Salmonella Vaccines for Animals and Birds and Their Future Perspective

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Abstract: Vaccines are the most powerful biologicals which have modulated the economic, social and cultural life of human beings. Certain diseases have haunted humanity for centuries but are now extinct due to vaccines. On the other hand, some diseases such as salmonellosis, that were uncontrollable in the past, still cause pandemics today. There are more than 2500 serovars of *Salmonella* and vaccines made from any one serovar do not confer cross-protection against another, no matter how much antigenic similarity there is between the two. *Salmonella* strains are able to cause disease and to adapt to different types of animals whilst still maintaining their zoonotic and interspecies transfer potential. Three major types of vaccines are being used to control salmonellosis: killed bacteria, subunit vaccines and live attenuated vaccines. Effective vaccines against some host adapted and common serovars have been developed but their use has led to the emergence of other serovars. The problem has become more complex because increased international trade and travel has helped *Salmonella* strains to cross continental boundaries. It seems unlikely that we will be able to develop an effective *Salmonella* vaccine in the near future that is able to control all forms of salmonellosis, even in a single animal species. Recent advances in *Salmonella* vaccines will be reviewed including the use of *Salmonella* as vector for delivery of multivalent DNA and recombinant vaccines for controlling salmonellosis and other infectious diseases as well as for the control of cancer.

Key Words: *Salmonella*, Vaccines, Competitive exclusion, DIVA, Marker, Live, Killed.

INTRODUCTION

Few scientific discoveries have had such an impact on world health as the discovery of vaccines. The phenomenon that individuals who recovered from some infectious diseases were resistant to subsequent re-infection was observed by Edward Jenner and Louis Pasteur and provided the impetus for the early development of vaccines. Thanks to the advances in immunology and molecular biology the field of Vaccinology has undergone considerable development during the last century mainly because of new techniques: attenuation and inactivation of pathogens, cell-culture of viruses, genetic engineering and acellular component identification. In recent years considerable progress has occurred in areas such as combination vaccines, new adjuvants, proteomics, reverse vaccinology and vaccines for noninfectious diseases. These various revolutions [1] have resulted in the appearance of many different types of vaccines such as whole cell inactivated vaccines, bacterins (*Pasteurella multocida*, *Salmonella*), live attenuated vaccines (tuberculosis and *Salmonella* Typhi infections), toxoids (tetanus and diphtheria toxoids, *Salmonella* toxoid), acellular vaccines or subcellular vaccines or subunit vaccines (pertussis, *Salmonella* infections), polysaccharide vaccines (*Haemophilus influenzae* type B, Vi capsular vaccine for *Salmonella* Typhi infection), recombinant protein vaccines (hepatitis B, antigens expressed in yeast cells and *Salmonella*), anti-idiotypic vaccines (hepatitis B, rabies, human immunodeficiency virus-HIV), synthetic peptide vaccines (hepatitis B, foot and mouth disease), DNA and mRNA vaccines, live vectored vaccines such as vaccinia- VRG, an oral rabies vaccine, pox and adenoviruses exploited as vectors).

Although, there is no systematic surveillance in operation in India and other south East Asian countries, salmonellosis, an important zoonotic disease, is an endemic problem in the region [2]. More than 2500 serovars of genus *Salmonella* have been identified, contributing to massive global losses in human and animal productivity as a result of diarrhoea [3-5]. A few strains, particularly host-adapted ones also cause heavy mortality in young, immunocompromised and stressed populations. Year after year, millions of people suffer with salmonellosis and about one third of the foodborne disease outbreaks in humans are caused by Salmonellae alone [6]. Transmission of salmonellosis is often associated with animal and plant products and more than 235 *Salmonella* serovars were found to be prevalent in India alone [7-11].

Of the many vaccines tried for control of salmonellosis, killed vaccines are serovar specific and produce only short lived immunity. Live vaccines may turn infective in immunocompromised individuals, in elderly and in infants as well as in healthy people because of the zoonotic potential of *Salmonella* [12].

Despite these limitations many different types of vaccines, broadly classified as killed vaccines or bacterins, subunit vaccines and live vaccines have been developed to control salmonellosis. Advantages and disadvantages of each type of vaccine are summarized in Table **1**.

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Table 1. Advantages and Disadvantages of Live and Inactivated Vaccines

Criteria	Live Vaccine	Inactivated Vaccine
Oral dosing	Good immunity	No or poor immunity
Duration of immunity	Long	Short
Requirement of adjuvant	No	Yes
Cross protection from related strains	Present	Rare
Safety on inoculation	Varies	Often safer
Horizontal spread of the vaccine strain	Possible	Not applicable
Vertical spread of vaccine strain	Possible	Not possible
Potential contamination	Possible	Remote chance
Stability and maintenance	Poor and difficult	Good and easy
CMI induction	Good	Poor
Secretary IgA and local mucosal immunity	Good	No
Reversion of vaccine strain to pathogenic	Possible	No
Persistence in the vaccinee	Yes	No
Interference from normal flora of vaccinee	Possible	No
Cost of the vaccine	Less	More
Requirement for immunomodulators	No	Yes
Vaccine marker	Genetic markers	Serological markers
Potential for vector vaccine development	Good	Poor
Potential for use in multivalent combination	Less	Good
Changes in growth conditions during production have impact on immunogenicity	Less	More

EXPECTED QUALITIES OF A VETERINARY *SALMONELLA* VACCINE

There is no ideal vaccine available for control of salmonellosis. Such a vaccine must be cheap, minimally reactive, induces mucosal immunity and has self-boostering quality. It should be a single dose oral vaccine, preferably live and invasive but still safe to induce durable immunity but not causing any disease in progeny of vaccinated animals either on vertical or horizontal transmission. However, an ideal vaccine should afford a life-long protection. Except for broilers and pig which are reared for a short duration of 2-3 months, no *Salmonella* vaccine affords protection even for 1 year. An ideal vaccine must be enabling differentiation of vaccinated from infected animals (DIVA vaccine). A good vaccine candidate must easily be distinguished from wild type *Salmonella* in a basal bacteriology laboratory by antigenic or genetic or phenotypic markers. Some of the identifiable phenotypic characters such as susceptibility to low or high temperature and requirement of some specific ingredients for growth (auxotrophic) have been incorporated into modern vaccine candidates along with their compatibility with growth promoting antibiotics, probiotics and prebiotics. However such phenotypic markers must be non-transferable to the wild type homologous or heterologous strains. A vaccine should not deteriorate on storage if killed and should be stable and non-reverting to pathogenic if live. It should not be interfering with colonization of normal mucosal flora necessary for pathogen exclusion mechanism in healthy individuals, should not cause development of tolerance on overuse, and must not be interfering with other vaccines to be used in tandem.

KILLED VACCINES

Initial work on development of *Salmonella* vaccine started in late nineteenth century with attenuated vaccine [13] for typhoid infection in human beings. Later, in 1956, Smith [14] developed 9R and 9S strains of *S. enterica* ssp. *enterica* serovar Gallinarum (*S.*Gallinarum) for control of fowl typhoid. Subsequently killed vaccines were used successfully with confidence of safety to stamp out salmonellosis from equines. Later, many different *Salmonella* serovars were used to produce killed bacterins for veterinary use such as *S.* Typhimurium [15, 16], *S.* Abortusequi [17-22], *S.* Dublin [23], *S.* Virchow [24], *S.* Gallinarum [25] and *S.* Enteritidis [26, 27].

In India, the most successful killed vaccine was made from formalin killed, alum precipitated *S.* Abortusequi in 1955 [17]. The vaccine was found to be much superior than earlier vaccines giving up to 86% protection in mice as compared to heat killed phenolized vaccine which could protect only 50% of the vaccinated population.

Incremental improvements in existing and new killed vaccines came after use of various adjuvants which enhanced not only immunogenicity but also increased length of the protection periods. Chrome alum [18], alhydrogel [20, 24], mineral oil [21, 24, 25], potash alum [22], Freund's incomplete [22] and Freund's complete [24] adjuvant (FCA) have

been used in different *Salmonella* vaccines. For inactivating *Salmonella* in killed vaccine agents such as heat [15, 16], ß-propiolactone (BPL), glutaraldehyde [20] and formaldehyde [16, 22-24] have been tried to preserve the antigenicity and increase the efficacy of vaccines. Another technique to improve antigenicity of the vaccine was to enhance expression of better immunogenic antigens on *Salmonella* during *in-vitro* growth of the vaccine candidate. It was attempted through mimicking *in-vivo* conditions as culturing the vaccine strain in iron-deficient medium [28], used to produce Selanvac, a commercially available *Salmonella enterica* subsp. *enterica* serotyope Enteritidis PT4 bacterin. Another approach to increase efficacy of vaccines was use of immunopotentiators such as thymulin, zinc [27], levamesol and vitamin E [29]. To make broad-spectrum *Salmonella* vaccines, the concept of multivalent vaccines came was applied. Cooper and Mac Farlane [30] vaccinated sheep with a bivalent *Salmonella* vaccine but immunity could not be enhanced beyond 3-4 months and complete protection was not achieved [12].

The importance of killed vaccines was evident in control of salmonellosis in equines and birds. In developed countries with sufficient infrastructure facilities , use of killed vaccines along with flock sanitation and regular infection-monitoring led to eradication of a few *Salmonella* serovars such as *S. enterica* subsp. *enterica* serotyope Abortusequi from equids and *S. enterica* subsp. *enterica* serotyope Pullorum/ Gallinarum from birds. But the same could not be repeated in resource-poor countries. In later years, the niche emptied by the host adapted strains of *Salmonella* in equids and birds was successfully filled with more dangerous and potentially zoonotic serovars as *S. enterica* subsp. *enterica* serotyope Typhimurium and *S. enterica* subsp. *enterica* serotyope Enteritidis in horses and birds, respectively [28].

Comparative analysis of live and killed vaccines revealed that killed vaccines are usually less effective for three reasons. Firstly, they only contain surface antigens that give an incomplete protective antibody response; secondly, they fail to elicit cell-mediated immune response, which is important for long-term protection from salmonellosis and finally; they fail to elicit production of secretory immunoglobulin (sIgA) response critical for protection of mucosal surfaces from colonization with the pathogen. Attempts to overcome all three problems, by culturing vaccine candidates under iron limiting microaeropilic conditions, through use of adjuvant to induce cell mediated immunity (CMI) and mucosal immunity (sIgA) gave only partial success [31].

FUTURE OF KILLED VACCINES

Despite many weaknesses (Table **1**) killed vaccines are often preferred. Meta-analytic review of three typhoid vaccines namely live attenuated, Vi subunit and whole-cell killed vaccines used in more than 1.8 million recipients has revealed that the killed vaccine afforded the best protection [32]. The major point of criticism that killed vaccines afford a short-lived immunity was of little relevance as the protection period could not be extended beyond six months even after use of most of the live *Salmonella* vaccines. The only exception was a recently developed *S. e.* Abortusequi vaccine, which was reported to afford protection for 11 to 14 months after booster inoculation.

