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Review Article

SALMONELLA VACCINES FOR VETERINARY USE: AN OVERVIEW

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SUMMARY

Salmonella infection does not appear to spare any animal species. It has evolved strains to adapt different animals still maintaining their zoonotic and interspecies transfer potential. Control of salmonellosis is important in view of urgent need to follow international and national standards of animal products to stay in market under WTO regime. To control salmonellosis, three major types of vaccines including killed bacterins, sub-unit vaccines and live attenuated vaccines have been used. Each type of vaccine has its advantages and disadvantages. The major hurdles appear to be multiplicity of the serovar and inability of serovar specific vaccine to protect against other serovars (even related antigenically). The researchers have developed effective vaccines against most common serovars but exclusion of one serovar makes space for emergence of another serovar. The complicated problem has been made more complex by WTO, which has opened ways of transportation of *Salmonella* serovars from one place to another which was otherwise impossible for the bacterium.

Key words: Salmonellosis, killed vaccines, sub-unit vaccines, attenuated vaccines, fowl typhoid

Salmonellosis is a group of diseases caused by more than 2500 serovars of genus *Salmonella* and has long been recognized as an important zoonotic disease of global economic significance in man and animals. Although, it is mainly associated with diarrhoea (Lax *et al.*, 1995), it also causes substantial economic losses (Peters, 1985) through mortality, decrease production and poor growth. Globalization of trade, the devastating effects of salmonellosis in poultry industry and increased susceptibility of AIDS patient to the salmonellosis has heightened the interest in this pathogen recently. The economic impact of salmonellosis falls upon the public sector, the food industry and on infected persons and their families. Importance of the disease can be gauged by the fact that even in a developed country like the USA, about 5.3 million people fall victims to salmonellosis every year. The US Center for Disease Control (CDC) in Atlanta, has records to show that salmonellae are responsible for approximately one third of the confirmed food borne disease outbreaks in humans (Daniel *et al.*, 2002). The total annual cost of salmonellosis in the USA was calculated at US $ 3.99 billion with an average cost per case of $ 1350 (Todd, 1989).

In India, more than 235 serovars of *Salmonella* have been recorded so far from humans, animals, birds and other sources. Though no systematic surveillance and monitoring system for *Salmonella* infection exists in our country, still on the basis of various reports of diarrhoeal diseases and common occurrence of *Salmonella* in foods of animal origin (milk, meats and their products), it appears to be one of the major causes of food borne infections (Singh *et al.*, 2005). Furthermore, sprouts, cereals, fruits and vegetables have also been found to be associated with *Salmonella* outbreaks (Daniel *et al.*, 2002)

Only a few scientific discoveries have had the impact on the health of the world as have vaccines. The phenomenon that individuals who recovered from some infectious diseases were resistant to subsequent reinfection was observed by Edward Jenner and Louis Pasteur, which provided the impetus for the early development of vaccinology. Since then, with advances in the field of immunology and molecular biology, vaccinology has undergone dimensional changes during the last century albeit at varying pace.

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To control salmonellosis, many workers have tried killed/live vaccines or bacterin-toxoid with varying success. There are some limitations with these vaccines, e.g., these are not effective against infections caused by wide variety of *Salmonella* serovars because of the marked variation in their surface antigens. Besides, live vaccines cannot be used in highly susceptible birds and there is danger of revival of pathogenicity of non-pathogenic organisms. However, from the consumer safety point of view, there is a school of thought that considers inactivated or sub-unit vaccines to be the safest. The benefits of developing effective killed or sub-unit vaccines over the use of live vaccines are enormous. The sub-unit vaccine developed should be targeted against the main virulence factors that play major role in pathogenesis of the disease. Different vaccines used for protection against salmonellosis include bacterins, toxoids and live attenuated vaccines.

Mukkur *et al.* (1987) reported that numerous attempts have been made to protect mice, ruminant animals and birds against *Salmonella* with killed, live or sub-unit vaccines. These vaccines have shown variable success only against homologous or related serovars. Since these vaccines have not been targeted against the main virulence factors, the success of these vaccines in the field remained questionable.

