Protective efficacy of maternal antibodies induced by *Salmonella* toxoid (vaccine)

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An attempt was made to evaluate the protective efficacy of maternal antibodies in chicks against salmonellosis. Layer chicks ageing 21 days were individually vaccinated with 100 μg of *Salmonella enterica* subspecies *enterica* serovar Weltevreden (BM 1643) toxoid adjuvanted with vitamin E subcutaneously. After 90 days of the primary vaccination the birds were given booster dose of the vaccine. The saline extract of the yolk of eggs laid by the vaccinated birds yielded agglutination and ELISA titres ranging from 43.2 ± 5.33 to 75.2 ± 6.26 and 4.987x10³ ± 0.54 to 5.89x10³ ± 0.56, respectively. Sera of chicks hatched from eggs laid by the vaccinated layers were also subjected to agglutination and ELISA. Agglutination and ELISA titres on the 5th day- post hatching (dph) were 21.6 ± 1.75 and 4.025 x 10² ± 0.59, while on the 10th dph titre were 13.6 ± 1.65 and 1.21x10² ± 0.60, respectively. It was also observed that only one out of 6 chicks died when challenged with 2 x 10⁴ CFU of *S. enterica* serovar Gallinarum at the age of 7 days showing 83.33 % protection. Thus it can be concluded that passive immunity confided by *Salmonella enterica* subspecies *enterica* serovar Weltevreden (BM 1643) toxoid can protect chicks against salmonellosis during their early days of life.

Keywords: Poultry birds, Toxoid vaccine, Salmonella

Salmonellosis is one of the major hatchery-borne bacterial diseases of poultry. Further, with the expansion of poultry industry, the importance of salmonellosis is being increasingly recognized. The disease is important not only for poultry industry but also from public health point of view, as poultry eggs and meat are the major sources of *Salmonella* infection to human beings.

Although salmonellosis has been thoroughly studied, not much has been achieved in the area of immunoprophylaxis against the disease. Many workers have tried killed, live attenuated and subunit vaccines with varying success, but so far no widely proclaimed vaccine is commercially available.

Recently, Mishra and Sharma reported development of a novel toxoid vaccine prepared from *S. enterica* serovar Weltevreden toxins (an enterotoxin and four cytotoxins). The vaccine adjuvanted with Freund’s complete adjuvant (FCA) afforded 100% protection against homologous challenge as well as against heterologous *Salmonella* serovar, viz., *S. Gallinarum*. Also, Barman et al. reported that vitamin E adjuvanted *Salmonella* toxoid afforded solid immunity against salmonellosis. Singh and Sharma reported that apparently healthy mice inoculated with the toxins truly mimicked natural *Salmonella* infection suggesting that the toxins play a significant role in the pathogenesis of salmonellosis.

It stands well documented that immunoglobulins transferred through eggs confer passive immunity to chicks. The immunoglobulins are partitioned in the egg: IgG in the yolk and IgA and IgM in the white. It has been suggested that he chick embryo may have received IgA and IgM by swallowing the egg white and thereby are conferred with a passive immunological protection similar to that provided to young mammals through colostrums.

In view of the above, the present investigation has been undertaken to study the protective efficacy of maternal antibodies induced by the *Salmonella* toxoid vaccine initially developed by Mishra and Sharma and later modified by Barman et al.

**Materials and Methods**

*Salmonella enterica* subspecies *enterica* serovar Weltevreden (BM-1643) and *S. serovar Gallinarum* (4211) were obtained from *Salmonella* laboratory in the department. The former had been isolated from a...
buffalo-meat sample and identified as a potent producer of an enterotoxin and four cytotoxins, while S. serovar Gallinarum had been isolated from poultry.

Partial purification of toxins—Salmonella serovar Weltevreden (BM-1643) toxins were partially purified as per protocol described by Malik et al. \(^5\). Briefly, the test organism was propagated in brain heart infusion broth (HI-Media, Mumbai) at 37°C on a shaker (200 rpm) for 18 hr. Bacterial pellet was obtained by centrifuging the growth at 10,000 g for 10 min. The pellet was re-suspended in PBS and polymyxin-B was added to it in the concentration of 0.1 mg ml\(^{-1}\). The supernatant was collected and designated as polymyxin-B extract (PBE). It was then precipitated with ammonium sulphate at 45% saturation level and centrifuged as above. To the supernatant additional salt was added so as to achieve 70% saturation level. The precipitated protein was collected after centrifugation as above.

