Immunological Studies on Local and Imported Mineral Oil Vaccines Against Paratyphoid in Broiler Chickens

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Abstract: Salmonellosis is one of the most important bacterial diseases affecting poultry. Its importance is derived from the loss in productivity in affected birds and the hazard it causes for public health. Vaccination is the best mean for controlling salmonellosis in birds. In the present study, the immunizing and protective efficacy of locally prepared and imported bivalent S. Typhimurium and S. Enteritidis formalin inactivated oil adjuvant vaccines have been studied. The 1st group was vaccinated with the locally prepared vaccine, the 2nd group was vaccinated with the imported vaccine and the 3rd was kept unvaccinated as a control group. The three groups were challenged with virulent S. Typhimurium and S. Enteritidis strains (10⁶ CFU/ml of each) 1ml orally, 3 weeks post boosting of the vaccine. The degree of protection was assessed according to the severity of the clinical signs, the mortality and fecal shedding of the challenge organisms. Blood samples were collected weekly and humeral immune response was measured against Salmonella strains using micro-agglutination test, tube-agglutination tests and ELISA. The locally prepared vaccine induced high protection rates in challenge test with reduced fecal shedding and higher antibody response compared with the imported one.

Key words: Salmonella Typhimurium • Salmonella Enteritidis • Vaccines • Broilers

INTRODUCTION

Salmonella bacteria are facultative intracellular pathogens that cause localized or systemic infections, in addition to their emphasis in chronic asymptomatic carrier state. They are of worldwide economic and public health significance [1, 2]. In poultry, which represents an important source of cheap protein throughout the world, avian Salmonellosis continues to cause economic losses in Egypt, where the poultry industries are continuing to intensify whereas open sided housing is common.

Control of Salmonella infections in poultry is posing itself as one of the difficult problems not only for those who are concerned with poultry industry, but also for public health hazard because of the fact that most of the serovars of salmonellae which poultry harbor can act as potential pathogens for man [1]. Previous studies all over the world showed many trials to control and eradicate Salmonellosis in poultry by vaccination. Live attenuated Salmonella vaccines may be hazardous because of the residual virulence due to insufficient attenuation [3]. Prevention of avian salmonellosis using inactivated vaccines has been reported by several authors to provide good protection with decrease or absence of the residual virulence [4-6].

The present work aimed to evaluate the immunizing and protective efficacy of two bivalent Salmonella Enteritidis and Salmonella Typhimurium formalin inactivated oil adjuvant vaccines; the first was prepared from local strains and the second was imported one. Evaluation was conducted by monitoring the humoral immune response developed against the vaccines using micro agglutination test, tube-agglutination tests and ELISA. In addition, determination of the fecal shedding of virulent S. Enteritidis and S. Typhimurium from the immunized broiler chickens following challenge was studied.
MATERIALS AND METHODS

Bacterial Strains: Standard local Salmonella Typhimurium and S. Enteritidis strains of chicken origin, kindly supplied by Central laboratories-Ministry of Health, Cairo, Egypt, were used in our experiments.

Diagnostic Antisera: Salmonella somatic (O) and Salmonella flagellar (H) antigens agglutinating sera (Welcome, Dartford, England) were used for identification of Salmonella isolates.

Salmonella Antigens: Salmonella antigens were kindly supplied by the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

Preparation of the Local Vaccine: Bulk cultures from S. Enteritidis and S. Typhimurium were prepared according to Charles et al. [7]. A separate final suspension from each of the selected strains was prepared and adjusted to 10^10 CFU/0.5ml of each according to Read and Muench [8]. Inactivation of vaccine strains was performed by addition of formaldehyde solution 37% to the bacterial suspension to obtain a final concentration of 0.4%. The inactivation was carried out under stirring for 24h at 24°C to complete the inactivation process. The inactivated cultures were neutralized with sodium meta-bisulfite then stored at temperature of 5-7°C.

The amount of inactivated bacterial cells suspension from each strain that represents 500 doses was calculated and centrifuged at 5000 r.p.m. for 20 minutes at 4°C. Then, the supernatant was discarded and the bacterial cell pellets was collected. The 50 ml of the final content containing 500 doses of the 2 inactivated Salmonella strains was gently and thoroughly mixed with 4% tween 80. This watery phase of the vaccine was emulsified with 200 ml of the oily phase (Mineral oil adjuvant+span80) according to Stone et al. [9] forming a total of 250 ml containing 500 doses from each of the vaccine immunogens (0.5 ml=1 vaccine dose). Thiomersal was added as a preservative in a concentration of 0.05 /liter.