Numerous vaccines used in animals for control of salmonellosis (Tables 1, 2, 3) can be grouped into three classes, viz., killed vaccines or bacterins, sub-unit vaccines and live attenuated vaccines.

KILLED VACCINES

Initial work on development of *Salmonella* vaccine (Table 1) started with the development of killed vaccines. Mendel *et al.* (1972) demonstrated that heat killed vaccines induced effective degree of immunity in mice with 100% protection and prevented extensive multiplication of the organism in the liver and spleen on intravenous or intraperitoneal challenge with *S.* Typhimurium. Nicolas *et al.* (1981) reported that cases of abortion decreased when sheep were vaccinated with formal and heat inactivated *Salmonella* vaccine without adjuvant. Liberal (1989) also found that formalized vaccine of *S.* Dublin significantly reduced the mortality in calves. According to Cooper and Mac Farlane (1974), vaccination of sheep with bivalent *Salmonella* vaccine significantly reduced the after effects of *Salmonella* challenge viz. pyrexia, severe scours, inappetance and *Salmonella* in blood and faeces. Ghosh (1989) found formal killed oil adjuvanted *S.* Virchow vaccine more effective than aluminium hydroxide gel and FCA adjuvanted vaccines. The above findings were confirmed by Mohrah and Zaki (1995) who reported that activated oil adjuvant vaccine of *S.* Gallinarum given subcutaneously at 2 weeks of age markedly prevented *Salmonella* colonization in birds on challenge with *S.* Gallinarum.

Gast *et al.* (1993) evaluated the efficacy of oil-emulsion bacterins for reducing faecal shedding of *Salmonella* Enteritidis by laying hens. However, the degree of protection afforded by vaccination was only partial but it was suggested that if used in conjunction with other flock sanitation and infection-monitoring strategies, vaccination with bacterins could potentially reduce the horizontal transmission of *Salmonella enterica* serotype Enteritidis (SE) within and between laying flocks.

S. Enteritidis grown under conditions of iron restriction, to simulate conditions in the host and to produce *in-vivo* antigens, has been used to prepare an inactivated vaccine which has been licensed in a number of European countries. Field trials in UK showed that the vaccine decreased *Salmonella* shedding and increased productivity of broiler breeders (Woodward *et al.*, 2002).

The protective effect of Selanvac, a commercial available iron-restricted *Salmonella enterica* subsp. *enterica* serotyope Enteritidis PT4 bacterin vaccine in laying birds was studied by Woodward *et al.* (2002). Immunization was done intramuscularly at day one and again at 4 weeks of age. The data gave compelling evidence that the vaccine is efficacious and may contribute to the reduction of layer infection and egg contamination.

Barbour *et al.* (2001) investigated the immunopotentiation of a developed killed SE

Table 1
Some important killed vaccines tried for control of salmonellosis in animals

Method of killing	Adjuvant	Used in	Immunity afforded	Comments
Heat killed				
S. Typhimurium	-	Mice	100% protection (homologous)	Immunity afforded is of short duration.
S. Abortusovis (Mendel *et al.*, 1972, Nicolas and Andrews, 1991)		Sheep	67% protection (homologous)	
Formal killed				
S. Dublin (Liberal, 1989)	-	Calves	Controlled diarrhoea	Afford serovar specific immunity.
S. Virchow (Ghosh, 1989)	Oil adjuvant FCA	Mice	100% protection (homologous) Oil adjuvant was better than FCA.	Afford only humoral response not sufficient to restrict a facultative intracellular pathogen.
S. Gallinarum (Morah and Zaki, 1995)	Oil adjuvant FCA	Chicken	100% protection (homologous) Oil adjuvant was better than FCA.	
S. Enteritidis (Gast *et al.*, 1993)	Oil-emulsion	Chicken	Partial protection	
S. Enteritidis (Barbour *et al.*, 2001)	Thymullin and Zinc	Chicken	Partial protection but better than oil adjuvant and non-adjuvanted vaccine.	Induce poor mucosal immune response required to control salmonellosis.
S. Abortusequi (Dhanda *et al.*, 1955)	Potash alum	Mice	86% protection in mice. Superior than phenolized vaccine (50 % protection).	
(Kataria, 1978), (Gupta *et al.*, 1987), (Kataria and Uppal, 1981)	Alhydrogel FICA Chrome-alum Oil adjuvant	Mice	Chrome-alum was best, 92.8% protection, Oil adjuvant was the most inferior one.	
Glutaraldehyde killed				
S. Abortusequi	Chrome-alum Potash alum Oil adjuvant	Mice	100% protection up to 21 days	Potash alum precipitated and β propiolactone inactivated vaccine gave better protection than any other preparation
0.1% β- propiolactone inactivated				
S. Abortusequi (Siddiqui, 1968, Kataria, 1978, Gupta *et al.*, 1987).	-	Mice	100% protection up to 21 days	

