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Development and evaluation of gamma irradiated toxoid vaccine of *Salmonella enterica* var Typhimurium

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ABSTRACT

Development of a single effective vaccine against non-typhoidal salmonellosis is very challenging due to the presence of hundreds of serovars of *Salmonella* which are antigenically different from each other. The *Salmonella* enterotoxin (Stn), a common virulence factor occurring amongst a wide range of serovars, used as a formalized toxoid vaccine has been found to be effective against homologous and heterologous serovars. However, the process of formalization has its own drawbacks. Gamma radiation (γ) on the other hand is widely used as a safe and convenient method of sterilization worldwide. In this experiment we used gamma rays to inactivate the partially purified Stn of *Salmonella enterica* serovar Typhimurium (DT 193). The toxoid obtained was tested for its immunogenicity and loss of toxicity and then used to formulate a gamma irradiated toxoid vaccine (ITST). The efficacy of the developed ITST was tested in Kuroiler, a Broiler breed, against homologous and heterologous challenges (*S.* Typhimurium and *S.* Gallinarum) administered intra-peritoneally and orally. Birds in groups challenged with *S.* Typhimurium by both routes recorded protective indices (PI) of 100% while birds in groups challenged intra-peritoneally with *S.* Gallinarum recorded PI of 83.33% and those challenged orally scored 100%. The overall protective index (PI) being 95.83%. The antibody titres calculated as geometric mean with standard error at $1{:}10^{-4}$ dilutions showed a steep rise after the first dose and peaked at week 6 post primary vaccinations. Thus the ITST was found very effective in protecting poultry against both the challenge organisms tested.

1. Introduction

Foodborne non-typhoidal salmonellosis (NTS) remains an important public health problem worldwide and is often transmitted through contaminated meat, meat products, poultry and eggs. The control of *Salmonella* infection in domestic birds and animals would remove a major source of this organism and reduce diseases in human (Rabsch et al., 2000; Mastroeni et al., 2001; Wallis, 2004). Attempts to develop an effective vaccine for prevention of NTS have been met with limited success due to the vast antigenic differences amongst the different serovars (Lax et al., 1995; Barrow et al., 2003). Bacterins in various forms (Hassan and Curtiss, 1990; Ferdous et al., 2009; Berghaus et al., 2011), live attenuated or recombinant organisms (Barrow et al., 1991; Babu et al., 2004; Wolfenden et al., 2010; Matsuda et al., 2011), and outer membrane proteins (Bouzoubaa et al., 1989; Toyata-Hanatani et al., 2009; Maripandi and Al-Salamah, 2010), have been tried as vaccines to control NTS; however, an effective vaccine conferring protection against multiple serovars is still lacking. Hope for the development of a broad spectrum *Salmonella* vaccine against multiple serovars arose when Mishra and Sharma (2001) demonstrated the efficacy of a formalized toxoid vaccine of *S.* Weltevreden against salmonellosis caused

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by different serovars of *Salmonella enterica* in poultry. The efficacy of the formalized toxoid vaccine was further established by subsequent studies carried by other workers (Barman et al., 2002, 2005; Bhattacharya et al., 2004). However, a large scale trial of the vaccine has not been possible due to the difficult, cumbersome and lengthy process of repeated washing and dialysis of formalin removal during toxoid production. Also, the possibility of a hazardous reaction between formalin and the toxic moiety cannot be rule out (Rahman et al., 1994). Therefore, the need exists for a safe and convenient method to inactivate the *Salmonella* enterotoxin for vaccine formulation.