Imported Vaccine: A commercial S. Enteritidis and S. Typhimurium imported vaccine; Gallimune SE+ST, Meriel Co was used.

Challenge Experiment: A total of 240, six-weeks old SPF Lohmann chickens were divided into 3 groups; 80 chickens each. The first group of experimental chickens was vaccinated with the local mineral oil adjuvanted vaccine and the second group was vaccinated with the Gallimune SE+ST oil adjuvanted vaccine, while the third group was used as a control (non-vaccinated). The chickens in each group were inoculated twice subcutaneously in the middle part of the neck with an initial dose at 6 weeks of age and a booster dose at 9 weeks of age with 0.5ml of the vaccines. The three groups then were challenged by oral administration of 1ml of S. Typhimurium and S. Enteritidis virulent strains suspension containing 10^8 CFU/ml of each three weeks after the booster dose. The inoculated chickens were observed for one month [10]. The degree of protection was assessed according to the severity of the clinical signs, the mortality and the recovery of the challenge organisms from fecal samples.

Blood samples (2-5ml/bird) were collected in sterile test tubes from wing vein before immunization, for three times after each vaccination and post challenge for three weeks (once/week) to measure and evaluate the developed immune response to the immunogenic components of the vaccines.

Fecal samples were collected before the start of the experiment and after challenge for one month (once/week) using sterile swabs which were inoculated into tetrathionate broth from all chickens including the vaccinated and the control ones and examined bacteriologically for shedding of Salmonellae.

Evaluation of the Developed Humoral Immune Response Against S. Enteritidis and S. Typhimurium in the Vaccinated Chickens: The developed humoral immune response against S. Enteritidis and S. Typhimurium in the vaccinated chickens was measured in the sera using the micro-agglutination test (MAT) according to Brown et al. [11] and the antibody titer was expressed as Geometric Mean Titer (GMT), tube agglutination test according to Cruickshank et al. [12] and ELISA according to Haider et al. [13]. Calculation of the antibody titers was performed according to the following formulae:

Calculation of S/P Ratio:

\[
\text{S/P} = \frac{\text{Mean of test sample} - \text{mean of negative control}}{\text{Mean of positive control} - \text{mean of negative control}}
\]

Calculation of Antibody Titer:

Log10 Titer=1.13Log (SP)+3.156

AntiLog= Antibody titer
RESULTS

Protective Efficacy of the Local and Imported Mineral Oil Adjuvanted Vaccines: The protection rates of the locally prepared and imported vaccines were 90 and 85%, respectively after 4 weeks post challenge (Table 1).

Fecal Shedding of Salmonellae from Chickens Vaccinated with the Different Vaccines after the Challenge with Virulent Salmonella Strains: The re-isolation rates of salmonellae from chickens vaccinated with the locally prepared vaccine in the 1st, 2nd and 3rd weeks post challenge were 13.5, 5.5 and 1.4% respectively compared to 19.4, 13.2 and 7.3%, respectively in those vaccinated with the imported vaccine. In the 4th week the fecal shedding disappeared in both groups. Regarding the control non-vaccinated birds the re-isolation rates were 83.9, 71.4, 56.2 and 18.75% in the 1st, 2nd, 3rd and 4th weeks post challenge, respectively (Table 2).

Chickens in both vaccinated groups suffered from mild white diarrhea, with pm lesions included slight enteritis. Chickens in the control group were suffered from profuse white watery diarrhea, depression and the birds were reluctant to move. The pm lesions included enteritis, cecal core, swelling of the liver, spleen and gallbladder with small necrotic foci in the liver. In some cases the pericardium was turbid and covered with yellowish white materials.

Evaluation of the Developed Humoral Immunity Against Salmonella Enteritidis and Salmonella Typhimurium in the Vaccinated Chickens: Micro-Agglutination Test: The GMT titer against Salmonella Enteritidis and Salmonella Typhimurium of both local and imported vaccines increased from (0), pre-vaccination level, to 113 and 92, against Salmonella Enteritidis and to 160 and 121, against Salmonella Typhimurium, at the 3rd week after the primary immunization in local and imported vaccines respectively. Moreover, a gradual increase was shown post boosting till reach 197 and 160, against Salmonella Enteritidis, compared with 215 and 160, against Salmonella Typhimurium, at the 3rd week post boosting, respectively. After challenge, the antibody titer had increased in in both groups vaccinated with local and imported vaccines reaching 299 and 260, against Salmonella Enteritidis, while reaching 394 and 320, against Salmonella Typhimurium, at the 3rd week post challenge, respectively. On the other hand, an abrupt increase of GMT titer was recorded in the control non-vaccinated group, where the titer against both of Salmonella Enteritidis and Salmonella Typhimurium increased from (0) to (35), (70) and (65) at 1st, 2nd and 3rd weeks post challenge, respectively (Fig. 1 & 2).

