions) and are harvested as intact viruses or bacteria. Such live vaccines also do not need to be administered with costly adjuvants since they possess the PAMPs needed to recruit innate immunity ([118, 153, 186]). It is thus likely that the further development of such live attenuated vaccines and especially live attenuated recombinant vaccines in which an attenuated bacterium or virus serves to deliver one or more protective antigens or the genetic information to specify these antigens from various pathogens to the immunized host will result in vaccines that are safe and cost effective for use throughout the developing and developed world. Only then can we expect to conquer known infectious diseases.

7. **Special concerns and needs in vaccine design and development.** Most infectious diseases are caused by pathogens that are reasonably genetically stable and host-specific such that a single widely administered vaccine can be used to effectively prevent widespread disease and especially epidemics. However, some infectious diseases have zoonotic reservoirs with differing potentials for transmission of the pathogen from the infected animal reservoir to humans. Thus Yersinia pestis, the etiologic agent of plague, that is carried by a significant number and diversity of rodent species in the U.S. southwest and Rocky Mountain states is not frequently transmitted to humans since the fleas associated with these rodents have no propensity to associate with humans. Thus, the infrequent plague infections in the U.S. are often transmitted to humans by outdoor cats that have caught a plague-infected mouse ([56, 108, 149, 150]). This was not the case in the Middle Ages when the European black rat was the reservoir for plague and had a flea that liked to bite humans as well as the rat ([103, 113, 151, 169, 181]) thus resulting in the fatal decimation of about one-quarter of the entire European human population. Epidemic spread from a zoonotic reservoir is thus often a consequence of other factors such as population density of the reservoir species, which can be influenced by food availability, and the existence of insect or other animal vectors. In this regard, influenza transmission poses additional problems. Influenza has a segmented genome with eight RNA molecules encoding all of its properties and these include the ability to infect many host species ([191]). However, many of the avian influenza strains remain mostly restricted to avian species as is true for equine influenza and human influenza strains that are mostly restricted to equines and humans, respectively ([10, 75, 79, 178]). Pigs are not restrictive influenza hosts such that they can be easily infected with avian, equine and human influenza strains as well as with those frequently associated with pigs ([32, 74, 164, 182]). Dual infection of pigs with multiple influenza strains thus enables formation of reassortment viruses that have genome segments derived from all the infecting viruses ([26, 27, 31]). Recent evidence of these occurrences was obtained upon thorough examination and sequencing of all the genome sequences in the H1N1 virus epidemic that commenced in Mexico in spring of 2008 and quickly circled the world ([36, 55]). As revealed by genome sequence analyses, certain segments were derived from avian, swine and human influenza strains and some of the combinations had been seen in influenza strains identified in earlier years ([170]). Fortunately, this H1N1 epidemic was not severe with no higher mortality than associated with the seasonal influenza strains that had already been circulating in the human population ([13, 67]). This, of course, was not true in 1918 when the new reassortment influenza virus had antigenic components and other virulence attributes not seen before by the human population and this epidemic claimed millions and millions of lives throughout the world ([8, 180]). In addition

to the occasional occurrence of new reassortment influenza viruses, antigenic variation occurs continuously due to mutations in the RNA genome segments encoding the surface-localized hemagglutinin and neuraminidase antigens that are essential for virus infection ([17, 48, 59, 72, 102, 163, 187]). Thus amino acid substitutions are accumulated over time and these often permit virus propagation in individuals infected or immunized in previous years with ancestor strains of the virus. Due to this antigenic drift, it is necessary to change the components of the influenza vaccine to be used each year. Typically the influenza vaccine is made up of one Type B influenza virus (that does not undergo much change from year to year) and two Type A virus strains that are subject to both annual antigenic drift and the occasional antigenic reassortment ([57]). Because of the time to manufacture new vaccines every year, decisions on the virus components are usually made in late winter in the year prior to administration of the vaccine the coming fall. This permits sufficient time for manufacture and safety testing. This antigenic drift is a problem for control of a number of infectious diseases caused by RNA viruses and the worst of these is HIV ([93, 158]) for which no effective vaccine has yet been developed. Influenza control is also complicated by the worldwide dissemination of avian influenza strains that can also contain reassortment viruses with genomic segments from porcine, equine and human influenza strains by migratory waterfowl ([127, 132, 191]). These birds act as vectors of virus from northern to southern hemispheres and reverse and ensure that genomic combinations are globally distributed on a regular basis. One aspect of influenza control would be the immunization of these migratory zoonotic reservoirs. This is problematic and not practical, however, unless a very inexpensive anti-influenza vaccine could be developed. Manufacture of annual influenza vaccines is also complicated by use of embryonated eggs for virus propagation since only about one dose of vaccine is produced per embryonated egg ([73, 132]). For this reason, annual influenza vaccine production only enables immunization of between one-quarter and one-third of the U.S., Canadian and European populations ([136]) with the rest of the world having an exceedingly limited supply of vaccine. The problem is compounded by the recommendation of vaccination preferably every year or at least every several years. It should be evident that it is impossible to produce sufficient influenza vaccine for the entire world population or even a quarter using embryonated chicken eggs. A further complication would arise if a reassortment virus arose causing epidemic disease that possessed avian influenza virus components and attributes. In this case, vaccine virus could not be productively manufactured in embryonated eggs since avian influenza strains kill the embryos so quickly ([128]) as to preclude much virus propagation. Use of cell culture to propagate vaccine viruses would be a solution but not one that would be very cost effective. Clearly, problems abound in attempting to devise a cost-effective means for prevention and control of both seasonal and epidemic influenza.

8. **Current problems in vaccine delivery.** Given that the majority of the global infectious disease burden is borne by those in the developing world and disproportionately by those less than five years of age ([193]), it behooves society to accelerate research to discover and develop vaccines that will be safe and effective in preventing any and all infectious diseases whether caused by bacteria, viruses, fungi or parasites and to be able to manufacture these vaccines at less than a dollar a dose in a thermostable form for reconstitution at time and place of use to be administered needle-free at a mucosal site. Such successes will also depend on achieving

a far better understanding of the pathogenesis of many pathogens, especially parasite pathogens causing orphan diseases of no concern in the developed world. In addition, many of these pathogens have multiple means to evade, suppress or modulate host immunity to their own benefit. In these cases, live attenuated vaccines derived from these pathogens will not likely be effective unless the means for this immune evasion can be discovered and inactivated in the live attenuated vaccine. Such discoveries only come from extensive research. It may therefore be best if one can generate recombinant poly-valent vaccines using a viral or bacterial vector to deliver multiple protective antigens or information encoding those antigens to the immunized animal or human host. Recent work in engineering vaccinia virus derivatives that lack the ability to preclude induction of host interferon-gamma that are totally attenuated yet replication proficient ([81]) offers a promising means to induce protective immunity to heterologous pathogens, especially if means for immunization at a mucosal site can be developed, offer great promise. Similarly, much has been learned during almost thirty years of effort by hundreds of laboratories to harness Salmonella enterica serotypes as effective vectors ([40, 53, 99]) for delivery of heterologous protective antigens especially from bacterial pathogens to induce protective immunity in animals. Future work, however, will need to focus on perfecting these technologies for use in humans and for delivery of means to induce immunity to viral, fungal and parasite pathogens.

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