Killed vaccines, whilst not very effective, are still the best for use where the disease is eradicated and are the preferred choice for eradication of an endemic strain from a herd or when dealing with an outbreak of salmonellosis. Under these instances, herd specific killed vaccines have been found to be more effective than the established live attenuated vaccines [33, 34]. Killed vaccines are criticized for their inability to induce good CMI and mucosal immunity which are thought to be more important in affording solid protection against salmonellosis than humoral antibodies. Recent studies in Africa on non-typhoidal *Salmonella* infections in children [35] revealed that protective antibodies play a greater role in protection in children against bacteremia caused by non-typhoidal salmonellosis than CMI. Therefore, a suitable killed vaccine which may cause formation of protective antibodies at early childhood is the need of the day.

SUB-UNIT VACCINE

Poor performance of killed vaccines forced researchers in 1980s to develop other types of *Salmonella* vaccines employing sub-cellular components of *Salmonella* and as a result several subunit vaccines came into being. Common sub-cellular components of *Salmonella* used for development of vaccines are: outer membrane proteins (OMPs), porins, toxins and ribosomal fractions. Such vaccines tried in different animals had variable success [12, 22, 36-45]. OMP of *S.* Gallinarum [36, 37] adjuvanted with mineral oil caused 100% clearance of challenge strain of *S.* Gallinarum in birds vaccinated with 400 μg OMP/bird. The immune response of OMPs from *S.* Heidelberg [38] and *S.* Gallinarum [39] could be improved through lipid-conjugation with immunostimulating complex (ISCOM) and bacterin, respectively. In India, Gupta and co-workers [22] found ribosomal fractions and non-ribosomal proteins (non-denatured bacterial cell envelops) of *S.* Typhimurium to be a potential vaccine candidate in a rabbit model.

Most of the subunit vaccines failed to afford significant protection [46] either in field or in experimental models except a toxoid vaccine [40] made from *S.* Weltevreden toxins which provided 100% protection in a mice model against lethal challenge with homologous toxins and *S.* Weltevreden. The toxoid afforded incomplete protection against heterologous *Salmonella.* Salt precipitated protein toxoid made from *S.* Abortusequi afforded better protection than conventional killed and other subcomponent vaccines [41, 45]. Mishra and Sharma [43] reported good efficacy of the toxoid vaccine against salmonellosis in poultry (Patents, US 6,605, 285 B2 and India-189049-96). A toxoid from *S.* Weltevreden prepared after polymyxin-B extraction, salt precipitation, dialysis, gel filtration and formalin inactivation and adjuvanted with FCA provided 100% protection. When the the toxoid was used without any adjuvant only 60-70% protection was recorded in vaccinated birds. Studies of Kumar [42] revealed immunopotentiation of the toxoid with use of vitamin E, the vaccination of birds with vitamin E-potentiated toxoid protected 75-90% of birds after homologous and heterologous lethal challenge, while toxoid potentiated with vitamin E plus selenium afforded only 70-80% protection. Aluminium hydroxide adjuvanted toxoid afforded 70% protection to birds on lethal challenge. Later on [44] toxoid prepared from purified pooled enterotoxin and

cytotoxins adjuvanted with saponin proved far better. The birds vaccinated with saponified toxoid at a dose of 75-100 μg per birds subcutaneously, protected 100% birds against homologous as well as heterologous challenges and a booster dose after 90 days of the primary immunization provided lifelong immunity to vaccinated birds. The vaccine prevented multiplication of the challenge organism in the internal organs and eventually checked shedding of the challenge strain. Nevertheless a major draw-back with subunit vaccines is their complicated protocols of manufacturing and high cost of production.

FUTURE OF SUBUNIT VACCINES

Hope for development of a broad spectrum *Salmonella* vaccine lies with sub-component and cytotoxin-I toxoid vaccines. Sharma and his group [40, 43] have not only revived interest in subcomponent vaccines, but also demonstrated their potential through development of an effective broad spectrum *S*. Weltevreden toxoid vaccine (Patents, US 6,605, 285 B2 and India-189049-96) for control of salmonellosis caused by different serovars of *Salmonella enterica* as *S*. Gallinarum, *S.* Pullorum and *S*. Enteritidis in poultry birds [43] and *S.* Abortusequi in equids [41] inducing an immunity lasting for more than 90 days. Moreover there is potential of development of recombinant toxoids, multivalent toxoids and combining the toxoids of *Salmonella* with toxoids or whole cell bacterins of other enteric and systemic infectious agents.

LIVE ATTENUATED VACCINES

Attenuated avirulent live *Salmonella* vaccine candidates have received considerable attention since the dawn of vaccinology due to solid immunity conferred by them on oral administration and recently because of their potential as mucosal vaccines and as prototype vaccine vectors for delivery of DNA vaccines and development of theracines [47]. Several live attenuated *Salmonella* strains, either with unknown mutations or with site specific mutations created through genetic deletions or insertions [14, 48-74], have been used in humans, birds and animals exploiting host-specific strains or wide host range *Salmonella* serovars (Table **2**).

On per-oral vaccination, *Salmonella* invade and multiply in the mucosa associated lymphatic tissues (MALT) and gut-associated lymphatic tissues (GALT) such as Peyer's patches and then reach systemic sites through mesenteric lymph nodes. This characteristic dissemination pattern allows *Salmonella* to stimulate cell-mediated, humoral and secretory antibody immune responses. A good live *Salmonella* vaccine should be totally avirulent both for animals and humans, highly immunogenic providing long lasting protection from invasion and colonization of *Salmonella* in internal organs and gastro-intestinal tract. The immunity induced by good live vaccine must be solid against different *Salmonella* serotypes. Suitable live vaccine candidates are genetically stable possessing two or more attenuating defined deletion mutations, and their invasion and dissemination in the body should remain unaffected by the diet of the host and such strains should be easy to grow, store and administer [12]. Thus during development of live vaccine candidates, major emphasis is laid on creating a strain capable of invasion in GALT and MALT which can survive and multiply for a period of time just sufficient for eliciting a protective response. This goal has been achieved by introducing mutations that make the bacteria dependent on normal body constituents (which are rapidly depleted on infection) for their multiplication i.e, the mutations are preventing the pathogen to propagate further. The *aro* mutations (blocking synthesis of aromatic amino acids) in *aro*A mutants of *S.* Typhimurium, *S*. Enteritidis, *S*. Dublin and *S*. Choleraesuis are examples of some successful live vaccine candidates. The immunogenicity of *aro* mutants is excellent but their long survival in the host remains a problem in some cases [61, 69]. Mutations that cripple the pathogens' ability to survive in a host by diminishing resistance to non-specific host defense mechanisms such as Δ*cya*, Δ*crp* (deficient in adenylate cyclase and the cAMP receptor proteins) and Δ*htr*A (lacking heat response proteins) have also been used to develop good vaccine candidates [64, 75]. Mutations in genes encoding for regulatory components such as, *pho*P/*pho*Q, a two component regulatory system which regulates genes for acid phosphatases and for the ability of *Salmonella* to survive in macrophages and sigma factor, a universal regulator for many genes [66] and DNA adenine methylase (Dam), which regulates the production of a number of adhesins required for *Salmonella* infection [71] have also been exploited to obtain successful *Salmonella* vaccine candidates.

Reviews of live *Salmonella* vaccines [12, 30, 76] have concluded that they are superior to killed and subunit vaccines in controlling *Salmonella* infections and revealed their potential as a prototype messenger for DNA vaccines and theracines. Studies revealing various aspects of environmental safety associated with live attenuated vaccines [75, 77] have further cleared the clouds over their safety and stability by showing that strains containing defined deletion mutations cannot revert back even after long co-existence with wild type parent or heterologous strains either *in vitro* or *in vivo*. Live *Salmonella* vaccines have also been proved effective and compatible with probiotics and prebiotics with added benefits [78].

Recent studies on *aro*A-*htr*A mutants of *S.* Abortusequi [75] proved their suitability as an oral vaccine in all types of equids with no apparent adverse effect. The mutant strain (S-30) has also been found safe through intra-vaginal and subcutaneous routes even in doses as high as 10^9 cfu per guinea pig. Safety testing in foals, pregnant mares and stallions revealed that the vaccine is safe through oral inoculation in doses as high as 4.2×10^{12} cfu/animal and the vaccine was immunogenic in doses as low as 1×10^{10} cfu/ animal. However, the vaccine produced unacceptable side-effects when inoculated through subcutaneous and intramuscular routes in equids and caused abscesses at the site of inoculation. Challenge tests in mares after 8 months of vaccination revealed that the vaccine afforded 100% protection against fatal challenge with wild type lethal strains inoculated in 100 times the abortion-causing dose (5.7×10^{10} cfu/ animal through intraperitoneal route). In the study, none of the immunized mare aborted and excreted the wild type strains after a challenge infection. Further studies on *aro*A$^-$*htr*A$^-$ vaccine strain of *S.* Abortusequi revealed that the strain could easily be differentiated from

Table 2. Live Attenuated Vaccines Candidates Used for Control of Salmonellosis in Experimental and Domestic Animals