Tube-Agglutination test: The GMT titer against Salmonella Enteritidis and Salmonella Typhimurium of both local and imported vaccines increased from (0), pre-vaccination level, to 113 and 92, against Salmonella Enteritidis and to 160 and 121, against Salmonella Typhimurium, at the 3rd week after the primary immunization in local and imported vaccines, respectively. Moreover, a gradual increase was shown post boosting till reach 197 and 160, against Salmonella Enteritidis, compared with 215 and 160, against Salmonella Typhimurium, at the 3rd week post boosting, respectively. After challenge, the antibody titer had increased in in both groups vaccinated with local and imported vaccines reaching 299 and 260, against Salmonella Enteritidis, while reaching 394 and 320, against Salmonella Typhimurium, at the 3rd week post challenge, respectively. On the other hand, an abrupt increase of GMT titer was recorded in the control non-vaccinated group, where the titer against both of Salmonella Enteritidis and Salmonella Typhimurium increased from (0) to (35), (70) and (65) at 1st, 2nd and 3rd weeks post challenge, respectively (Fig. 3 & 4).

ELISA Test: The antibody titer against Salmonella Enteritidis and Salmonella Typhimurium of both local and imported vaccines increased from 162.8, pre-vaccination level, to 843.5 and 595.5, against Salmonella Enteritidis and to 1351.5 and 941.7, against Salmonella Typhimurium, at the 3rd week after the primary immunization, respectively. Moreover, a gradual increasing was shown post boosting till reach 2232.2 and 1611.4, against Salmonella Enteritidis, compared with 2712.9 and 2213.4, against Salmonella Typhimurium, at the 3rd week post boosting in both groups vaccinated with local and imported vaccines, respectively. After challenge, the antibody titer had increased in both vaccinated groups reaching 2247.5 and 1416 against Salmonella Enteritidis, while reaching 2539.3 and 2213.4, against Salmonella Typhimurium, at the 3rd week of challenge in both groups vaccinated with local and imported vaccines, respectively. On the other hand, an abrupt increase of antibody titer was recorded in the control non-vaccinated group, where the antibody titer against Salmonella Enteritidis and
Table 1: Protective efficacy of local and imported mineral oil adjuvanted vaccines in chickens challenged with virulent *S. Enteritidis* and *S. Typhimurium* strains

| Route of challenge | Chicken groups | No. of birds | 1st week | 2nd week | 3rd week | 4th week | Dead/Total | Survived/Total | Mortality rate | Protection%st | Protection%nd | Protection%rd | Protection%th |
|--------------------|----------------|--------------|----------|----------|----------|----------|------------|--------------|----------------|---------------|---------------|---------------|---------------|---------------|
| Orally             | Local vaccine  | 80           | 6        | 2        | 0        | 0        | 8/80       | 72/80        | 10%            | 90%           |               |               |               |               |
|                    | Imported vaccine | 80          | 8        | 4        | 0        | 0        | 12/80      | 68/80        | 15%            | 85%           |               |               |               |               |
|                     | Control group   | 80           | 24       | 28       | 12       | 0        | 64/80      | 16/80        | 80%            | 20%           |               |               |               |               |

*Protection % = (Survived × 100)/total number*

Table 2: Results of fecal shedding from vaccinated chickens by local and imported vaccines after the challenge with virulent *Salmonella* strains:

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local vaccine group</td>
<td>10/74 (13.5%)</td>
<td>4/72 (5.5%)</td>
<td>1/72 (1.4%)</td>
<td>0/72 (0%)</td>
</tr>
<tr>
<td>Imported vaccine group</td>
<td>14/72 (19.4%)</td>
<td>9/68 (13.2%)</td>
<td>5/68 (7.3%)</td>
<td>0/68 (0%)</td>
</tr>
<tr>
<td>Control non vaccinated group</td>
<td>47/56 (83.9%)</td>
<td>20/28 (71.4%)</td>
<td>9/16 (56.2%)</td>
<td>3/16 (18.75%)</td>
</tr>
</tbody>
</table>

