

An Overview: Vaccination to control fowl typhoid in Commercial layers, Sri Lanka

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Poultry production and consumption in Sri Lanka, has been dramatically increased during last two decades and Salmonellosis was reported as one of the prevalent diseases in commercial layers. Both *S.Gallinarum* as well as *S.Pullorum* is causing severe economical impact to the industry, while *S.Typhimurium* and *S.Enteritidis* are also important in the public health aspects. Vaccination against Salmonellosis is widely practiced in several countries in the world to control the infection: In Sri Lanka, killed vaccine is permitted only for commercial layer, while breeder birds, commercial broilers are prohibited by regulation. Both Live attenuated and killed vaccine have many benefits, and proven results for controlling of none host- specific *Salmonella* in poultry and also in reducing the occurrence of human food born infections. Both vaccines were considered as potential to control the host specific *Salmonella* in poultry by reducing the mortality and faecal shedding to the environment. Evidencely, live vaccines are capable of controlling the human infections caused by non host specific *Salmonella* as a result of cross immunization in poultry. Since, Both vaccine given positive effect as well as negative effect to control the Salmonellosis in chicken and further studies are encouraged relevant to local situation.

INTRODUCTION

The poultry sector in Sri Lanka, is a fast growing industry in livestock engaged over 500 000 families directly or indirectly. They depend on commercial chicken farming and associated industry like animal feed, Processing, sales and pharmaceuticals. The total poultry population has been augmented during recent past. It was 9.5 million in 1998 and 13.8 million in year 2007(SL.Livestock statistic, 2007). The commercial eggs production was 915 millions in 2007 and annual production of day old chicks also was increased from 14.5 million to 79.9 million from 1990 to 2007 respectively. However, Per capita consumption of poultry meat and consumption of egg was 4.85 Kg and 2.55kg in year 2007, respectively (SL. Livestock statistic, 2007). Expenditure for import of Veterinary Pharmaceutical, Vaccine and chemicals has been increased by 71.4 times for 9 years parallel to the growth of the industry. (SL. Livestock statistic, 2007)All the information emphasized that economical value of the poultry sector, related social back grown and political influence according to the number people involve in the industry.

Salmonellosis re-emerged in 1998 as a common bacterial disease in poultry, Sri Lanka. Most of the commercial layer flocks in the country are in risk of getting infected with this gram negative organism at present. Government already has taken necessary actions to control the disease commencing from the Breeder flocks. This national control program consists of routine culling of Sero-positive birds, monitoring of hatcheries and improvement of bio-security measures in breeder farms. However, there is no definitive protocol declared for commercial operations (Comm. Layer and broiler) so far. Vaccination against *Salmonella* is an alternative to control disease prohibited in Breeders to avoid misleading on detection of infected birds. However,

Salmonella Killed vaccine is allowed to use only in commercial layers under strict post vaccination monitoring by regional Veterinary investigation Centers, while live vaccine are not allowed to import or to use in the field. The objective of this overview is to critically evaluate application of globally available *Salmonella* vaccines in commercial operations to control the Salmonellosis in poultry industry.

Over 2500 serovars have been identified in Genus *Salmonella*, Most of which belongs to the Species *Salmonella bongori* and *S.enterica*. Based on pathogenesis, *S.enetrica* can be divided into two broad groups.(Chao et al,2007) Group I consist of a large number of serovars, including *S.Typhimurium* and *S. Enteritidis*, which can cause paratyphoid in birds. The organism is colonized in the alimentary tract of food animals and cause gastrointestinal disease in a broad range of host as well as in humans. Therefore, these groups of organisms produce systemic diseases under circumstances like Laying and after some viral infestation. Group II consists of a small number of serovars that may cause systemic typhoid like infection in restricted host species. This group of bacteria colonizes in the intestine poorly and produce systemic disease and do not contaminate carcass surface hence rarely involve in food poisoning (Chao.M.R. et al,

2007). While, there are number of serovars present under this group, *S.Gallinarum* is the most important in chicken. Two biovars as *S. Pullorum* and *S.Gallinarum* have been described in the same serovar, causes Pullorum disease and fowl typhoid respectively (OIE Manual, 2007, Gupta et al, 2008).