Type of Mutation for Attenuation	Strains/ Serovars [Reference]	Test Animals	Comments
Cell wall lipo-polysachcharide chains (Rough strain)	9R (rough) *S.* Gallinarum [14, 48-52]	Chicken	Afforded protection against virulent parent up to 12 to 32 weeks (when given after $NaHCO_3$) and cross protection against *S.* Typhimurium and *S.* Enteritidis. Adjuvants interfere with protection. Effectiveness decreases after two months of vaccination.
Adaptation in another nonspecific host or some specific growth-medium	Strain 51 of *S.* Dublin [53-56]	Chicken, calves	Cleared the vaccine strain from 99% birds but could not clear *S.* Typhimurium Gave better protection in calves than killed *S.* Dublin bacterin. Calves had a little diarrhoea and febrile reaction to vaccine. Both cell mediated and humoral immune responses were induced.
*gal*E mutant	S.Typhimurium [57], *S.* Choleraesuis and *S.* Typhi [58]	Mice, calves	Significantly reduced faecal shedding of the homologous challenge but there was no significant humoral immune response.
*aro*A mutant	*S.* Dublin and *S.* Choleraesuis [59], *S.*Typhimurium [60], *S.* Enteritidis [61], *S.* Abortusequi [62, 63]	Mice, Chicken, Calves, guinea pigs	Excellent immunogenicity but prolonged carriage. Oral vaccination protected against intravenous challenge. Vaccination induced transient diarrhoea. Vaccine strain could be detected in blood. Induced pyrexia on parentral inoculation.
Δcya Δcrp mutant	*S.* Typhimuruim [64]	Mice	Protection up to 4 months days post vaccination on challenge with 10^9 CFU, strong mucosal, humoral and cellular immune response.
*Pho*P$^-$*pho*Q$^-$ mutant	*S.* Typhimurium [65, 66]	Mice	Found immunogenic, their frequency of reversion to virulent forms is relatively high rendering them unsafe
*v*Pla$^-$ mutant	*S.* Typhimurium [67]	Mice	Highly immunogenic but reminiscent virulence was detected.
*nuo*G mutant	SG9NGK- *S.* Gallinarum [68]	Chicken	Afforded more than 75 % protection.
*aro*A$^-$*sec*C$^-$ mutant	*S.* Gallinarum [69]	Chicken	Conferred 100% protection against homologous challenge.
*htr*A$^-$ mutants	*S.* Abortusequi [63, 70]	Guinea pigs	Afforded 80-100% protection on oral, intravaginal, and parentral inoculation
dam mutant	F98- *S.* Typhimurium [71]	Chicken	Highly attenuated, elicited cross-protection immune response against *S.* Enteritidis too.
dam$^-$*pho*A$^-$ mutants	ZJ111, *S.* Typhimurium [72]	Chicken	Safe and effective against homologous challenge.
dam$^-$*aro*A$^-$ mutants	*S.* Typhimurium [73, 74]	C57BL/6J mice	Safe and effective against homologous challenge.
*aro*A$^-$*htr*A$^-$ mutants	*S-30*, *S.* Abortusequi [63, 75]	Mice, guinea pigs and equines	Safe through oral, intra-vaginal and subcutaneous routes but reactogenic through subcutaneous and intramuscular routes, 100% protection up to 11 months in guinea pigs and pregnant mares. Safe in pregnant animals as well as in foals.

wild type *S.* Abortusequi through simple bacteriological, biochemical and immunological methods, indicating its DIVA potential [79].

FUTURE OF LIVE VACCINES

Although the utility of live vaccines in eradication of salmonellosis is limited, there is vast potential for their use as vector for DNA vaccines and as recombinant antigens [12, 80-90] which might lead to evolution of multivalent vaccines in coming decades (Table **3**). Recombinant DNA technology combined with defined gene deletion method for attenuation has made it feasible to develop vaccines against a broad range of human and animal pathogens. The vector potential of *Salmonella* vaccine strains have been exploited for expression of a number of antigens of bacterial, viral, protozoan and eukaryotic antigens [12, 82]. Besides, *Salmonella* vaccines are foreseen as one of the most potent vectors for oral delivery of multivalent DNA and plasmid-vectored vaccines [47, 88].

The use of live *Salmonella* vaccines as theracines for delivery of therapeutic molecules in nano-medicine and cancer therapy is increasing rapidly. Live vaccine strains of *Salmonella* naturally accumulate in tumors due to slightly anoxic environment of tumors, and continue to thrive there protected from the immune system which further deplete oxygen to levels lethal to tumor cells and this might lead to cure from cancer [89]. As tumors require a supply of blood in order to continue to grow and spread, one approach to control tumor growth might be cutting off the blood supply to the tumor rather than direct killing of the cancer cells. Exploiting the above two facts, i.e., affinity of *Salmonella* towards tumor tissues and requirement of fast growing blood vessels in tumors can lead to effective cancer therapy. The principle has been confirmed experimentally and a *S.* Typhimurium-based DNA vaccine expressing vascular endothelial growth factor (VEGF) receptor-2 (Flk1) and IL-12, has been used to first trigger an immune response to the VEGF antigen and then to stimulates T-cell to seek out and

Table 3. Some Important Antigens Expressed in *Salmonella* Mutants (CVD908 *aro*C$^-$*aro*D$^-$ Mutant of *S.* Typhi, *aroA*$^-$, *dam*$^-$*aroA*$^-$ and *dam*$^-$*pho*P$^-$ Mutants of *S.* Typhimurium etc.)

Diseases/ Pathogens/ Source of Antigens	Antigens Expressed in/ Carried on *Salmonella* [References]
Eukaryotic Pathogens	
Eimeria tenella	Antigen 5401 [72]
Plasmodium falciperum	Circum sporozoite protein (CSP) [105]
Onchocerca volvulus	Gutathione S-transferase [106]
Echinococcus granulosus	Surface antigen [107]
Leishmania mexicana	protein gp63 [108]
Plasmodium berghei	Merozoite Surface Protein-1 [109]
Leishmania major	T-cell epitope [110] and gp63 [111]
Schistosoma haematobium	glutathione S-transferase [112]
Bacteria	
Anthrax (*Bacillus anthracis*)	Protective antigen (PA) [90]
Pertussis (*Bordetella pertussis*)	P-69, FHA, PTX51 [113, 114]
Tetanus (*Clostridium tetani*)	Tetanus toxin fraction C [114]
Pneumococcal infections (*Pneumococcus* species)	Alpha-helical region of PspA (pneumococcal surface protein A [82]
Streptococcus mutans	Saliva-binding region (SBR) [85]
Escherichia coli	LTB, CFA1, Ki capsule, K88, CST [115-118]
Neiseria meningitidis	28 kDa outer membrane protein (OMP) [119]
Tularemia (*Francicella tularensis*)	17 kDa OMP [120]
Cholera (*Vibrio cholerae*)	Cholera toxin B subunit (CTB), O antigen [121, 122]
Yersinia spp.	Invasin, F1 capsular antigen [123], invasin [132]
Typhoid (*S.* Typhi)	Vi antigen [124]
Leprosy (*Mycobacterium leprae*)	Many antigens (85A, EAST6, Pst 3, Hsp 65) [80]
Mycobacterium bovis	30-kDa antigen [125]
Dysentery (*Shigella* sp.)	Many 'O' antigens [81, 126]
Diphtheria (*Corynebacterium diphtheriae*)	Diphtheria toxin [127]
Helicobacter pylori	HpaA and UreB [128]
Streptococcosis (*Streptococcus* sp.)	M protein, pneumolysin toxin [85], colonization and virulence antigens [129], antigen A [130]
Listeriosis	Protective antigens and haemolysins [131]
Campylobacter infection (*C. jejuni*)	Surface antigen [133]
Brucellosis (*Brucella abortus*)	31-kilodalton protein antigen [134]
Viruses	
Rabies	Glycoprotein [135]
Herpes simplex virus	Glycoprotein D [136]
Melanoma virus	NYESO-1 antigen [137], melanoma differentiation antigens Gp100 [138]
Hepatitis B virus	HBs antigen [83, 139]
Measles virus	HA antigen [81]
Human papillomavirus	Type 16 E7 epitopes [139]
Influenza A virus	Nucleoprotein [140]
Simian papilloma virus	SIV capsid antigen [141]
Transmissible gastroenteritis virus	Coronavirus S protein [142]
Murine fibrosarcoma virus	Murine fibrosarcoma antigen [143]
FMD Virus	FMDV antigen expressing DNA [144]
Human immunodeficiency Virus	HIV-1 gag antigen [145]
Antigens for Tumor Control	Endoglin (CD 105) an antigen having therapeutic value in breast cancer, CCL-21 (a secretory chemokine) to cure lung cancer, murine vascular endothelial growth factor (VEGF) receptor-2 (flk1), IL-2 etc. [73-74, 84, 86-89]
Antigens for Contraception	Fox sperm LDH-C4 antigen [146] and human sperm surface antigen [147]

destroy the blood vessels that feed growing tumors. One such vaccine [89] has prevented tumor growth in vaccinated mice after two weeks on challenge injection of melanoma, colon cancer cells and lung cancer cells. The therapy with another attenuated *Salmonella* Typhimurium vaccine strain encoding Flk1 and interleukin-12 has also been reported to be effective against tumors. The benefits of such recombinant vaccines lie in their multivalent nature which might work against many types of tumor cells simultaneously. The use of vaccines as therapy to treat cancer could potentially have several advantages over conventional therapies that directly target tumors [84, 89]. A DNA vaccine encoding secretory chemokine CCL21 and an inhibitor of apoptosis protein survivin could be delivered orally through doubly attenuated *S.*Typhimurium (*dam*$^-$ and *aro*A$^-$) mutant. The vaccine enhanced activation of antigen-presenting dendritic cells, and also $CD8^+$ T cells to produce an effective immune response against the survivin self-antigen. Vaccination resulted in eradication or suppression of pulmonary metastases of non-small cell lung carcinoma both in prophylactic and therapeutic trials in C57BL/6J mice [74]. Another oral *Salmonella* based anti-cancer DNA vaccine to specifically target tumor cells has been developed through cloning and expression of endoglin (CD 105), a tumor specific antigen. The vaccine led to suppression of pulmonary metastases of D2F2 breast carcinoma cells in a syngeneic mouse tumor model [87]. Other factors potentially able to break the immunotolerance to cancer antigens such as murine ubiquitin peptide epitopes gp10025-33 and TRP-2181-188 have also been cloned in and delivered by an oral *Salmonella* vaccine. The protective immunity against tumors obtained through *Salmonella* vectored vaccine is mediated by MHC class I antigen-restricted CD8+ T cells that secrete TH1 cytokine IFN and induce tumor rejection and growth suppression. The vaccine has been found experimentally successful in mice exposed to lethal challenge with B16G3.26 murine melanoma cells [73].

NON-VACCINE IMMUNOLOGICALS

Although live attenuated vaccines are being claimed as the most effective means of immunoprophylaxis against *Salmonella,* they do have a few drawbacks. Firstly, vaccinated animals continue to shed the vaccine strain for some time, making it difficult to differentiate between vaccinated and infected animals unless specific diagnostic methods are used. Secondly, the possibility of reversal of attenuated *Salmonella* vaccine strains to virulent forms cannot be ruled out. Thirdly, the live vaccines give protection against homologous *Salmonella* serovars from which the vaccine has been prepared, thus leaving the vaccinated animal potentially susceptible to thousands of other *Salmonella* serovars. Moreover, live attenuated *Salmonella* vaccines while protecting against virulent *Salmonella,* paradoxically may induced profound immuno-suppression against non-*Salmonella* antigens and may also suppress lymphoproliferative response to mitogens [34]. Thus, in the quest for novel means of controlling salmonellosis, Lowry and coworkers [91] found that immunoprophylactic use of a few lymphokines in young turkey poults and broiler chicks can reduce the horizontal transmission of *Salmonella* in poultry. Thus the study suggested the possibility of using non-vaccine immunologicals as part of a preventive strategy against *Salmonella* in poultry.