Fig. 1: Geometric means of antibody titer against *Salmonella Enteritidis* in sera of chicken vaccinated with local and imported mineral oil adjuvanted vaccines measured by micro-agglutination test

Fig. 2: Geometric means of antibody titer against *Salmonella Typhimurium* in sera of chicken vaccinated with local and imported mineral oil adjuvanted vaccines measured by micro-agglutination test

Fig. 3: Geometric means of antibody titer against *Salmonella Enteritidis* in sera of chicken vaccinated with local and imported mineral oil adjuvanted vaccines measured by tube-agglutination test

Fig. 4: Geometric means of antibody titer against *Salmonella Typhimurium* in sera of chicken vaccinated with local and imported mineral oil adjuvanted vaccines measured by tube-agglutination test
Table 3: Comparative results of overall mean of the different tests used for evaluation of both local and imported mineral oil adjuvated vaccines

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Protection %</th>
<th>Micro-agglutination test</th>
<th>Tube-agglutination test</th>
<th>ELISA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre S.E</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>162.8</td>
</tr>
<tr>
<td>Pre S.T</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>162.8</td>
</tr>
<tr>
<td>Local vaccine</td>
<td>90%</td>
<td>171</td>
<td>197</td>
<td>2232.2</td>
</tr>
<tr>
<td>S.E</td>
<td>211</td>
<td>215</td>
<td>2712.9</td>
<td></td>
</tr>
<tr>
<td>S.T</td>
<td>160</td>
<td>160</td>
<td>1611.4</td>
<td></td>
</tr>
<tr>
<td>Imported vaccine</td>
<td>85%</td>
<td>171</td>
<td>160</td>
<td>2213.4</td>
</tr>
<tr>
<td>S.E</td>
<td>160</td>
<td>160</td>
<td>1611.4</td>
<td></td>
</tr>
<tr>
<td>S.T</td>
<td>0</td>
<td>0</td>
<td>206.3</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>20%</td>
<td>0</td>
<td>0</td>
<td>206.3</td>
</tr>
</tbody>
</table>

S.E: *Salmonella* Enteritidis S.T: *Salmonella* Typhimurium

Comparative results of overall mean of the different tests used for evaluation of both local and imported mineral oil adjuvanted vaccines. Concerning the antibody titers against *Salmonella* Enteritidis at the 3rd week post boostering in chickens vaccinated with local vaccine by micro-agglutination test, tube-agglutination test and ELISA were 171, 197 and 2232.2, respectively, compared with 160, 160 and 1611.4, respectively in chickens vaccinated with imported vaccine, while in control non-vaccinated group, the antibody titers during 1st, 2nd and 3rd week post boostering were 0, 0 and 206.3, respectively in both local and imported vaccines (Table 3).

On the other hand, the antibody titers against *Salmonella* Typhimurium at the 3rd week post boostering in chickens vaccinated with local vaccine by micro-agglutination test, tube-agglutination test and ELISA were 211, 215 and 2712.2, respectively, compared with 171, 160 and 2213.4, respectively in chickens vaccinated with imported vaccine. On the other hand, in control non vaccinated group, the antibody titers at the 3rd week post boostering were 0, 0 and 206.3, respectively in both local and imported vaccines (Table 3).

**DISCUSSION**

*Salmonellae* are responsible for considerable losses in the poultry industry through the death of birds and loss in production and it is estimated to cost poultry farmers in some countries like the United States of America up to 114 million US$ annually [14, 15]. Globally avian salmonellosis is also associated with massive public health problems. In August (2010) in USA 380 million eggs had been condemned due to avian salmonellosis.
caused by S. Enteritidis in addition; hundreds of people have been sickened in a Salmonella outbreak linked to infected eggs.

Salmonella are facultative intracellular pathogens causing localized or systemic infections, in addition to chronic asymptomatic carrier state. Domestic poultry constitutes the single largest reservoir of Salmonella serovars. Perales and Audicana [16] reported that the number of Salmonella infected poultry flocks and human beings has been increased substantially in several countries. Although more than 2000 Salmonella serovars have been identified worldwide, only about a dozen serovars accounting for more than 65% of the isolates reported from human beings and poultry [17].