Pullorum disease is an acute, exclusive disease of young chicken, while backyard flocks as well as game birds act as reservoir of this infection, Wild birds may act as vectors.(Feberwee. et al,2001) Diagnosis is based on isolation, identification of bacteria and detection of specific antibodies in the serum or blood.(OIE Manual, 2007)*Salmonella Pullorum* can be introduced into eggs by both vertical and horizontal transmission, the organism persist in both spleen and reproductive tract for a long period(OIE Manual, 2007)This organism can be organized in ovaries and oviduct of hen and infect eggs directly as with sexual maturation.(OIE Manual, 2007)This is an economically threatening diseases in developing countries through mortality, morbidity and reduction of eggs production.(Berchieri et al,2001) However, The Pullorum disease has been eliminated in developed world by culling of positive birds together with stringent management practices.(Berchieri et al,2001) Fowl typhoid caused by *S.Gallinarum* is a septicemic disease in poultry and has a severe economical impact in the industry through mortality and reduced production. (Berchieri et al,2001) It is more frequent in growing and mature birds, although it has been seen in young chicks as a results of transmission from infected eggs.(Barrow et al,1991,Paiva et al,2009) Primary lesions of pathogenesis are observed in spleen and liver but macroscopic changes also can be seen in heart, kidney and reproductive organs.(Haider et al,2007) Incubation period of the disease is 5-6 days in domestic chicken.(Carina et al,2006) Fowl typhoid has been eradicated in many areas in the world as consequence of modified management practices like highly intensified and enclosed housing system.(Berchieri et al,2001) In contrast,The disease is still important in countries where industry was recently intensified and countries under tropical environment. It is difficult to maintain environmental hygiene inside poultry houses in such environment. (Barrow et al, 1991) Further more, it was known that stringent management procedures and concept of eradication was the key to control the Salmonellosis in commercial poultry.(Berchieri et al,2001) Maintaining the disease free status is a challenging exercise due to the expanding nature of the industry. Fact was proven with that number of *Salmonella* outbreaks reported in the world as a result of injudicious introduction of infected birds.(Meeusen et al,2007)According to a recent study done by Gupata et al, Poultry feed which was contaminated with "Ochratoxine A" was more susceptible to *Salmonella* infection than non contaminated feed. Therefore, Quality of feed need to be evaluated at any level of poultry operation, which is not constant in local conditions specially with raining (Gupta et al,2008).

Infections with *Salmonella* Serovar *Typhimurium* or *Enteritidis* lead to prolong high titer of specific antibodies as a consequence of the persistence of these serovars in the GIT, a phenomenon that is not found with *S.Gallinarum*. (Rabsch et al, 2000) However, the role of anybody is not yet clearly defined in primary avian *Salmonella* infection. Both cell mediated and humoral antibody respond peak at 3 to 4 week post infection, declined rapidly after that. More prolonged antibody response and T lymphocyte proliferation was observed in Pullorum infection than *S.Gallinarum* infection in chicken(Rabsch et al, 2000).

Public health importance

Salmonellosis has created major political issues by which the general public had been made aware of this kind of infections, specially caused by the *S. Enteritidis* and *S.Typhimurium*. (Jackson et al, 2009) Further more, *S. Enteritidis* became a major concern for food safety in Europe and in America by 1980. (Rabsch et al, 2000) Consequently, Poultry was found as one of the leading sources of *Salmonella* illnesses, in trace back studies. Most of these outbreaks were associated with food that containing undercooked eggs. The pathogenic serovar of *Salmonella* spp. could be changed with geographical location. According to the page identification studies, Majority of human cases reported in UK caused by *S. Enteritidis* strain PH 4, while strain PH 8 and PH 13a had been identified frequently in USA. (Rabsch et al, 2000)In addition, *S.Typhimurium* is also an often reported, uniquely important serovar in Europe among egg and meat consumers.(EFSA,2004) It was also

shown that reported *S. Enteritidis* cases in poultry were inversely related to the number of *S. Gallinarum* reported, suggesting mutual balance between two serovars in chicken. This relationship between two serovars in poultry, prompted hypothesis that *S. Enteritidis* filled the ecological niche vacated by eradication of *S. Gallinarum* in domestic fowl (Wigley et al, 2005). In contrast, overall *Salmonella* infection in poultry and food born *Salmonella* infection in human have been increased over the last 15 years, although different resolution were made to control the disease so far. (Meeusen et al, 2007)

Vaccination

Veterinary vaccines are expensive biological commodity, comprises approximately 23% of the global market for animal health products. (Meeusen et al, 2007) This sector has been grown consistently for last few years as a result of new technology and continuous development of drug resistance by various pathogens. Basically, Vaccination aims to facilitate the development of naturally acquired immunity by inoculation of nonpathogenic but still immunogenic component or closely related organism. (Meeusen et al, 2007) Furthermore, the main objective of livestock vaccine is to improve overall production of the primary producer, and cost benefit resulting from the vaccination is the bottom line for this industry. (Meeusen et al, 2007) However, Vaccination has become a better alternative method to control a disease in poultry, as a outcome of recent banning of antibiotic and growth promoters from commercial flocks. (Meeusen et al, 2007) Emerging multiple antibiotic resistance serovar including *S. Typhimurium* and *S. Enteritidis* would be threatening issue in human near future, has been identified from different gram negative bacterial spp. (Chamber et al, 2002).