The precipitate was desalted by dialyzing against phosphate buffer saline (PBS) using dialyzing bag # 250-11 (Sigma, USA), concentrated by dialyzing against 30% polyethylene glycol (PEG-20,000, Merck, Germany) and gel filtered through Sephadex G-100 column (Pharmacia Fine Chemicals, Sweden) according to the protocol described by Malik et al.\(^5\). Contents of the peaks I (enterotoxin) and II (cytotoxin) were pooled and then protein concentration was determined by Bradford’s method.\(^6\) The toxin thus purified did not contain detectable amount of LPS as assessed according to Wright and Roberts.\(^7\)

Preparation of formalized toxoid—Toxoid was prepared according to Mishra and Sharma\(^4\) by treatment of the toxin pool with an equal volume of 0.2% formalin for 48 hr at 37°C. To remove the traces of formalin, the preparation was dialyzed against PBS. Then the sample was brought to original volume by concentrating against 30% PEG-20,000. Antigenicity of the above preparation was tested by agar gel precipitation test using known anti-toxoid serum raised in rabbit. Also, toxoid was tested for Vero-cytotoxicity to confirm the inactivation of cytotoxins. The toxoid was stored at -20°C for further use as vaccine.

Vaccination—Apparently healthy Salmonella free, 21-day-old white leghorn layer chicks were procured from the Instructional Poultry Farm (IPF) of the University and divided into two groups of 20 chicks. The chicks belonging to group ‘A’ were individually vaccinated with one ml of the vaccine containing 100 μg of Salmonella toxoid adjuvanted with vitamin E (100 IU) subcutaneously in the abdominal region. After 90 days of primary vaccination, all the birds were again given one ml of the above vaccine subcutaneously as booster dose. The chicks belonging to group ‘B’ were kept as unvaccinated control and received 1 ml PBS only. Before the start of laying, two Salmonella free cocks procured from the IPF of the University were kept with each of the above groups. Eggs from these birds were collected at regular intervals and were tested serologically. Some eggs were incubated for hatching of chicks after 15 days of start of laying.

Assessment of passive transfer of immunoglobulins—The presence of antibodies in egg-yolk was detected as per protocol of Nicholas and Andrews\(^8\) with slight modification. Briefly, the egg-yolk was separated from the white and mixed with equal volume of normal saline solution. Then a homogenous suspension was made by shaking on vortex shaker and allowed to settle overnight. Next day, the suspension was centrifuged at 10,000 g for 30 min and the supernatant was collected. For estimation of level of maternal antibodies in chicks, sera were collected on 5\(^{th}\), 10\(^{th}\) and 14\(^{th}\)-day posthatching (dph). Thereafter, both sera samples and saline extract of egg-yolk were subjected to agglutination test using heat killed bacterial cells (2×10\(^9\) cells ml\(^{-1}\)) of Salmonella serovar Weltevreden and Single Dilution ELISA using the toxoid as coating antigen and 0.05 M carbonate-bicarbonate buffer (pH 9.6) each @ 50 μl per well.

Challenge study—To assess the protective efficacy of passively transferred immunity, six chicks from each of the above two groups (A and B) were challenged with 2×10\(^9\) CFU of Salmonella serovar Gallinarum intraperitoneally\(^9\), on the 7\(^{th}\) and 14\(^{th}\)-day posthatching (dph).

After challenge, birds were observed for clinical changes and their cloacal swabs were collected for bacteriological examination on 2, 5, 10 and 15 days after challenge. The survivors were sacrificed on the 21\(^{st}\) day post challenge. The bird died of challenge infection and the sacrificed ones were necropsied and pathological changes were recorded. The liver, spleen and caeca were collected for bacteriological examination.\(^1\)
Results

Agglutination test and ELISA were performed to determine the passively transferred antibodies through eggs. Agglutination titres recorded in the yolk extract of eggs laid during the 1st and 2nd weeks of laying were 75.2 ± 6.26 and 73.6 ± 6.61, respectively, which declined to 43.2 ± 5.33 and 41.6 ± 3.36, in the yolk of eggs laid during the 3rd and 4th weeks. The corresponding ELISA titres during the 4 weeks were 5.134x10^3 ± 0.41, 5.898x10^3 ± 0.56, 4.987x10^3 ± 0.5 and 5.095x10^2 ± 0.41, respectively. No seropositivity was detected in the egg-yolk of the control group.

The sera collected from 5-day-old chicks yielded agglutination titre of 21.6 ± 1.75. The titre declined to 13.6 ± 1.65 and 12.4 ± 1.36, respectively, in the sera of 10 and 14 days old chicks. Similar trend was seen in ELISA also with titres being 4.025x10^2 ± 0.64, 1.219x10^2 ± 0.60 and 1.854x10^2 ± 0.86 on the 5th, 10th and 14th day of age, respectively. However, control chicks showed no seropositivity.