VACCINES FOR POULTRY SALMONELLOSIS

In poultry, salmonellosis is a multi-etiology zoonotic infection. Fowl typhoid and pullorum disease are caused by *S.* Gallinarum and *S.* Pullorum, bird-specific, host-adapted serovars, respectively. Most of the early attempts to produce an effective killed vaccine to control salmonellosis in poultry were of little practical value [92, 93]. A live rough strain (9R) vaccine developed in the early 1950s [14] was protective and, unlike killed vaccines, it did not interfere in the disease eradication programme, even where the disease is to be eradicated by using the whole-blood agglutination test. At most, 10% vaccinated birds may turn reactors in the test [50, 94]. Gupta and Mallick [49] reported better protection with adjuvanted 9R vaccine but their findings were soon contradicted [51]. Although requirement of oral dosing of sodium bicarbonate [48] before 9R vaccine delivery to birds was cumbersome, cross protection conferred by 9R vaccine against *S.* Typhimurium [95-97] was an added advantage. The 9R strain possesses some virulence [50, 98-100] therefore its use in young chicken of certain susceptible breeds is often a problem. Protection level by 9R vaccine is often questioned (~ 60% against challenge with virulent *S.* Gallinarum) and OMP based subunit vaccine was reported to be a better option [37, 50]. The use of rough strains as vaccines is further jeopardized because of frequent isolation of rough strains from food animals [7, 8, 101].

Elimination (curing) of a virulence plasmid from a *S.* Gallinarum strain greatly reduced virulence of *S.* Gallinarum, but the strain was able to persist in the reticuloendothelial system of the chicken for some time to induce good protective immune response [102]. However, the plasmid cured strain could not be exploited as vaccine because all vaccinated birds reacted positively by whole blood agglutination test for fowl typhoid. The vaccine faced strong criticism due to its inapplicability in regions where whole blood test is used as screening method for control of salmonellosis in poultry. In 3-week-old chickens, the plasmid-cured derivative was virtually avirulent, but residual virulence was enough to cause disease in newly hatched chickens, thus the vaccine could not be used in the field. Besides, acquisition of the plasmid from wild strains present in intestines of vaccinated birds remained a potential threat.

Short term and incomplete protection afforded by killed vaccines, long term persistence and fecal excretion of live vaccine candidate (9R and plasmid cured strains) in immunized birds failed to curtail worldwide prevalence of fowl typhoid. Besides, consistently increasing frequency of isolation of antibiotic resistant *S.* Gallinarum and foodborne zoonotic *Salmonella*e are pressing hard for evolution of improved *Salmonella* vaccine. Singh and Sharma [40] reported 100% protection in mice vaccinated with *S.* Weltevreden formalized toxoid, and similar methodology was used to develop a successful toxoid vaccine which provided long lasting immunity to birds against salmonellosis [42-44]. The same subunit toxoid vaccine also protected the chicks in early age due to maternal transfer of *Salmonella* antibodies in egg yolk.

USE OF *SALMONELLA* VACCINES FOR COMPETITIVE EXCLUSION OF THE PATHOGENS

In the late 20th century, *Salmonella* enteritidis has emerged as a major egg-borne zoonotic infection probably due to overuse of *S.* Gallinarum vaccines. Meta-analysis of epidemiological data of poultry salmonellosis revealed an inverse relationship between the incidence of *S.* Gallinarum infection in chickens and egg-associated *S.* Enteritidis infections in humans from England, Wales, and the United States. The findings indicated that *S.* Enteritidis might have emerged as a result of a gap filling mechanism, having probably filled the ecologic niche vacated by *S.* Gallinarum as a result of extensive use of *S.* Gallinarum vaccines in poultry. Increased colonization of *S.* Enteritidis in birds could be associated with increased excretion of the pathogen by the birds in the environment and increased prevalence of the pathogen in poultry products leading to a marked increase in human infections from *S.* Enteritidis [103]. *Salmonella* Enteritidis is unlikely to be eliminated from poultry by relying solely on the test-and-slaughter method of disease control because, unlike *S.* Gallinarum, *S.* Enteritidis can be reintroduced into flocks from its rodent reservoirs. Instead, vaccination would be effective in excluding *S.* Enteritidis from domestic fowl because it would eliminate one of the risk factors (loss of flock immunity against the O9-antigen), which had probably contributed to the emergence of *S.* Enteritidis as a foodborne pathogen. In fact, much of the decline in human *S.* Enteritidis cases in England and Wales since 1994 has been attributed to the use of an *S.* Enteritidis vaccine in poultry [104]. However, serologic evidence suggesting that *S.* Gallinarum is more immunogenic than *S.* Enteritidis indicates that a more effective approach for eliciting protection in chickens would be immunization with a live attenuated *S.* Gallinarum vaccine. Colonization of avirulent *S.* Gallinarum in birds shall restore the natural balance (exclusion of *S.* Enteritidis by a natural competitor) that existed before human interventions implemented early in the 20th century and may also exclude the threat of rodents and human mediated transmission of *S.* Gallinarum (as the pathogen is host restricted serovar) in flocks desired to be free from antibodies or the bacteria, which is not possible with the use of *S.* Enteritidis vaccine.

FUTURE OF *SALMONELLA* VACCINES AS MUCOSAL VACCINES

Mucosal vaccines are the ultimate targets for control of many diseases. It is now quite clear that mucosally administered immunogens when delivered with appropriate adjuvants or in an appropriate form, can stimulate the most effective systemic immune response against not only those pathogens invading through mucosal lining but more effectively against those which have predilection site and invasion sites remote from the gut. *Salmonella* being a gut invading bacteria efficiently deliver required antigens while moving in the body. Therefore, oral vaccine against tetanus developed through cloning of DNA coding for an immunogenic non-toxic fraction of tetanus toxin into an attenuated *Salmonella* strain proved more efficacious than any other type of tetanus vaccine. Many antigens of immunogenic potential for control of parasitic [72, 105-112], bacterial [80-82, 85-86, 90, 113-134] and viral [81, 83, 135-145] infections, tumor growth [73, 74, 84, 86-89] and also for contraception [146, 147] have already been expressed in attenuated *Salmonella* strains, a step towards development of multivalent mucosal vaccines (Table **3**). Studies revealed that *Salmonella* vaccines as vector for DNA and plasmid-vectored vaccines bear a vast potential. Live oral *Salmonella* vaccines may be the future prototype vaccine vector for passenger immunogens in humans and animals [47] and mucosal delivery of a battery of antigens [105-150]. Live attenuated *Salmonella* may overcome limitations with conventional methods of DNA immunization because of plasmid stability conferred on oral DNA delivery by the use of attenuated *Salmonella* vaccine strains. Studies have shown success of BRD509, *S.* Typhimurium oral vaccine candidate, transformed with plasmid having a DNA vaccine cassette comprising the C fragment of tetanus toxin under control of the cytomegalovirus (CMV) promoter. The cloning plamids including pBBR122, pACYC184, pRSF1010/CAT, pBR322 and pAT153 were stably retained by BRD509 and induced a tetanus toxin-specific neutralizing antibody response following oral delivery [151].

Mucosal vaccines can be administered intranasally, intravaginally, and orally. The immunity acquired is not limited to the local mucosal site of administration, so intranasal administration can produce antibody at alternative mucosal sites as well as systemically. Combining DNA and mRNA vaccines with a mucosal route delivery may be even more ideal for any disease control programme [152].

FUTURE AS MARKER AND DIVA VACCINES

Marker vaccines include those attenuated vaccine candidate strains of pathogens which can easily be identified while circulating and isolated from vaccinated or unvaccinated populations by having easily identifiable phenotypic marker, such as heavy metal or selected antibiotic resistance markers and fluorescence generating genes. Apart from the rough strains used in poultry birds, most of the genetically engineered vaccines for control of *Salmonella* infection have at least one suitable marker. These types of vaccines induce protective responses easily be differentiated from those immune responses caused by natural infections. The OIE (World Animal Health Organization, WAHO) has recommended that a vaccine must have both, differentiating infected from vaccinated animals (DIVA) and marker(s) qualities before being released for wide spread veterinary use. It is much more pertinent in cases where prevalence of causative agent is high as for *Salmonella* infections. The success of such vaccines has come true in control of avian influenza in Italy. In planning *Salmonella* as vector for delivery of various antigens of parasitic, bacterial and viral origin will automatically take care of DIVA strategy in vaccines designed for delivery of multiple antigens. Steps towards development of DIVA vaccines essentially require an antigenic differentiation in parent and the mutant vaccine candidate, recently *S.* Abortusequi S-30 oral double mutant (*aro*A$^-$*htr*A$^-$) vaccine candidate developed for control of salmonellosis in equids has been shown to posses the DIVA potential [79].

CONCLUSION

Extensive work on killed vaccines for successful control of salmonellosis caused by host adapted serovars like *S.* Typhi (human typhoid), *S.* Gallinarum (fowl typhoid) and *S.* Abortusequi (abortion in equines) revealed that they confer

only short lived immunity. Killed vaccines also failed to afford desired protection in genetically susceptible animals, which are better protected by use of suitable live vaccine [12, 153] but the opposite is also true [35], thus development of either killed or live vaccine is not the end point in field of *Salmonella* vaccinology. Research groups are currently developing live attenuated vaccine strains of *Salmonella* not only to control salmonellosis but to exploit their potential as mucosal multivalent vaccine candidates. The most common problems with development of live vaccines include 1) attenuation through a genetic lesion in the same gene which in different *Salmonella* serovars may have different attenuating effect [34] and 2) different attenuated strains even of same serovar having deletion of the same gene may not be having similar vaccine potential [60]. Besides, the same mutant found effective in one animal species may not have the same performance in others, for instance the same *aro*A mutant strain of *S*. Typhimurium on oral vaccination gave good results in human but failed to protect chicken, however when route of inoculation was changed to intramuscular (im) it was found useful indicating that route of inoculation might have great impact on outcome of vaccination results [154, 155]. Moreover protective response of live *Salmonella* vaccine can vary in the population due to many factors such as competition of the vaccine strain with resident flora in different host species leading to altogether different outcomes, variability of predilection sites for invasion or colonization of the same serovar in different animals and finally competition with resident *Salmonella* and trapping of vaccine by cross reacting sIgA. It has been observed that sIgAs against an antigenically related *Salmonella* serovar cross protects the niche from colonization by the vaccine strain which may adversely effect the outcome of vaccination. Thus, if *Salmonella* is to be used both as vaccine and as a vector to carry DNA or plasmid for development of multivalent vaccines there is an urgent need to develop specific strains for different animal populations. It is apparent that considerably more research is required to develop vaccines able to protect the target animal from a wide range of common *Salmonella* serovars and this is likely to be achieved in the near future by use of biogenetic engineering tools.