It has been accepted worldwide that practical possibility of Vaccination to prevent or reduce *Salmonella* infection in poultry (Barrow et al, 2007). In big poultry producing countries (Ex: Brazil) Commercial vaccine are commonly used in layers as well as broilers to control the outbreaks. (Paiva et al, 2009)

Primary objective of *Salmonella* vaccination can be varied from country to another, and also identified Serovar in the industry. However, objective in the European Union, where these host specific bacteria had been eradicated, is to control food-born infection by eliminating risk through out the food chain. (Nasar et al, 1994), The intentions of vaccination in Sri Lanka is to control flock mortality by non motile *Salmonella* infection as well as to minimize food born infection.

It is accepted that cell mediated immunity is more important than humoral response against *Salmonella* infection. (Barrow et al, 2007) The efficacy of *Salmonella* vaccine is gathered by level of intestinal and systemic colonization, morbidity, mortality rate (Young et al, 2005) In contrast, other factors like challenge strain, the rout of administration, the infective dose, the age of birds and species/line of birds also are included for the efficacy. (Woodwards et al, 2002., Young et al, 2007)

Killed vaccine

Salmonella killed vaccines derived from *S. Enteritidis* and *S. Typhimurium* are used mainly to control non host specific *Salmonella* infection in poultry. (Barrow et al, 2007) With these vaccines, decrease in mortality, variable effect on fecal shedding, colonization in intestine and other internal organs have been observed against host and non host specific *Salmonella* spp. (Meesen et al, 2007., Wolfgang et al, 2000) It was also shown that shedding of non-host specific *Salmonella* through egg shell and egg content of the vaccinated flock was lower than non-vaccinated birds. The vaccine is safe because there was no reversion to virulence, no spreading in the environment and good enough to protect chicken when applied in large scale poultry production. (Nasar et al, 1994. Young et al, 2007)

Woodward et al has shown the effects of *S. Enteritidis* killed vaccine ("Salenvac"- Intervet) against experimental intravenous challenge of same serovar. The study further confirmed that reduced egg contamination and low level of systemic phase of *Salmonella Enteritidis* infection in layers. (Woodward et al, 2002) Also the number of pathogenic *S. Enteritidis* organism that inhabited in the caeca was significantly lowered by "Salenvac" vaccination (Woodward et al, 2002). The protection was satisfied and long last up to 59 weeks of challenge and reported a strong humoral response against *S. Enteritidis*. Although the protection against *S. Gallinarum* was and not described in "Salenvac", short term cross protection was reported for *S. Gallinarum* by oral route just three weeks after vaccination in local conditions. (Priyantha et al, 2008) In another study, it has been proven that low growth of *Salmonella Enteritidis* in eggs and eggs surface shown by the killed vaccination (Chanter et al, www.intervet.com)

Killed bacterin synthesized from *S. Gallinarum* also is used widely in the South Asian region to control *S. Gallinarum* infection in domestic fowl.

That vaccine has given better protection against experimental infection of *S. Gallinarum* than inactivated vaccines derived from *S. Enteritidis* and *S. Typhimurium* (Haider et al, 2007).

Some disadvantages were reported in killed bacterin, like labor cost for administration and post vaccination stress due to tissue reaction at the site of injection, which is caused by releasing of bacterial cell wall endotoxin subsequent to vaccine antigen metabolism in birds. In addition, Killed vaccine may be destroyed and eliminated from the host rapidly, therefore considered as unable to induce activation of cytotoxic T cells. (Wolfgang et al, 2000)

Live vaccine

Live vaccine derived from avirulent strains of *Salmonella* were given attention to produce, because of the accumulated evidence that such strains of *Salmonella* were more immunogenic in poultry than killed or subunit vaccines. (Tan et al, 2007., www.Intervet.com) It has been shown that live attenuated vaccines many times more effective limiting mortality and bacterial excretion than killed bacterin. (Barrow et al, 1991) According to a study done by Young et al (2007), *Salmonella Typhimurium* colonization in visceral organ (Liver and Spleen) of offspring broilers can be reduced by live SG9R vaccination at Breeder birds. (Silva et al, 1981) Although the study was limited to *S. Typhimurium*, combination of live and killed vaccine made of *Salmonella Gallinarum* has given better protection against *S. Enteritidis* in mortality and organ invasion. (Tan et al., 2009, www.Intervet.com)