vaccine by thymulin and zinc in meat chicken breeders. The group, which had received zinc and thymulin, had the earliest and highest immune response to SE. In addition, immunopotentiated group had the highest mean thymus weight index, and the highest mean lymphocyte count in the thymus cortex.

Killed vaccines have not been found very effective because of the following three reasons. Firstly, they only contain surface antigens that give an incomplete antibody response. Secondly, they fail to elicit cell-mediated immune response, which is important for long-term protection. Thirdly, they fail to elicit secretory IgA responses, which are potentially important in protecting mucosal surfaces.

In India, the most successful killed vaccine was developed by Dhanda *et al.* (1955) using

formal killed alum precipitated *S.* Abortusequi culture. The vaccine was proved to be much superior than earlier vaccines giving upto 86% protection in mice model as compared to heat killed phenolized vaccine giving only 50% protection.

Afterwards, various adjuvants have been incorporated to enhance efficacy of killed vaccines to make them to afford long lasting immunity. In series of such attempts, formalized alhydrogel adsorbed vaccine (Kataria, 1978) conferred 92.8% protection in mice although immunity remained short lived. Oil adjuvant vaccines of *S.* Abortusequi in single and double dose have also been tried (Kataria and Uppal, 1981) but proved inferior to alhydrogel adsorbed or chrome alum precipitated vaccine. Incorporation of Freund's incomplete adjuvant was also not able to enhance efficacy of killed vaccine (Gupta *et al.,* 1987).

Although, chrome salt vaccines are shown to be less toxic, more stable and more potent than heat killed or oil adjuvant *S.* Abortusequi vaccines. Study on ß-propiolactone (BPL) and glutaraldehyde as inactivating agents showed that 0.1% BPL inactivated chrome alum vaccine afforded greater protection than glutaraldehyde treated vaccine (Kataria, 1978). In further studies, formalized potash alum vaccine afforded better protection than formalized vaccine and chrome alum vaccines used earlier (Siddiqui, 1968, Kataria, 1978, Gupta *et al.,* 1987).

SUB-UNIT VACCINES

Many workers have used *Salmonella* proteins (Table 2) for prevention of salmonellosis. Bouzoubaa *et al.* (1987, 1989) used *S.* Gallinarum outer membrane proteins with and without mineral oil and compared it with killed vaccine for protection against salmonellosis. The birds, which received *Salmonella* proteins with mineral oil, showed 100% protection and the vaccine was found to be more effective than killed vaccine. Bouzoubaa *et al.* (1987) extracted outer membrane protein of *S.* Gallinarum using urea and vaccinated chickens at 8 and 12 weeks of age with protein extract at level of 50, 100, 200 or 400 µg/100 gm live body weight. Protein extract was in aqueous suspension or with mineral oil adjuvant. Two other groups of chickens were also vaccinated with formalin killed whole cell bacterin of the same organism, one group with adjuvant and another group without adjuvant. The chickens were challenged at 15 weeks of age with live *S.* Gallinarum. The mortality rate was highest in the group that received bacterin without adjuvant and the birds that received 400 µg protein extract with adjuvant showed 100% clearance of *S.* Gallinarum.

Lee *et al.* (1997) reported that the protection of OMP or whole-cell combined with OMP against challenge with *S.* Gallinarum (>75%) was greater than that of the whole-cell vaccine (45-50%). The chickens vaccinated with OMP or whole-cell combined with OMP complex had better delayed type hypersensitivity (DTH) than those vaccinated with whole-cells.