In the 20th century, S. Typhimurium has been recognized as the most wide range host adaptable Salmonella species. In 1982, noticeable increase (27%) in S. Enteritidis infection in human beings was observed (3248 isolates compared with 2554 isolates in 1981). Further increases in S. Enteritidis infection in human beings have been reported recently (5549 isolations in 1985 and 6952 in 1987) according to Barbour et al. [18].

The costs or impracticality of improvements in hygiene and management together with the increasing problems of antibiotic resistance suggest that vaccination in poultry will become more attractive as an adjunct to the existing control measures. Vaccination appears to be the most specific control measure and has contributed in the eradication of S. Enteritidis and S. Typhimurium [19]. For this reason considerable efforts have been made to develop Salmonella vaccine, which would induce protective immunity in chickens and reduce the public health hazards [20].

Evaluation of the protective value of the locally prepared and imported vaccines formulations was performed by applying the challenge test according to Paiva et al. [10]. This test is considered the master test for determination of the protective value of a vaccine [4].

The protective value against virulent Salmonella strains; post oral challenge, in chickens vaccinated with the locally prepared vaccine reached to (90%) which was higher than the protection value in chickens vaccinated with imported vaccine (85%). The achieved protection values by both vaccine formulations are accepted to pass the vaccine for use according to Heddleston [21] and Egyptian Veterinary Codex- CLEVB [22]. The protective value of the locally prepared vaccine (contained local isolates) was higher than that of the imported one and these results are in agreement with that reported by Haider et al. [13].

Fecal shedding of Salmonella organisms in the 1st group of chickens (vaccinated with locally prepared vaccine) reached 1.4 % which was lower than that in the 2nd group (vaccinated with the imported vaccine); 7.3% while the non-vaccinated control group at 3rd week post challenge revealed fecal shedding of 56.2 %. Similar fecal shedding rates were reported by Khamis [23] and Sayd [24].

Concerning the locally prepared mineral oil adjuvant vaccine, the geometric mean titer (GMT) of micro-agglutination test against S. Enteritidis and S. Typhimurium reached to 121 and 160 after the 3rd week from the primary vaccination, respectively, while it reached 171 and 211 after 3rd week post booster dose, respectively. On the other hand the imported mineral oil adjuvant vaccine produced GMT of 61 and 121 for micro-agglutination test against S. Enteritidis and S. Typhimurium after the 3rd week from the primary vaccination, respectively and 160 and 171 after 3rd week post booster dose, respectively. These results coincide with that proved by Nagraja et al. [17], Khamis [23] and Gast and Beard [25].

The GMT of tube-agglutination test against S. Enteritidis and S. Typhimurium in locally prepared vaccine reached 113 and 160 after the 3rd week from primary vaccination, respectively and 197 and 215 after 3rd week post booster, respectively. While the imported vaccine, produced GMT of tube-agglutination test against S. Enteritidis and S. Typhimurium of 92 and 121 after the 3rd week from the primary vaccination, respectively and 160 and 160 after 3rd week post boostering, respectively. Similar results about the enhancement of anti-Salmonella antibodies production by the use of mineral oil adjuvants have been reported by Nakamura et al. [26] who recommended the use of formalin inactivated oil emulsion vaccine for protection against S. Enteritidis infection in chickens as they observed a high titer of anti-S. Enteritidis antibodies by the tube agglutination test using a specific O-antigen prepared from the same Salmonella strain to make the bacterin. Williams and Whitemore [27] and Kumar et al. [28] found that the tube agglutination test and serum plate agglutination test are superior in detecting S. Typhimurium and Arizona hinshawii infection in chickens and turkeys, respectively.

The ELISA antibody titers against S. Enteritidis and S. Typhimurium reached 834.5 and 1351.5 after the 3rd week from primary vaccination with the locally prepared vaccine respectively and 2232.2 and 2712.9 after the 3rd week post boostering, respectively. While, the imported vaccine produced ELISA antibody titers against S. Enteritidis and
Salmonella Typhimurium of 595.5 and 941.7 after the 3rd week from the primary vaccination, respectively and of 1611.4 and 2213.4 after 3rd week post boosting, respectively. These results coincide with those obtained by several authors [5, 6, 29].

In conclusion, it is deduced that the difference in the effect of the local and the imported mineral oil adjuvant vaccines depend on the immune response in chickens after vaccination and challenge with higher antibody response in the local vaccine than the imported one. This may be referred to the fact that the local vaccine was produced by locally isolated Salmonella Enteritidis and Salmonella Typhimurium strains and this point and other points need more investigations.

REFERENCES