It was also shown that *S. Typhimurium* live vaccine was capable to control the invasion of visceral organs by non-host specific *Salmonella* and diminished colonization within gastrointestinal tract (Lahman information, Lohman. www.worldpoultry.net/news). A live vaccine produced by Lohmann's ("Avipro MEGANVAC") is a modified *S. Typhimurium* strain. (Revolledo et al, 2006). According to the manufactures information, it has given better protection against *S. Typhimurium*, *S. Enteritidis* as well as *S. Heidelberg* in chicken. It also provides resistance to colonize pathogenic *S. Enteritidis* in the digestive track including crop and caeca. (Lahman. www.worldpoultry.net/news/Lohmannsvaccine- to reduce salmonella-id 3767). However, Scientific evidence or information made independence research relevant to particular vaccine was not found. Chacana et al observed cross immunization by live *Salmonella Enteritidis* vaccine on fecal shedding, mortality and organ invasion against *S. Gallinarum* for 3 months post vaccination at 28 weeks of age ("TAD *Salmonella VAC E*"). However, protection efficacy was not confirmed for longer duration. (chacana et al, 2006)

Salmonella Gallinarum strain (Rough) 9(SG9R) vaccine has been one of the most popular live vaccine in poultry developed by the H. William smith of SG 9R strain. (Barrow et al, 2007) The rough strain provides sufficient immunity in chicken, without showing pathogenicity for day old chicks. (Feberwee, A, 2001(10)) This strain of bacteria has a semi-rough lipopolysaccharide structure; however, the nature of its attenuation was not mentioned. Drop of egg production and mortality reported with smooth strain was not reported with rough strain in chicken. Further more smooth strain cause mortality in day old chicks and mark drop in egg production in layers. (Barrow et al, 2007) In contrast, It has been observed mild form of systemic infection in the chicken, (Barrow et al, 1990) induces a mild form of systemic disease and both cellular and humoral immune response, peak level of reaching soon after bacterial clearance. (Paiva et al, 2009) In addition, live attenuated vaccine of *Salmonella Gallinarum* was many times effective against *Salmonella Enteritidis/Gallinarum* infection in limiting mortality and bacterial excretion than killed bacterin (Chao et al, 2007). Cross protection for *S. Enteritidis* was a definitive advantage and no fecal spreading of *S. Enteritidis* was reported (OIE, 2007). Therefore, Primary vaccination at 6 weeks and booster vaccination at 16-18 of week of age provide better protection than single or no vaccination. (Paiva et al, 2009) Study done by the Chacana and Terzole (2006) have confirmed that, elicited cross immunization against fowl typhoid by vaccine derived from *Salmonella Enteritidis*. The vaccine was also shown potential for lack of fecal shedding and low organs invasion by the *S. Gallinarum* pathogenic strain for prolong duration. (Chacana et al, 2006) The Protection against mortality or organ invasion in highly susceptible chicken exposed to virulent strain of *Salmonella* may be limited by 9R vaccine (Paiva et al, 2009). The vaccine causes a mild form of systemic *Salmonellosis* although the strain does not cause significant mortality and sometimes vaccine itself resulted and reported systemic disease with pathological changes in liver, spleen. The vaccine strain may persistence for several weeks at these sites. (Paiva et al, 2009) Colonization of *Salmonella* in offspring can be reduced by vaccinating broiler breeder with combination of SG9R live vaccine and autogenous bacterin (Young J.L. et al, 2007). Efficacy and safety of the 9R vaccine for the prevention of fowl typhoid was confirmed by various studies. The immunity was long in duration comparatively maintained for six

month after vaccination (Young J.L. et al, 2007).

The live SG 9R vaccine was not protected for intestinal colonization by *S. Typhimurium*, which is a common inhabitant in the environment (Silva et al, 1981). Although it provides a better protection by expression of all appropriate antigens and stimulate both cell mediated and humoral immunity. (Silva et al, 1981) also the protection against mortality rate and organ invasion in highly susceptible chicken exposed to virulent strain of *S. Gallinarum* may be severely limited by live 9R vaccination. (Young et al, 2005) Further more, SG9R vaccine given better protection in *Salmonella* free flocks while in infected flocks, protection may varied and not successful. (Personal communication, Intervet technical services). However, scientists are still arguing on excretion of vaccine strain, possible environment contamination and possibility to reversion of rough mutant to smooth stage either by mutation or any other. In addition, vaccine was not proven protection against some wild strain which was intermittently reported in country as local outbreaks caused mortality in commercial chicken (Priyantha et al, 2007).

CONCLUSION

Vaccination is only an alternative to control *Salmonellosis* in chicken and other precaution like bio-security, good management practices must be taken to consideration first. There are pros and cons available with both live and killed vaccine, depend on the requirement in the country either to control the human infection or to control the mortality by fowl typhoid in layers. However, immediate action is preferable by the government since the *Salmonellosis* is common disease, farmers who suffered more and more losses in layer farming in Sri Lanka. Therefore, Selection of vaccine regime can be determined by after thorough understanding in the field. By the time, temporary solution is also urgent to control the current outbreak in the field and author also concluded more epidemiological survey and research on *Salmonellosis* in local situation. It was also concluded that vaccination may be preferred by the consumers than broad range of antibiotic and its residues in eggs and meat, in which antibiotic are used as medication in the field.

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