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REFERENCES

[1] Plotkin, S.A. Six revolutions in vaccinology. *Ped. Infect. Dis. J.*, **2005**, *24*, 1-9.

[2] Kumar, R.; Sazawal, S.; Sinha, A.; Sood, S.; Bhan, M.K. In *Round Table Conference Series on Water Borne Diseases*. 12th ed. Ranbaxy Science Foundation. New Delhi: India, **1997**, vol. *2*, pp. 31-36.

[3] Lax, A.J, Barrow, P.A.; Jones, P.W.; Wallis, T.S. Current perspectives in salmonellosis. *Br. Vet. J.*, **1995**, *151*, 351-377.

[4] Peters, A.R. An estimation of the economic impact of an outbreak of *Salmonella dublin* in a calf-rearing unit. *Vet. Record.*, **1985**, *117*, 667-668.

[5] Ghosh, S.S.; Gupta, B.R.; Verma, J.C. Economic loss due to an outbreak of *Salmonella virchow* in an organized poultry farm in Nagaland. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, **1987**, *8*, 145-148.

[6] Daniel, N.A.; Mackinnon, L.; Rowe, S.M.; Bean, N.H.; Griffin, P.M.; Mead, P.S. Foodborne disease outbreaks in United State schools. *Ped. Infect. Dis. J.*, **2002**, *21*, 623-38.

[7] Verma, J.C.; Singh, B.R. In *Emerging Health Problems in Animals*, Proceedings of 1st Indian Veterinary congress, IVRI, Izatnagar, India, February 18-19, 2000; Indian Association for Advancement of Veterinary Research. Izatnagar: India, **2000**, pp. 28-38.

[8] Verma, J.C.; Singh, V.P.; Singh, B.R.; Gupta, B.R. Occurrence of *Salmonella* serotypes in animals in India VII. *Indian J. Comp. Microbiol. Immuol. Infect. Dis*., **2001**, *22*, 51-55.

[9] Singh, B.R.; Singh, P.; Verma, A.; Agrawal, S.; Babu, N.; Chandra, M.; Agarwal, R.K. A study on prevalence of multi-drug resistant (MDR) *Salmonella* in water sprinkled on fresh vegetables in Bareilly, Moradabad, and Kanpur (Northern Indian cities). *J. Public Health*, **2006**, *14*, 125-131.

[10] Teotia,U.V.S. Agrawal, R.K.; Pant, S.; Singh, B.R.. Raj, H. How to control food borne Salmonellosis . http://www.nio.org/past_events/comits/hum _patho mar_syst.jsp (assessed August 11, 2007).

[11] National *Salmonella* Centre (Veterinary) Izatnagar. *Salmonella* Serovars in India. http://upgov.up.nic.in/ivri/nsc (assessed June 12, 2006).

[12] Singh, B.R.; Singh, Y.; Agarwal, M.C.; Agarwal, R.K.; Sharma, V.D. *Salmonella* vaccines for veterinary use: an overview. *Haryana Vet.*, **2005**, *44*, 1-12.

[13] Wright, A.E.; Sample, D. Typhoid vaccines. *Br. Med. J.*, **1997**, *1*, 256-262.

[14] Smith, H.W. The use of live vaccines in experimental *Salmonella gallinarum* infection in chickens with observations on their interference effect. *J. Hyg*., **1956,** *54*, 419-432.

[15] Mendel, H.; Hash, P.; Hino, S. Degree of immunity induced by killed vaccines to experimental salmonellosis in mice. *Infect. Immun.*, **1972**, *5*, 83-90.

[16] Nicholas, R.A.J.; Andrews, S.J. Degree of immunity induced by killed vaccines to experimental salmonellosis in mice. *Vet. Record.*, **1991**, *128*, 98-100.

[17] Dhanda, M.R.; Lall, Y.M.; Singh, M.M. Studies on *Salmonella abortusequi* vaccine. *Indian J. Vet. Sci.*, **1955**, *25*, 245-249.

[18] Siddiqui, I.H. *A study of Salmonella abortusequi.* Thesis. Agra University, Agra, **1968**.

[19] Gupta, B.R.; Mittal, K.R. Active mouse protection test to evaluate the potency of *Salmonella abortusequi* vaccine. *Indian Vet. J.*, **1975**, *52*, 903-905.

[20] Kataria, J.M. *Studies on Salmonella abortusequi vaccines*. Thesis. Rohilkhand University, Bareilly, **1978**.

[21] Kataria, J.M.; Uppal, P.K. Studies on beta-propiolactone and glutaraldehyde inactivated *Salmonella abortusequi* vaccines. *Indian Vet. J.*, **1981**, *58*, 839-845.

[22] Gupta, B.R.; Singh, U.S.; Verma, J.C. Immunogenicity of whole cell vacine and *Salmonella* fractions of *Salmonella abotusequi*. A comparative study. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, **1987**, *8*, 50-54.

[23] Liberal, M.H.T. Control of salmonellosis in young calves by vaccination. *J. Braz. Pesargrorio*, **1989**, *1*, 11-17.

[24] Ghosh, S.S. Comparative efficacy of four vaccines against *Salmonella virchow* in chicks in India. *Res. Vet. Sci.*, **1989**, *47*, 280-282.

[25] Mohrah, I.M.; Zaki, M.M. Trials to prepare potent vaccine against *Salmonella gallinarum-pullorum* infection. *Vet. Med. J. Giza*, **1995,** *43*, 97-102.

[26] Gast, R.K.; Stone, H.D.; Holt, P.S. Evaluation of the efficacy of oil-emulsion bacterins for reducing fecal shedding of *Salmonella enteritidis* by laying hens. *Avian Dis.*, **1993**, *37*, 1085-1091.

[27] Barbour, E.K.; Hamadeh, S.K.; Bejjani, N.E.; Faroon, O.M.; Eid, A.; Sakr, W.; Bouljihad, M.; Spasojevic, R.; Safieh, G.B. Immunopotentiation of a developed *Salmonella enterica* serotype Enteritidis vaccine by thymulin and zinc in meat chicken breeders. *Vet. Res. Commun.*, **2001**, *25*, 437-447.

[28] Woodward, M.J.; Gettinby, C.; Breshlin, M.F.; Corkish, J.D.; Houghton, S. The efficacy of salenvac, a *Salmonella enterica* subsp. *enterica* serotype Enteritidis iron restricted bacterin vaccine, in laying chickens. *Avian Pathol.*, **2002**, *31*, 383-392.

[29] Singh, B.R.; Verma, J.C. A study on factors influencing 'O' and 'H' agglutinin titres of hyper immune serum raised in rabbits

against *Salmonella* and *E. coli*. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, **2003**, *24*, 49-53.

[30] Cooper, B.S.; Mac Farlane, D.J. Single or double vaccination schedules in sheep against experimental infection with *Salmonella typhimurium* or *S. bovismorbificans. N Z Vet. J.*, **1974**, *22*, 95-99.

[31] Baljer, G.; Hoerstke, M.; Dirksen, G.; Sailer, J.; Mayr, A. Efficacy of local and parenteral vaccination against calf salmonellosis with inactivated vaccines. *J. Vet. Med.*, **1986**, *33*, 206-212.

[32] Engles, E.A.; Falagas, M.E.; Lau, J. Bennish, M.L. Typhoid fever vaccines: a meta-analysis of studies on efficacy and toxicity. *Br. Med. J.*, **1998**, *316*, 110-116.

[33] Weber, A.; Bernt, C.; Bauer, K.; Mayr, A. Control of bovine salmonellosis under field conditions using herd specific vaccines. *Tierztliche Praxis*, **1993**, *21*, 511-516.

[34] Barrow, P.A.; Wallis, T.S. In *Salmonella in Domestic Animals*; Wray, C.; Wray, A. Eds.; CABI Publishing: London, **2000**, pp. 323-240.

[35] MacLennan, C.A.; Gondwe, E.N.; Msefula, C.L.; Kingsley, R.A.; Thomson, N.R.; White, S.A.; Goodall, M.; Pickard, D.J.; Graham, S.M.; Dougan, G.; Hart, C.A.; Molyneux, M.E.; Drayson, M.T. The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of *Salmonella* in African children. *J. Clin. Invest.*, **2008**, *118*, 1553-1562.

[36] Bouzoubaa, K.; Nagaraja, K.; Newman, J.A.; Pomeroy, B.S. Use of membrane proteins from *Salmonella gallinarum* for prevention of fowl typhoid infection in chickens. *Avian Dis.*, **1987**, *31*, 499-504.

[37] Bouzoubaa, K.; Nagaraja, K.; Kabbaj, F.Z.; Newman, J.A.; Pomeroy, B.S. Feasibility of using proteins from *Salmonella gallinarum* vs 9 R live vaccine for the prevention of fowl typhoid in chickens. *Avian Dis.*, **1989**, *33*, 385-391.

[38] Charles, S.C.; Nagaraj, K.V.; Sivanandan, V. A lipid conjugated immunostimulating complex subunit vaccine against *Salmonella* infection in turkeys. *Avain Dis.*, **1993**, *37*, 477-484.

[39] Lee, H.; Kim, S.; Kim, K.; Mo, I.; Woo, Y.; Kwon, Y.; Kim, T.; Lee, H.S.; Kim, S.J.; Kim, K.S.; Mo, I.P.; Woo, Y.K.; Kwon, Y.K.; Kim, T.J. Immunogenicity of outer membrane protein extracted from *Salmonella gallinarum* in chickens. *Natl. Vet. Res. Inst.*, **1997**, *37*, 555-568.

[40] Singh, B.R.; Sharma, V.D. Solation and characterization of four distinct cytotoxic factors of *Salmonella* Weltevreden. *Zentralbl. Bakteriol.*, **1999**, *289*, 457-474.

[41] Vasava, K.A. *Studies on comparative efficacy of various vaccines against Salmonella Abortusequi infection*. Thesis. Deemed University, Indian Veterinary Research Institute: Izatnagar, **1999**.

[42] Kumar, S. *Immunopotentiation of toxoid against salmonellosis in poultry*. Thesis, Govind Ballabh Pant University of Agriculture and Technology: Pantnagar, **1999**.

[43] Mishra, R.S.; Sharma, V.D. Comparative efficacy of various toxoids against salmonellosis in poultry. *Vet. Res. Commun.*, **2001**, *25*, 337-344.

[44] Barman, T.K.; Sharma, V.D.; Kumar, S. Optimization of dose of *Salmonella toxoid* vaccine in poultry. *Indian Vet. J.*, **2002**, *79*, 106-110.

[45] Vasava, K.A.; Singh, B.R.; Verma, J.C. Detection of cytotoxigenicity among strains of *Salmonella enterica* subspecies *enterica* serovar Abortusequi by an indirect ELISA. *Indian J. Vet. Res.*, **2004**, *13*, 31-34.