Charles *et al.* (1993) observed that incorporation of outer membrane protein (OMP) from *S.* Heidelberg into lipid-conjugate, immunostimulating complex (ISCOM) resulted into greater immune response than OMP alone in turkey.

In India, Gupta *et al.* (1987) tried ribosomal fractions and non-ribosomal proteins as potential vaccines capable to mount good 'O' and 'H' agglutinin titres in rabbit model. Non-denatured bacterial cell envelopes of *S.* Typhimurium and some other serovars have also been reported as useful vaccine candidates. However, salt precipitated protein toxoid made from *S.* Abortusequi could afford better protection than conventional killed and other subcomponent vaccines (Vasava, 1999).

LIVE ATTENUATED VACCINES

Attenuated avirulent live *Salmonella* vaccines (Table 3) have received considerable attention as mucosal vaccines. Following oral vaccination, *Salmonella* replicates in gut mucosa associated lymphoid tissue and thereafter disseminates via mesenteric lymph nodes to systemic sites like spleen. This characteristic dissemination pattern allows *Salmonella* to induce wide spectrum immune response including cell-mediated, humoral and secretory antibody-antigen responses (Lax *et al.*, 1995).

Table 2
Some important sub-unit vaccines tried for control of salmonellosis in animals

Preparations	Adjuvant	Animals	Protection	References
Outer membrane proteins of *S.* Gallinarum	With and without mineral oil	Chicken	Better than heat killed, when given with oil, 100% protection in birds receiving 400 mg protein extract	Bouzoubaa *et al.* (1987, 1989)
OMP or whole-cell combined with OMP of *S.* Gallinarum	-	Chicken	Combined type afforded 100% protection, OMP (75%), heat killed (50%)	Lee *et al.* (1997)
OMP of *S.* Heidelberg	ISCOM	Turkeys	Better than OMP alone	Charles *et al.* (1993)
Ribosomal fractions and non-ribosomal proteins of *S.* Typhimurium	-	Mice	Combined fractions were better than whole cell vaccine	Gupta *et al.* (1987)
Salt precipitated protein toxoid of *S.* Abortusequi	-	Mice	Better protection than killed, subcomponent and conventional chrome alum	Vasava, (1999)
S. Weltevreden formalized toxoid	Without adjuvant	Mice	100% protection in mice against homologous and heterologous challenge	Singh and Sharma (1999)
Formalin inactivated Polymyxin-B extract of *S.* Weltevreden culture	FCA	Poultry	Adjuvanted formalized toxoid gave 100% protection, while toxoid alone gave 60-70% protection	Mishra and Sharma (2001)
Formalin inactivated Polymyxin-B extract of *S.* Weltevreden culture	Vitamin E, vitamin E plus selenium, & aluminum hydroxide	Chicks	Toxoid plus vitamin E alone protected 75-90% and better than FCA, vitamin E and selenium protected 70-80%. Aluminium hydroxide adjuvanted toxoid could protect 70%	Kumar (1999)
Toxoid from purified pooled of enterotoxin and cytotoxins of *S.* Weltevreden	75 µg and 100 µg per bird	Chicks	Vaccine conferred solid protective immunity for at least 3-4 months and a booster dose was recommended after 90 days	Barman *et al.* (2002)

A safe efficacious live *Salmonella* vaccine should be (i) totally avirulent for both animals and man, (ii) highly immunogenic with long lasting immunity, blocking *Salmonella* invasion of internal organs and reducing colonization of the intestinal tract by diverse *Salmonella* serotypes, (iii) genetically stable with two or more attenuating deletion mutations (iv) unaffected by the diet of the host and (v) easy to grow, store and administer.