The vaccinated birds did not show any clinical sign after challenge infection. Out of 6 chicks derived from vaccinated birds, only one chick died following challenge with 1 x 10^5 CFU of S. serovar Gallinarum at the age of 7 days, thus showing 83.33% protection while 66.67% protection was recorded in 14-days old chicks (Table 1). On the other hand, all the chicks belonging to the control group stood dejectedly with closed eyes and developed diarrhoea. All the chicks of the control groups shed the challenge organism and eventually succumbed to the challenge infection whereas only one out of the 6 chicks derived from the vaccinated group was found to shed *Salmonella* between 2 and 10 days.

On necropsy, chicks died of the challenge infection showed necrosis and blackish discoloration of the liver with ballooning and congestion of the caeca. *Salmonella* was invariably isolated from the caeca of dead chicks. The survivors of the challenge infection were sacrificed on 21 dpc and subjected to necropsy. Neither was any lesion seen in any organs nor *Salmonella* was recovered on cultural examination.

Discussion

In the present study, attempts were made to elucidate whether immunoglobulins generated by *Salmonella* serovar Weltevreden vaccine in layers are passively transferred to chicks. *Salmonella* serovar Weltevreden was used to prepare the toxoid as it produces all the toxins produced by *Salmonella*, and that too in sufficient amount. *Salmonella* serovar Gallinarum was used as challenge organism as it is the most common *Salmonella* serovar found in poultry birds and secondly, to study the efficacy of the vaccine against heterologus serovars.

In the present study, the challenge dose of *Salmonella* was kept exceptionally high (2x10^9 CFU) and the birds were challenged intraperitoneally so as to ensure establishment of the infection in unprotected birds. The organisms in the alimentary canal encounter a number of natural barriers and antimicrobial factors which interfere with their infectivity and pathogenicity. Thus the establishment of the infection on oral challenge and consequently determination of the efficacy of the vaccine remain uncertain.

In an earlier study, the birds vaccinated with the toxoid orally showed 90% protection against oral challenge^15,16/. The findings of the passive transfer of immunity to chicks hatched from eggs laid by the vaccinated bird and its protective efficacy against live *Salmonella* challenge were encouraging. As expected, the egg yolks were found sero-positive by both agglutination test and ELISA with high titres ranging from 41.6 ± 3.36 to 75.2 ± 6.26 and 4.987x10^2 ± 0.54 to 5.899x10^2 in the egg-yolk, respectively.

Comparatively lower agglutination (12.4 ± 1.36 to 21.6 ± 1.75) and ELISA (1.219x10^2 ± 0.6 to 4.025x10^2 ± 0.64) titres were recorded in the sera of chicks

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<th>Age of chick</th>
<th>Birds challenged</th>
<th>Nos. of chicks challenged</th>
<th>Shedder/survivor on different days of cloacal swabs culture (days post challenge)</th>
<th>Protection percent</th>
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<td>7 days</td>
<td>Vaccinated</td>
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<td>Control</td>
<td>6</td>
<td>6/6</td>
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<td>14 days</td>
<td>Vaccinated</td>
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ageing between 5-15 days. Bringman et al.\textsuperscript{18} also detected antibodies against *Salmonella* serovar Enteritidis in eggs and sera of chicken flocks by using agglutination, ELISA and immuno-blot. It was expected that chicks hatched from eggs laid by vaccinated birds would be protected in their early days against salmonellosis. Attempts made to elucidate the protective efficacy of the maternal antibody also yielded promising results. The protection percentage against challenge was higher (83.33\%) at the age of 7 days than in two-week-old chicks (66.67\%). This confirms the observation of Miller et al.\textsuperscript{19} who also reported that bacterin toxoid induced humoral immune response and afforded good protection against salmonellosis in cows. The chicks hatched from unvaccinated control bird could not withstand the challenge and finally succumbed to infection.

Significantly, survivors of the vaccinated group sacrificed on 21\textsuperscript{st} day did not yield *Salmonella* on bacteriological examination. This proved that maternal antibodies effectively prevented multiplication of the organism in the internal organs (liver, spleen and caeca) and consequently checked shedding of the organism in faeces. This is important not only for poultry industry but also from public health point of view as it will help in preventing transmission of salmonellosis from carrier birds to man via food chain.

Thus, it can be concluded that passive immunity from birds vaccinated with *Salmonella* serovar Weltevreden vaccine can protect chicks against salmonellosis during their early days when they are highly prone to infection. Toxoid is the most suitable vaccine for protection against salmonellosis as it induces immunity against all the serotypes involved in the pathogenesis of the organism\textsuperscript{13}. Killed, live or live attenuated vaccines are effective against homologous or closely related serovars but toxoid can protect against heterologous strains also as the bacterial toxins are often immunologically conserved within bacterial species, thus providing a strong possibility for formulating cross-protective vaccines\textsuperscript{20}.

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**References**