[46] Mukkur, T.K.S.; McDowell, G.H.; Stockers, B.A.D.; Lascelles, A.K. Protection against experimental salmonellosis in mice and sheep by immunization with aromatic dependent *Salmonella typhimurium*. *J. Med. Microbiol.*, **1987**, *24*, 11-19.

[47] Lewis, G.K. Live-attenuated *Salmonella* as a prototype vaccine vector for passenger immunogens in humans: are we there yet? *Expert Rev. Vacc.*, **2007**, *6*, 431-440.

[48] Gupta, B.R.; Mallick, B.B. Immunization against fowl typhoid. 1. Live oral vaccine. *Indian J. Anim. Sci.*, **1976**, *46*, 502-505.

[49] Gupta, B.R.; Mallick, B.B. Immunization against fowl typhoid. 2. Live adjuvant vaccine. *Indian J. Anim. Sci.*, **1976**, *46*, 546-551.

[50] Silva, E.N.; Snoeyenbos, G.H.; Weinack, O.M.; Smyser, C.F. Studies on the use of 9R strain of *Salmonella gallinarum* as a vaccine in chickens. *Avian Dis.*, **1981**, *25*, 38-52.

[51] Kahraman, M.; Ozian, C. Immunogenicity of four fowl typhoid vaccines prepared with R strain of *Salmonella gallinarum*. *Vet. Fak. Derg. Ankar*, **1985**, *32*, 330-335.

[52] Cameron, C.M.; Buys, S.B. Production and application of a live *Salmonella gallinarum* vaccine. *Onderstepoort J. Vet. Res.*, **1979**, *46*, 185-189.

[53] Verma, J.C. Studies on salmonellosis with special reference to phage typing of *Salmonella* isolates and immunization of calves against *Salmonella dublin* infection. Thesis, Agra University: Agra, **1969**.

[54] Knivett, V.A.; Stevens, W.K. The evaluation of live *Salmonella* vaccine in mice and chickens. *J. Hyg.*, **1971**, *69*, 233-245.

[55] Knivett, V.A.; Tucker, J.F. Comparison of oral vaccination or Furazolidone prophylaxis on *Salmonella typhimurium* infection in chicks. *Br. Vet. J.*, **1972**, *128*, l0l-105.

[56] Chaturvedi, G.C.; Sharma, V.K. Cell mediated immunoprotection in calves immunized with rough *Salmonella dublin*. *Br. Vet. J.*, **1981**, *137*, 412-418.

[57] Pritchard, D.G.; Nivas, S.C.; York, M.D.; Pomeroy, B.S. Effect of a gal E mutant of *Salmonella typhimurium* on experimental salmonellosis in chickens. *Avian Dis.*, **1978**, *22*, 562-575.

[58] Baljer, G.; Hoerstke, M.; Dirksen,G.; Seitz, A.; Sailer, J.; Mayr, A. Comparisn of efficacy of oral immunization with heat inactivated and live avirulent (*gal*E) *Salmonella typhimurium* against salmonellosis in calves. *Zentralblatt Fur Vet. Med. B.*, **1981**, *28*, 759-767.

[59] Stocker, B.A.D.; Hoiseth, S.K.; Smith, B.P. Aromatic dependent *Salmonella* sp. as live vaccine in mice and calves. *Dev. Biol. Standard.*, **1883**, *53*, 47-59.

[60] Smith, B.P.; Reina-Guerra, M.; Hoiseth, S.K. Aromatic dependent *Salmonella typhimurium* as modified live vaccine for calves. *Am. J. Vet. Res.*, **1984**, *45*, 59-66.

[61] Cooper, G.L.; Venables, L.M.; Nicholas, R.A.J.; Cullen, G.A.; Hormaeche, C.E. Vaccination of chickens with-chicken derived. *Salmonella enteritidis* phage type 4 *aro*A$^-$ live oral *Salmonella* vaccines. *Vaccine*, **1992**, *10*, 247-254.

[62] Alam, J. *Studies on Immunogenesity and safety of aroA mutant of S. Abortusequi*. Thesis, Deemed University, Indian Veterinary Research Institute: Izatnagar, **2001**.

[63] Singh, B.R.; Alam, J.; Hansda, D. Alopecia induced by *salmonellosis* in guinea pigs. *Vet. Record*, **2005**, *156*, 516-518.

[64] Curtiss, R.; Kelly, S.M. *Salmonella typhimurium* deletion mutants lacking adenylate cyclase and cyclic AMP receptor protein are avirulent and immunogenic. *Inf. Immun.*, **1987**, *55*, 3035-3043.

[65] Miller, S.I.; Kukral, A.M.; Mekalanos, J.J. A two component regulatory system (phoP phoQ) controls *S.* Typhimurium virulence. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 5054-5058.

[66] Miller, S.I.; Mekalanos, J.J. Constitutive expression of the *phoP* regulon attenuates *Salmonella* virulence and survival within macrophages. *J. Bacteriol.*, **1990**, *172*, 2485-2490.

[67] Gulig, P.A.; Curtiss, R. Plasmid associated virulence of *Salmonella typhimurium*. *Infect. Immun.*, **1987**, *55*, 2891-2901.

[68] Li, Z.; Turner, A.K.; Dougan, G.; Barrow, P.A. Protection of chickens against experimental fowl typhoid using a *nuo*G mutant of *Salmonella* serotype Gallinarum. *Vaccine*, **1998**, *16*, 9-10.

[69] Barrow, P.A.; Lovell, M.A.; Stocker, B.A.D. Protection against experimental fowl typhoid by parenteral administration of live SL5828, an *aro*A-*ser*C (aromatic dependent) mutant of a wild-type *Salmonella gallinarum* strain made lysogenic for P22 sie. *Avian Pathol.*, **2000**, *29*, 423-431.

[70] Hansda, D. *Studies on Immunogenesity and safety of Double mutant (aroA-htrA) of S. Abortusequi*. Thesis, Deemed University Indian Veterinary Research Institute: Izatnagar, **2001**.

[71] Dueger, E.L.; House, J.K.; Heithoff, D.M.; Mahan, M.J. *Salmonela* DNA adenine methylase mutants prevent colonization of newly hatched chickens by homologous and heterologous serovars. *Int. J. Food Microbiol.*, **2003**, *80*, 153-159.

[72] Du, A.; Wang, S. Efficacy of a DNA vaccine delivered in attenuated *Salmonella* typhimurium against *Eimeria tenella* infection in chickens. *Int. J. Parasitol.*, **2005**, *35*, 777-785.

[73] Xiang, R.; Lode, H.N.; Ta-Hsiang, C.; Ruehlmann, J.M.; Dolman, C.S.; Rodriguez, F.; Whitton, J.L.; Overwijk, W.W.; Restifo, N.P.; Reisfeld, R.A. An autologous oral DNA vaccine protects against murine melanoma. *Annal. N Y Acad. Sci.*, **2005**, *1056*, 366-378.

[74] Xiang, R.; Mizutani, N.; Luo, Y.; Chiodoni, C.; Zhou, H.; Mizutani, M.; Ba, Y.; Becker, J.C.; Reisfeld, R.A. A DNA Vaccine targeting survivin combines apoptosis with suppression of angiogenesis in lung tumor eradication. *Cancer Res.*, **2005**, *65*, 553-561.

[75] Singh, B.R. Project CGP III-327 Report. *Development of double defined deletion mutant vaccine for control of Salmonella Abortusequi infection in equines*. IVRI, Izatnagar: India, **2005**.

[76] Cooper, G.L. Salmonellosis infections in man and chicken pathogenesis and the development of live vaccine - a review. *Vet. Bull.*, **1994**, *64*, 123-143.

[77] Barbezange, C.; Humbert, F.; Rose, V.; Lalande, F.; Salvat, G. Some safety aspects of *Salmonella* vaccines for poultry distribution and persistence of three *Salmonella* Typhimurium live vaccines. *Avian Dis.*, **2000**, *44*, 968-976.

[78] Methner, U.; Berndt, A.; Steinbach, G. Combination of competitive exclusion and immunization with an attenuated live *Salmonella* vaccine strain in chickens. *Avian Dis.*, **2001**, *45*, 631-638.

[79] Chandra, M.; Singh, B.R.; Srivastava, S.K.; Chaudhry, P.; Agrawal, R.K.; Sharma, A. Comparative analysis of protein profiles of wild virulent (E156) and *aro*A-*htr*A double deletion mutant vaccine strain (S30) of *Salmonella enterica subspecies enterica* serovar Abortusequi under *in vivo* and *in vitro* growth conditions. *Indian J. Exp. Biol.*, **2008**, *46*, 621-625.

[80] Parida, S.K.; Huygen, K.; Ryffel, B.; Chakraborty, T. Novel bacterial delivery system with attenuated *Salmonella typhimurium* carrying plasmid encoding Mtb antigen 85A for mucosal immunization: Establishment of proof of principle in TB mouse model. *Science*, **1998**, *281*, 565-568.

[81] Fennelly, G.J.; Khan, S.A.; Abadi, M.A.; Wild, T.F.; Bloom, B.R. Mucosal DNA vaccine immunization against Measles with a highly attenuated *Shigella flexneri* vector. *J. Immunol.*, **1999**, *162*, 1603-1610.

[82] Kang, H.Y.; Srinivasan, J.; Curtiss, R. Immune responses to recombinant pneumococcal PspA antigen delivered by live attenuated *Salmonella enterica* serovar Typhimurium vaccine. *Infect. Immun.*, **2002**, *70*, 1739-1749.

[83] Zheng, B.J.; Hon, Ng.M.; Chan, K.W.; Tam, S.; Woo, P.C.Y.; Park, Ng.S.; Yuen, K.Y. A single dose of oral DNA immunization delivered by attenuated *Salmonella typhimurium* down-regulates transgene expression in HBsAg transgenic mice. *Eur. J. Immunol.*, **2002**, *32*, 3294-3304.

[84] Keke, F.; Hongyang, Z.; Hui, Q.; Jixiao, L.; Jian, C. A combination of flk1-Based DNA vaccine and an immunomodulatory gene (IL-12) in the treatment of murine cancer. *Cancer Biother. Radiopharm.*, **2004**, *19*, 649-657.

[85] Salam, M.A.; Katz, J.; Zhang, P.; Hajishengallis, G.; Michalek, S.M. Immunogenicity of *Salmonella* vector vaccines expressing SBR of *Streptococcus mutans* under the control of a *T7-nirB* (dual) promoter system. *Vaccine*, **2006**, *24*, 5003-5015.

[86] Xu, C.; Li, Z.S.; Du, Y.Q.; Gong, Y.F.; Yang, H.; Sun, B.; Jin, J. Construction of recombinant attenuated *Salmonella typhimurium* DNA vaccine expressing H pylori *ure*B and IL-2. *World J. Gastroenterol.*, **2007**, *13*, 939-944.