In poultry, vaccination with two live attenuated vaccine strains of *S.* Gallinarum (smooth) and 9R (rough) afforded protection against virulent parent strain 9R, whereas the killed strain failed to induce immunity (Smith, 1956). Cameron and Buys (1979) also found live attenuated *S.* Gallinarum vaccine from rough mutant strain 5503, effective for 2 months thereafter protection level fell to 50%. Puka (1987) reported that a live vaccine of *S.* Gallinarum of turkey origin attenuated by 300 passages in a medium containing 0.016% acriflavin was harmless for chicks aged 5-10 days. This vaccine had no adverse effect on egg

Table 3
Some important live attenuated vaccines tried for control of salmonellosis in animals

Method of attenuation	Strain-Serovar	Animal	Protection and reversion	References
Rough strain	9R (rough) of *S.* Gallinarum	Chicken	Afforded protection against virulent parent	Smith (1956)
Rough strain	5503 of *S.* Gallinarum	Chicken	Effective for 2 months thereafter protection level fell to 50%.	Cameron and Buys (1979)
Other host adaptation and specificity	Strain 51 of *S.* Dublin	Chicken	Cleared the vaccine strain from 99% but not the *S.* Typhimurium. In calves, vaccine induced short lived diarrhoea	Verma (1969), Knivett and Stevens (1971), Knivett and Tucker (1972)
*gal*E mutant	*S.* Choleraesuis *S.*Typhi *S.*Typhimurium	Mice	Significantly reduced faecal shedding of the homologous challenge but there was no significant humoral response	Pritchard *et al.* (1978)
*aro*A mutant	*S.* Typhimurium, *S.* Enteritidis, *S.* Dublin and *S.* Choleraesuis	Mice Chicken	Excellent immunogenicity but prolonged carriage. Oral vaccination protected against intravenous challenge	Cooper *et al.* (1992)
Rough + *aro*A mutant	*S.* Enteritidis	Mice	Not protected the gut following oral challenges	Barrow *et al.* (1991)
Δcya Δcrp mutant	*S.* Typhimurium	Mice	Protection up to 4 months post vaccination on challenge with 10^9 CFU, strong mucosal, humoral and cellular immune response	Curtiss and Kelly (1987)
*Pho*P-*pho*Q-mutant	*S.* Typhimurium	Mice	Found immunogenic, their frequency of reversion to virulent forms is relatively high rendering them unsafe	Miller and Mekalanos (1990)
*v*Pla⁻ mutant	*S.* Typhimurium	Mice	Highly immunogenic but reminiscent virulence	Gulig and Curtiss (1987)
*nuo*G mutant	SG9NGK of *S.* Gallinarum	Chicken	>75 % protection	Li *et al.* (1998)
*aro*A-*sec*C mutant	*S.* Gallinarum	Fowl	Total protection	Barrow *et al.* (2000)
dam mutant	F98 of *S.* Typhimurium		Highly attenuated, elicited cross-protection immune response against *S.* Enteritidis	Dueger *et al.* (2003)
*aro*A-*htr*A mutants	S30 of *S.* Abortusequi	Mice, guinea-pigs and equines	Safe through oral, intra-vaginal and subcutaneous routes in mice and guinea-pigs but reactogenic through subcutaneous and intramuscular routes in equines. 100% protection up to 11 months in guinea-pigs and pregnant mares.	Singh *et al.* (2005)

production and protected fowls against challenge infection. Knivett and Stevens (1971) found that the chickens vaccinated with *S.* Dublin strain 51 cleared the vaccine strain from 99% of the birds in 9-40 days and when challenged orally with *S.* Typhimurium, the colonization of liver was reduced by up to 3 $\log_{10}$. However, faecal shedding of *S.* Typhimurium was not reduced (Knivett and Tucker, 1972).

In most cases, classical means of genetic manipulation, including transposon mutagenesis and generation of deletions following transposon excision have been used, but recombinant methods to generate deletion have also been employed to

develop attenuated *Salmonella* strain. Common mutations that render *Salmonella* both avirulent and immunogenic in mice and other animals are:

***gal*E mutant**: *Salmonella* strains with a *gal*E mutation are reversibly rough and sensitive to galactose. Although *gal*E mutants are avirulent and immunogenic, however, *gal*E mutants of *S.* Choleraesuis and *S.* Typhi retain some virulence. *S.* Typhimurium *gal*E live attenuated oral vaccine developed by Pritchard *et al.* (1978) significantly reduced faecal shedding of the homologous challenge but there was no significant humoral response.