The FDA in December, 1997 approved the use of gamma rays as means of irradiation. Today it is the most widely used source of irradiation for sterilization purposes in hospitals, research laboratories, food, pharmaceuticals, biotechnology industries, etc., due to its high penetrative power which require less exposure time and is very safe. Almost all radiation facilities in the world use Cobalt-60 (WHO, 1987) as it poses low risk to environment, decaying into non-radioactive nickel. Gamma rays have been previously used with success to detoxify many biological toxins like cholera exotoxins I, III and IV (Nedugova et al., 1984a,b,c), staphylococcal enterotoxin A (Modi et al., 1990), crotoxin (Do Nascimento et al., 1996), crotamine (Boni-Mitake et al., 2001), scorpion venom (Abib and Laraba-Djebari, 2003) and *Salmonella* endotoxin (Previte, 1968; Nerkar et al., 1977; El Sabbagh et al., 1982; Naidu et al., 1998). Toxin molecules treated with gamma rays were found to completely lose their toxicity while retaining the immunological properties which renders them eligible vaccine candidates (Nedugova et al., 1984a,b,c; Hati et al., 1990; Do Nascimento et al., 1996). Thus, this study was undertaken to use gamma rays as a means of inactivation of *Salmonella* enterotoxin (Stn) and to formulate and assess the efficacy of gamma irradiated toxoid vaccine against experimental salmonellosis in poultry.

2. Materials and methods

2.1. Salmonella strains

A known enterotoxigenic isolate of *S. enterica* serovar Typhimurium (DT 193), used for vaccine preparation, and *S. enterica* serovar Gallinarum (SG 46), isolated from poultry used as heterologous challenge, were obtained from Assam Agricultural University, Guwahati, Assam, India for the study. The cultures were revived on brilliant green agar (BGA) plates (Oxoid, UK) at 37 °C, checked for purity and maintained on nutrient agar (HiMedia, India) slants at 4 °C.

2.2. Preparation of partially purified Salmonella enterotoxin

The partially purified *Salmonella* enterotoxin (PPT) was prepared according to Rahman et al. (1994). Briefly, *S.* Typhimurium (DT 193) isolate was cultured in brain heart infusion (BHI) broth (Hi-Media, India) for 18 h (200 rotations per min, 37 °C). Thereafter, the broth culture was centrifuged at 10,000 rpm (20 min, 4 °C), the supernatant collected, made bacteria-free by membrane filtration (Millipore, 0.22 μm) and subjected to ammonium sulphate [$(NH_4)_2SO_4$] precipitation at 60% saturation at 4 °C. The precipitate was collected by centrifugation (10,000 rpm, 30 min, 4 °C) and made ammonium sulphate ions free by dialysis (Snake Skin Dialysis Tubing, MWCO 10,000, Pierce). The ion-free precipitate solution thus obtained was concentrated to about half the original volume by dialysing against 30% (w/v) polyethylene glycol-6000. The concentrated enterotoxin was then centrifuged (10,000 rpm, 30 min, 4 °C), supernatant collected, its protein content determined (Lowry et al., 1951) and designated as the partially purified toxin (PPT).

2.3. Gamma irradiation of the PPT

Five aliquots of 5 ml each of the PPT were taken in cryo vials and subjected to five different doses of gamma rays *viz*., 10 kGy, 25 kGy, 40 kGy, 60 kGy and 80 kGy at a dose rate of 5 kGy/h (Table 1). The cryo vials containing the PPT were placed vertically and securely in the gamma chamber (Gamma chamber 5000, BRIT, DAE, India) and irradiated for different time periods according to the required doses of gamma rays. The temperature inside the gamma chamber was maintained at 40–42 °C. After completion of irradiation, each vial was carefully removed from the gamma chamber and immediately stored at −20 °C for further use. The facility at the Division of Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre (BARC), Mumbai, India was utilized for the purpose.

2.4. Enterotoxicity and immunogenicity test

The PPT and all the gamma irradiated toxoid preparations were tested for their enterotoxicity and immunogenicity. Rabbit ligated ileal loop (RLIL) and Chinese hamster ovary (CHO) cell assays as described by De and Chatterjee (1953) and Rahman et al. (1994),

Table 1
Preparation of gamma irradiated toxoid of *Salmonella* enterotoxin (Stn).

Partially purified toxin (PPT) of	Gamma irradiation (^{60}Co) (dose rate = 5 kGy/h)	
	Doses	Irradiation time (h)
S. Typhimurium (DT 193)	10 kGy	2
	25 kGy	5
	40 kGy	8
	60 kGy	12
	80 kGy	16