[87] Reisfeld, R.A. An oral DNA vaccine encoding endoglin eradicates breast tumors by blocking their blood supply. http://handle.dtic.mil/100.2/ADA474671 (assessed August 11, 2008).

[88] Cochlovius, B.; Stassar, M.J.; Schreurs, M.W.; Benner, A.; Adema, G.J. Oral DNA vaccination: antigen uptake and presentation by dendritic cells elicits protective immunity. *Immunol. Lett.*, **2002**, *80*, 89-96.

[89] Touchette, N. DNA vaccine starves tumors in mice. www.genomenewsnetwork.org (assessed August 11, 2008).

[90] Stokes, M.G.M.; Titball, R.W.; Neeson, B.N.; Galen, J.E.; Walker, N.J.; Stagg, A.J.; Jenner, D.C.; Thwaite, J.E.; Nataro, J.P.; Baillie, L.W.J.; Atkins, H.S. Oral administration of a *Salmonella* enterica-based vaccine expressing Bacillus anthracis protective antigen confers protection against aerosolized *B. anthracis*. *Infect. Immun.*, **2007**, *75*, 1827-1834.

[91] Lowry, V.K.; Tellez, G.I.; Nisbet, D.J.; Garcia, G.; Urquiza, O.; Stanker, L.H.; Kogut, M.H. Efficacy of *Salmonella enteritidis* immune lymphokines on horizontal transmission of *Salmonella arizonae* in turkeys and *Salmonella gallinarum* in chickens. *Int. J. Food Microbiol.*, **1999**, *48*, 139-148.

[92] Hall, W.J.; MacDonald, A.D.; Legenhausen, D.H. Studies on fowl typhoid. II. Control of the disease. *Poult. Sci.*, **1949**, *28*, 789-801.

[93] Wilson, J.E. Fowl typhoid-the effect of vaccination on the natural and experimental disease. *Vet. Record*, **1956**, *68*, 664-668.

[94] Barrow, P.A.; Berchieri, A. Jr.; Al-Haddad, O. Serological response of chickens to infection with *Salmonella gallinarum* and *S. pullorum* detected by enzyme-linked immunosorbent assay. *Avian Dis.*, **1992**, *36*, 227-236.

[95] Padmnabhan, V.D.; Mittal, K.R. Cross protection against *Salmonella typhimurium* infection in mice. *Indian J. Microbiol.*, **1980**, *24*, 270-276.

[96] Padmnabhan, V.D.; Mittal, K.R.; Gupta, B.R. Cross protection against fowl typhoid immunization trial and humoral immune response. *Dev. Comp. Immunol.*, **1981**, *5*, 301-303.

[97] Chandran, N.J.D.; Moses, D.; Balprakashan, R.A. Cell mediated immune response in chicks against salmonellosis. *Cheiron*, **1983**, *12*,194-197.

[98] Gordon, R.F.; Garside, J.S.; Tucker, J.F. The use of living attenuated vaccines in the control of fowl typhoid. *Vet. Record*, **1959**, *71*, 300-305.

[99] Gordon, W.A.M.; Luke, D. A note on the use of the 9R fowl typhoid vaccine in poultry breeding flocks. *Vet. Record*, **1959**, *71*, 926-927.

[100] Feberwee, A.; Vries, T.S.de.; Hartman, E.G.; Wit, J.J.de.; Elbers, A.R.W.; Jong, W.A.de. Vaccination against *Salmonella enteritidis* in Dutch commercial layer flocks with a vaccine based on a live *S. gallinarum* 9R strain: evaluation of efficacy, safety and performance of serological *Salmonella* tests. *Avian Dis.*, **2001**, *45*, 83-91.

[101] Singh, B.R. *Salmonella* serovars prevalent in animals in India. http://upgov.up.nic.in/ivri/nsc (assessed January 10, 2006).

[102] Barrow, P.A.; Simpson, J.M.; Lovell, M.A.; Binns, M.M. Contribution of *Salmonella gallinarum* large plasmid toward virulence in fowl typhoid. *Infect. Immun.*, **1987**, *55*, 388-392.

[103] Rabsch, W.; Hargis, B.M.; Tsolis, R.M.; Kingsley, R.A.; Heinz, K.H.; Tschäpe, H.; Bäumler, A.J. Competitive exclusion of *salmonella* enteritidis by *Salmonella* gallinarum in poultry. Emerging infectious Diseases.http://www.cdc.gov/ncidod/EID/vol6no5/rabsch.htm (assessed January 10, 2004).

[104] Ward, L.R.; Threlfall, J.; Smith, H.R.; O'Brien, S.J. *Salmonella enteritidis* epidemic. *Science*, **2000**, *287*, 1753-1756.

[105] Sadoff, J.C.; Ballou, W.R.; Baron, L.S.; Majarian, W.R.; Brey, R.N.; Hockmeyer, W.T.; Young, J.F.; Cryz, S.J.; Ou, J.; Lowell, G.H. Oral *Salmonella* typhimurium vaccine expressing circumsporozoite protein protects against malaria. *Science*, **1988**, *240*, 336-338.

[106] Catmull, J.; Wilson, M.E.; Kirchhoff, L.V.; Metwali, A.; Donelson, J.E. Induction of specific cell-mediated immunity in mice by oral immunization with *Salmonella* expressing Onchocerca volvulus glutathione S-transferase. *Vaccine*, **1999**, *17*, 31-39.

[107] Chabalgoity, J.A.; Moreno, M.; Carol, H.; Dougan, G.; Hormaeche, C.E. *Salmonella typhimurium* as a basis for a live oral *Echinococcus granulosus* vaccine. *Vaccine*, **2000**, *19*, 460-469.

[108] Gonzalez, C.R.; Noriega, F.R.; Huerta, S.; Santiago, A.; Vega, M.; Paniagua, J.; Ortiz-Navarrete, V.; Isibasi, A.; Levine, M.M. Immunogenicity of a *Salmonella typhi* CVD 908 candidate vaccine strain expressing the major surface protein gp63 of *Leishmania mexicana mexicana*. *Vaccine*, **1998**, *16*, 1043-1052.

[109] Toebe, C.S.; Clements, J.D.; Cardenas, L.; Jennings, G.J.; Wiser, M.F. Evaluation of immunogenicity of an oral *Salmonella* vaccine expressing recombinant *Plasmodium berghei* merozoite surface protein-1. *Am. J. Trop. Med. Hyg.*, **1997**, *56*, 192-199.

[110] White, A.P.; Collinson, S.K.; Burian, J.; Clouthier, S.C.; Banser, P.A.; Kay, W.W. High efficiency gene replacement in *Salmonella enteritidis*: chimeric fimbrins containing a T-cell epitope from *Leishmania major*. *Vaccine*, **1999**, *17*, 2150-2161.

[111] Xu, D.; McSorley, S.J.; Chatfield, S.N.; Dougan, G.; Liew, F.Y. Protection against *Leishmania major* infection in genetically susceptible BALB/c mice by gp63 delivered orally in attenuated *Salmonella typhimurium* ($AroA^-$ $AroD^-$). *Immunology*, **1995**, *85*, 1-7.

[112] Lee, J.J.; Sinha, K.A.; Harrison, J.A.; de Hormaeche, R.D.; Riveau, G.; Pierce, R.J.; Capron, A.; Wilson, R.A.; Khan, C.M. Tetanus toxin fragment C expressed in live *Salmonella* vaccines enhances antibody responses to its fusion partner *Schistosoma haematobium* glutathione S-transferase. *Infect. Immun.*, **2000**, *68*, 2503-2512.

[113] Anderson, R.; Dougan, G.; Roberts, M. Delivery of the pertactin/P.69 polypeptide of *Bordetella pertussis* using an attenuated *Salmonella typhimurium* vaccine strain: expression levels and immune response. *Vaccine*, **1996**, *14*, 1384-1390.

[114] Barry, E.M.; Gomez-Duarte, O.; Chatfield, S.; Rappuoli, R.; Pizza, M,. Losonsky, G.; Galen J. and Levine, MM. Expression and immunogenicity of pertussis toxin S1 subunit-tetanus toxin fragment C fusions in *Salmonella* typhi vaccine strain CVD 908. *Infect. Immun*., **1996**, *64*, 4172-4181.

[115] Ascon, M.A.; Hone, D.M.; Walters, N.; Pascual, D.W. Oral immunization with a *Salmonella typhimurium* vaccine vector expressing recombinant enterotoxigenic *Escherichia coli* k99 fimbriae elicits elevated antibody titers for protective immunity. *Infect. Immun*., **1998**, *66*, 5470-5476.

[116] Covone, M.G.; Brocchi, M.; Palla, E.; Dias da Silveira, W.; Rappuoli, R.; Galeotti, C.L. Levels of expression and immunogenicity of attenuated *Salmonella enterica* serovar Typhimurium strains expressing *Escherichia coli* mutant heat-labile enterotoxin. *Infect. Immun*., **1998**, *66*, 224-231.

[117] Morona, R.; Morona, J.K.; Considine, A.; Hackett, J.A.; van den Bosch, L.; Beyer, L.; Attridge, S.R. Construction of K88- and K99-expressing clones of *Salmonella typhimurium* G30: immunogenicity following oral administration to pigs. *Vaccine*, **1994**, *12*, 513-517.

[118] Schriefer, A.; Maltez, J.R.; Silva, N.; Stoeckle, M.Y.; Barral-Netto, M.; Riley, L.W. Expression of a pilin subunit BfpA of the bundle-forming pilus of enteropathogenic *Escherichia coli* in an aroA live *Salmonella* vaccine strain. *Vaccine*, **1999**, *17*, 770-778.

[119] Niebla, O.; Álvarez, A.; Martín, A.; Rodríguez, A.; Delgado, M.; Falcón, V.; Guillen, G. Immunogenicity of recombinant class 1 protein from *Neisseria meningitidis* refolded into phospholipid vesicles and detergent. *Vaccine*, **2001**, *19*, 3568-3574.

[120] Sjostedt, A.; Sandstrom, G.; Tärnvik, A. Immunization of mice with an attenuated *Salmonella typhimurium* strain expressing a membrane protein of *Francisella tularensis*. A model for identification of bacterial determinants relevant to the host defence against tularemia. *Res. Microbiol*., **1990**, *141*, 887-91.

[121] Newton, S.M.; Jacob, C.O.; Stocker, B.A. Immune response to cholera toxin epitope inserted in *Salmonella* flagellin. *Science*, **1989**, *244*, 70-72.

[122] Russell, M.W.; Wu, H.Y. Distribution, persistence, and recall of serum and salivary antibody responses to peroral immunization with protein antigen I/II of *Streptococcus mutans* coupled to the cholera toxin B subunit. *Infect. Immun*., **1991**, *59*, 4061-4070.