***aro*A mutant**: *Salmonella* mutants blocked in the synthesis of aromatic amino acids (*aro*A) which also have a requirement of PABA needed for folic acid synthesis, have received greater attention and have been widely used to attenuate *S.* Typhimurium, *S.* Enteritidis, *S.* Dublin and *S.* Choleraesuis. The immunogenicity of these mutants has been found excellent. Barrow *et al.* (1991) found that a rough strain of *S.* Enteritidis Δ*aro*A given by oral and intramuscular routes, did not protect the gut following oral challenges. Cooper *et al.* (1992) found that *aro*A mutants of *S.* Enteritidis PT4, strains SeLA5 and Se267 showed reduced virulence up to 6 logs in Balb/c mouse models, did not kill 18-20 day-old chicks when infected intravenously and did not multiply in the tissue. Oral vaccination of chicks conferred significant protection against intravenous challenge, with virulent strain and reduced intestinal shedding on oral challenge. But the vaccine could not generate an effective humoral response and it was suggested that cell mediated immunity was the cause of protection.

***aro*A-*sec*C mutant**: Barrow *et al.* (2000) studied the protection against experimental fowl typhoid by the parentral administration of live SL5828, an *aro*A-*sec*C mutant of a wild *Salmonella* Gallinarum strain made lysogenic for P22 phage. There was evidence of protection in the contents, mucosa and lymphoid tissue of the alimentary tract in addition to that which occurred in the liver and spleen indicating that the use of such a strain would be useful adjunct to control schemes for fowl typhoid.

Δ*cya*, Δ*crp* mutant: Strains with Δcya and Δcrp mutations which lack adenylate cyclase and the cAMP receptor proteins are avirulent and immunogenic. These mutants grow slowly and display diminished resistance to non-specific host defence mechanisms. Δcya, Δcrp mutants of *S.* Typhimuruim were 1000 times less virulent than their wild type parents for mice (Curtiss and Kelly, 1987) and single vaccination with 10^7 Δcya, Δcrp cells induced protection up to thirty days post challenge with 10^9 colony forming units (CFU) of the wild types parent. Protective immunity was also apparent at 8 days after immunization and lasted for more than 4 months. These strains produced strong mucosal, humoral and cellular immune responses against LPS and protein antigens.

***pho*P/*pho*Q mutant**: The *pho*P/*pho*Q two component regulatory system regulates genes for acid phosphatases and for the ability of *Salmonella* to survive in macrophages. Although mutants have been found immunogenic, their frequency of reversion to virulent forms is relatively high rendering them unsafe (Miller and Mekalanos, 1990).

vPla⁻ mutant: Elimination of virulence plasmid yields the strain vPla- that colonizes intestine and GALT on oral administration but fails to colonize lymphoreticular organs (Gulig and Curtiss, 1987). Though these strains are highly immunogenic and avirulent, some vaccinated animals display some disease symptoms.

***nuo*G mutant**: *S.* Gallinarum *nuo*G mutant (named SG9NGK) colonized the caeca of chickens less efficiently than *S.* Gallinarum parent strain and was less invasive and showed no evidence of multiplication in the liver or spleen (Li *et al.*, 1998). A single oral immunization with live bacteria (SG9NGK) reduced mortality in 2-weeks-old chickens following challenge with virulent *S.* Gallinarum from 75% to less than 8%.

Dam mutant: *Salmonella* mutants lacking DNA adenine methylase (Dam) are highly attenuated for virulence. Dam regulate the production of a number of adhesions in *E. coli* and *Salmonella* as well as several genes required for *Salmonella* infection. A *Salmonella enterica* serovar Typhimurium Dam mutant vaccine strain was attenuated 100,000 fold for virulence in day-old chicks. Vaccination of chicks elicited cross-protective

immune response and reduced colonization (10-10,000 folds) of *Salmonella*, after challenge, in gastrointestinal tract and visceral organs. Protection was evident both aganist homologous (*S.* Typhimurium F98) and heterologous (S. enterica serovar Enteritidis 4973 and *S. enterica* 0: 06, 14, 24; e, h-) *Salmonella* serovars commonly implicated in *Salmonella* infection of poultry (Dueger *et al.*, 2003).

Though a large number of mutants have been tried to develop live oral *Salmonella* vaccines, only the *aro*A, Δcya, Δcrp, Δcdt and Dam mutations have been introduced in useful vaccines demonstrating avirulence, immunogenicity and safety not only in mice but also in animals and man.

Cooper (1994) reviewed the applications of live vaccines against *Salmonella* and concluded that live vaccines are more effective in controlling *Salmonella*. Other workers viz. Barbezange *et al.* (2000) analyzed the safety characteristic of three commercially available live *Salmonella* vaccine strains (VacT, Zoosaloral and Chi3985) in relation to their persistence in individual animals and also within a flock and in the environment. Strain VacT remained in the environment of inoculated animals for 4-5 weeks. Comparatively, both Zoosaloral and Chi-3985 vaccine strains persisted longer in the environment (8 wk atleast). Six weeks after the inoculation, VacT was not recovered from the internal organs such as liver and spleen, and it disappeared from the digestive tract between 6^{th} and 10^{th} weeks. Of the 3 vaccine strains, chi 3985 showed the greatest colonization of both systemic and digestive organs.

Methner *et al.* (2001) studied the combination of a competitive exclusion (CE) culture (Aviguard) and the immunization with a live *Salmonella* Typhimurium vaccine to determine any advantages in using both methods. They found that the administration of the live *Salmonella* vaccine prior to or simultaneously with the CE culture revealed the best protective effect because such combination ensures an adequate persistence of the vaccine strain as a prerequisite for the expression of an exclusion effect in very young chicks and the development of strong immune response affording protection in older birds.

Though live attenuated vaccines are being worked upon most seriously and are being claimed as the most effective measures of immunoprophylaxis against *Salmonella,* they too have some drawbacks. Firstly, they cause shedding of the vaccine strain on vaccination making it difficult to differentiate vaccinated and infected animals. Secondly, the possibility of reversal of attenuated forms to virulent forms cannot be ruled out. Thirdly, these vaccines give protection against the homologous *Salmonella* strains from which the vaccine has been prepared thus leaving the animal exposed to the rest *Salmonella* serovars. Lastly, it has been found that live attenuated *Salmonella* vaccines while protecting against virulent *Salmonella,* paradoxically induce profound immunosuppression against non-*Salmonella* antigens and suppress lymphoproliferative response to mitogen.

Lowry *et al.* (1999) found that the immunoprophylactic administration of *Salmonella* Enteritidis immune-lymphokines to young turkey poults and broiler chicks significantly reduced the horizontal transmission of *Salmonella* in poultry. These results suggest the possibilities of using a non-vaccine immunological based preventive strategy against *Salmonella* in poultry. Recent studies (Singh *et al.*, 2005) on *aro*A-*htr*A mutants of *S.* Abortusequi have shown that this may be a suitable oral vaccine for inoculating any kind of equine with no apparent danger. The mutant strain (S-30) has been found safe through oral, intra-vaginal and subcutaneous routes in $>10^9$ CFU per animal in guinea pigs but was not safe through intramuscular and intra-peritoneal routes in pregnant as well as in infant guinea pigs. 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 was immunogenic in doses as low as 1×10^{10} cfu/ animal. However, vaccine was reactogenic when inoculated through subcutaneous and intramuscular routes inducing abscess formation at the site of inoculation. Challenge test in mares after 8 months of vaccination revealed that vaccine affords 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). None of the immunized animals aborted and excreted the wild type strains after a fatal challenge.

VACCINES FOR FOWL TYPHOID (POULTRY SALMONELLOSIS)