[123] Bullifent, H.L.; Griffin, K.F.; Jones, S.M.; Yates, A.; Harrington, L.; Titball, R.W. Antibody responses to *Yersinia pestis* F1-antigen expressed in *Salmonella typhimurium aroA* from *in vivo*-inducible promoters. *Vaccine*, **2000**, *18*, 2668-2676.

[124] Cao, Y.; Wen, Z.; Lu, D. Construction of a recombinant oral vaccine against *Salmonella* typhi and *Salmonella* typhimurium. *Infect. Immun*., **1992**, *60*, 2823-2827.

[125] Hess, J.; Grode, L.; Hellwig, J.; Conradt, P.; Gentschev, I.; Goebel, W.; Ladel, C.; Kaufmann, SHE. Protection against murine tuberculosis by an attenuated recombinant *Salmonella typhimurium* vaccine strain that secretes the 30-kDa antigen of *Mycobacterium bovis* BCG. *FEMS Immunol. Med. Microbiol*., **2006**, *27*, 283-289.

[126] Su, G.F.; Brahmbhatt, H.N.; Wehland, J.; Rohde, M.; Timmis, K.N. Construction of stable LamB-Shiga toxin B subunit hybrids: analysis of expression in *Salmonella typhimurium aroA* strains and stimulation of B subunit-specific mucosal and serum antibody responses. *Infect. Immun*., **1992**, *60*, 3345-3359.

[127] Gomez-Duarte, O.G.; Galen, J.; Chatfield, S.N.; Rappuoli, R.; Eidels, L.; Levine, M.M. Expression of fragment C of tetanus toxin fused to a carboxyl-terminal fragment of diphtheria toxin in *Salmonella typhi* CVD 908 vaccine strain. *Vaccine*, **1995**, *13*, 1596-1602.

[128] Corthésy-Theulaz, I.E.; Hopkins, S.; Bachmann, D.; Saldinger, P.F.; Porta, N.; Haas, R.; Zheng-Xin, Y.; Meyer, T.; Bouzourène, H.; Blum, A.L.; Kraehenbuhl, J.P. Mice are protected from *Helicobacter pylori* infection by nasal immunization with attenuated *Salmonella typhimurium phoPc*^{c} expressing urease A and B subunits. *Infect. Immun*., **1998**, *66*, 581-586.

[129] Curtiss, R.3rd.; Goldschmidt, R.M.; Fletchall, N.B.; Kelly, S.M. Avirulent S*almonella typhimurium* delta cya delta crp oral vaccine strains expressing a streptococcal colonization and virulence antigen. *Vaccine*, **1988**, *6*, 155-160.

[130] Doggett, T.A.; Jagusztyn-Krynicka, E.K.; Curtiss, R.3rd. Immune responses to Streptococcus sobrinus surface protein antigen A expressed by recombinant *Salmonella typhimurium*. *Infect. Immun*., **1993**, *61*, 1859-1866.

[131] Igwe, E.I.; Geginat, G.; Rüssmann, H. Concomitant cytosolic delivery of two immunodominant listerial antigens by *Salmonella enterica* serovar typhimurium confers superior protection against murine listeriosis. *Infect. Immun*., **2002**, *70*, 7114-7119.

[132] Simonet, M.; Fortineau, N.; Beretti, J.L.; Berche, P. Immunization with live aroA recombinant *Salmonella* typhimurium producing invasin inhibits intestinal translocation of Yersinia pseudotuberculosis. *Infect. Immun*., **1994**, *62*, 863-867.

[133] Sizemore, D.R.; Warner, B. <http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TD4-4GR32V2-4&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=ec5c9685a9255e4835deb0da8d> Lawrence, J.; Jones, A.; Killeen, K.P. Live attenuated *Salmonella typhimurium* vectoring *Campylobacter* antigens. *Vaccine* <http://www.sciencedirect.com/science/journal/0264410X>, **2006**, *24*, 3793-3803.

[134] Stabel, T.J.; Mayfield, J.E.; Tabatabai, L.B.; Wannemuehler, M.J. Swine immunity to an attenuated *Salmonella typhimurium* mutant containing a recombinant plasmid which codes for production of a 31-kilodalton protein of *Brucella abortus*. *Infect. Immun*., **1991**, *59*, 2941-2947.

[135] Chatfield, S.N.; Strugnell, R.D.; Kennedy, R.C. Live *Salmonella* vaccines and carriers of foreign antigenic determinants. *Vaccine*, **1987**, *7*, 495-498.

[136] Chabalgoity, J.A.; Khan, C.M.; Nash, A.A.; Hormaeche, C.E. A *Salmonella typhimurium htrA* live vaccine expressing multiple copies of a peptide comprising amino acids 8-23 of herpes simplex virus glycoprotein D as a genetic fusion to tetanus toxin fragment C protects mice from herpes simplex virus infection. *Mol. Microbiol*., **1996**, *19*, 791-801.

[137] Nishikawa, H.; Sato, E.; Briones, G.; Chen, L.M.; Matsuo, M.; Nagata, Y.; Ritter, G.; Jager, E.; Nomura, H.; Kondo, S.; Tawara, I.; Kato, T.; Shiku, H.; Old, L.J.; Galan, J.E.; Gnjatic, S. *In vivo* antigen delivery by a *Salmonella typhimurium* type III secretion system for therapeutic cancer vaccines *J. Clin. Invest.*, **2006**, *116*, 1946-1954.

[138] Cochlovius, B.; Stassar, M.J.J.G.; Schreurs, M.W.; Benner, A.; Adema, G.J. Oral DNA vaccination: antigen uptake and presentation by dendritic cells elicits protective immunity. *Immunol. Lett*., **2002**, *80*, 89-96.

[139] Londono, L.P.; Chatfield, S.; Tindle, R.W.; Herd, K.; Gao, X.M.; Frazer, I.; Dougan, G. Immunisation of mice using *Salmonella typhimurium* expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. *Vaccine*, **1996**, *14*, 545-552.

[140] Tite, J.P.; Gao, X.M.; Hughes Jenkins, M.; Lipscombe, M.; O'Callaghan, D.; Dougan, G.; Liew, F.Y. Anti-viral immunity induced by recombinant nucleoprotein of Influenza A virus. III. Delivery of recombinant nucleoprotein to the immune system using attenuated *Salmonella typhimurium* as a liver carrier. *Immunol*., **1990**, *70*, 540-547.

[141] Steger, K.K.; Valentine, P.J.; Heffron, F.; So, M.; Pauza, C.D. Recombinant, attenuated *Salmonella typhimurium* stimulate lymphoproliferative responses to SIV capsid antigen in rhesus macaques. *Vaccine*, **1999**, *17*, 923-932.

[142] Smerdou, C.; Urniza, A.; Curtiss, IIIR.; Enjuanes, L. Characterization of transmissible gastroenteritis coronavirus S protein expression products in avirulent *S. typhimurium Δcya Δcrp* persistence, stability and immune response in swine. *Vet. Microbiol*., **1996**, *48*, 87-100.

[143] Paglia, P.; Medina, E.; Arioli, I.; Guzman, C.A.; Colombo, M.P. Gene transfer in dendritic cells, induced by oral DNA vaccination with *Salmonella* typhimurium, results in protective immunity against a murine fibrosarcoma. *Blood*, **1998**, *92*, 3172-3176.

[144] Mingqiu, L.; Xiaofeng, N.; Jingran, Y.; Weiyao, Y.; Zhaoxin, Z. Immune response induced by oral DNA vaccination against FMDV delivered by attenuated *Salmonella choleraesuis* C500. *Front. Biol. China*, **2006**, *1*, 110-114.

[145] Kotton, C.N.; Lankwski, A.J.; Scott, N.; Sisul, D.; Li, M.C.; Raschke, K.; Borders, G.; Boaz, M.; Spentzou, A.; Galan, J.E.; Hohmann, E.L. Safety and immunogenicity of attenuated *Salmonella* enterica serovar Typhimurium delivering an HIV-1 gag antigen *via* the *Salmonella* Type III secretion system. *Vaccine*, **2006**, *24*, 6216-6224.

[146] Bird, P.; Hayes, C.; de Jersey, J.; Bradley, M. Construction and immunological assessment of *Salmonella typhimurium* expressing fox sperm LDH-C4. *Reprod. Fertil. Dev.*, **1998**, *10*, 225-232.

[147] Srinivasan, J.; Tinge, S.; Wright, R.; Herr, J.C.; Curtiss, R. 3rd. Oral Immunization with attenuated *Salmonella* expressing human sperm antigen induces antibodies in serum and the reproductive tract. *Biol. Reprod.*, **1995**, *53*, 462-471.

[148] Sirard, J.C.; Niedergang, F.; Kraehenbuhl, J.P. Live attenuated *Salmonella*: a paradigm of mucosal vaccines. *Immunol. Rev.*, **1999**, *171*, 5-26.

[149] Thole, J.E, van Dalen, P.J.; Havenith, C.E.; Pouwels, P.H.; Seegers, J.F.; Tielen, F.D.; van der Zee, M.D.; Zegers, N.D.; Shaw, M. Live bacterial delivery systems for development of mucosal vaccines. *Curr. Opin. Mol. Ther.*, **2000**, *2*, 94-99.

[150] Dietrich, G.; Spreng, S.; Favre, D.; Viret, J.F.; Guzman, C.A. Live attenuated bacteria as vectors to deliver plasmid DNA vaccines. *Curr. Opin. Mol. Ther.*, **2003**, *5*, 10-19.

[151] Michelle, E.G.; Webster, D.E.; Wesselingh, S.L.; Strugnell, R.A. Impact of plasmid stability on oral DNA delivery by *Salmonella enterica* serovar Typhimurium. *Vaccine*, **2007**, *25*, 1476-1483.

[152] Lutwick, L.I. Commentary- Vax to the Future. http:/www. medscape.com/view publications/91 (Assessed January 5, 2004).

[153] Curtiss, R.; Kelly, S.M.; Hassan, J. O. Live oral avirulent *Salmonella* vaccines. *Vet. Microbiol.*, **1993**, *37*, 397-405.

[154] Barrow, P. A.; Hassan, J. O.; Berchieri, A. Reduction in faecal excretion of *Salmonella typhimurium* strain F98 in chicken vaccinated with live and killed *Salmonella typhimurium* organisms. *Epidemiol. Infect.*, **1990**, *104*, 413-426.

[155] Subhabphant, W.; York M. D.; Pomroy, B. S. Use of two vaccines (live G30D or killed RW16) in the prevention of *Salmonella typhimurium* infection in chickens. *Avian Dis.*, **1983**, *27*, 602-615.

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