Fowl typhoid is still a disease of worldwide significance. It has largely been eradicated from those countries which have had an intensive poultry industry for many years and is now of particular economic importance in those countries which are beginning to intensify their industry, e.g. countries in Latin America, South East Asia, the Middle East, Indian subcontinent and South Africa. Because of the economic importance of fowl typhoid, many studies have been carried out on the feasibility of vaccination to control it. Early studies (Hall *et al.*, 1949) showed that killed vaccines were of little practical use. Barrow *et al.* (1987) found that elimination (curing) of plasmid from a strain greatly reduced its virulence and may be used as a vaccine candidate because the plasmid-cured derivative of *S.* Gallinarum could persist in the reticuloendothelial system of the chicken for some time to induce good immune resposnse. However, this vaccine face criticism due to inapplicability of the vaccine where whole blood test is used as screening method for control of salmonellosis in poultry. Besides, in 3-week-old chickens, the plasmid-cured derivative was virtually avirulent, while in newly hatched chickens, there was some residual virulence and acquisition of the plasmid from wild strains present in intestines of vaccinated birds remained a potential threat (Barrow and Lovell, 1989). Smith (1956) developed two attenuated vaccine strains, one of which (a rough strain, 9R) was protective and did not induce the production of significant amounts of serum agglutinins. This latter characteristic is of particular importance where the disease is to be eradicated by using the whole-blood agglutination test. Efficacy of rough stain vaccine (9R) was observed to be enhanced by the use of adjuvant to make it able for affording protection upto 32 weeks of age (Gupta and Mallick, 1976b). Sodium bicarbonate fed prior to oral vaccination with 9R vaccine also improved its efficacy (Gupta and Mallick, 1976a). However, Silva *et al.* (1981) contradicted the utility of adjuvant, rather reported interference. Many researchers have shown the value of the 9R strain even for providing cross protection (Padmanabhan and Mittal, 1980, Padmanabhan *et al.*, 1981, Chandran *et al.*, 1983). However, a number of authors have indicated that the strain still possesses some virulence for some breeds of chicken (Gordon and Luke, 1959, Gordon *et al.*, 1959, Silva *et al.*, 1981), although there is no evidence for reversion to full virulence in the field. Furthermore, incomplete protection induced by the 9R vaccine strain (Barrow *et al.*, 1990) and long term persistence in immunized birds are the additional problems of the vaccine. Low protection with 9R vaccine particularly when young chicks are vaccinated, has complicated the problem (Gordon *et al.*, 1959, Silva *et al.*, 1981, Barrow *et al.*, 1990). The worldwide prevalence of fowl typhoid and emergence of antibiotic resistance in *S.* Gallinarum (due to extensive prophylactic use of antibiotics) necessitate for continuing work on the development of improved vaccines. Inefficiency of the most of the killed vaccines and live vaccines to afford complete and cross protection against different *Salmonella* serovar involved in fowl salmonellosis emphasized the need of further research in broad spectrum vaccine development.

Salmonella Weltevreden formalized toxoid afforded 100% protection against challenge with homologous toxin and its producer strain but incomplete protection against heterologous *Salmonella* strains (Singh and Sharma, 1999, Singh *et al.*, 2001). Mishra and Sharma (2001) reported the efficacy of toxoid vaccine against salmonellosis in poultry. Kumar (1999) studied the immunopotentiating efficacy of vitamin E, Vitamin E plus selenium and aluminum hydroxide so as to find a safe, potent and cheap replacement to FCA. The toxoid plus vitamin E alone protected a sizable number (75-90%) of birds on homologous and heterologous challenge while toxoid plus vitamin E and selenium protected 70-80%. Aluminium hydroxide adjuvanted toxoid could protect 70% of the birds on both the

challenges while control birds could not survive against the challenge and died within 15-20 days post challenge.

Barman *et al.* (2002) reported that the toxoid prepared from purified pooled enterotoxin and cytotoxins can be adjuvanted with saponin. The birds vaccinated with this toxoid at dose rate of 75 µg and 100 µg per bird subcutaneously provided 100% protection against homologous as well as heterologous challenges. The primary dose of vaccine conferred solid protective immunity for at least 3-4 months and a booster dose was recommended after 90 days of the first dose to augment the immunity. The vaccine prevented multiplication of the challenge organism in the internal organs and eventually checked its shedding. The chicks hatched from eggs of vaccinated birds possessed passive immunity and thus could be protected against salmonellosis in their early days of life.

CONCLUSION

From the continuous work of researcher all over the globe, it has become apparent that there is need of still more research particularly for development of vaccines by use of modern tools of biogenetic engineering which may protect the target animal from most of the common *Salmonella* serovars (if not from all). Use of genetically modified strains as vaccine has already shown the way and a few successful vaccines are in use in human beings and animals.

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