Protective Efficacy of Salmonella Local Strains Representing Groups B, C, D and E in a Prepared Polyvalent Formalin Inactivated Oil Adjuvant Vaccine in Layers

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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 local strains (S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis) and imported ones (S. Typhimurium and S. Enteritidis) in a prepared polyvalent and bivalent formalin inactivated oil adjuvant vaccines had been studied. A total of 150, six-weeks old SPF Lohmann layer chickens were divided into 3 groups; 50 chickens each. The 1st group was vaccinated with the polyvalent locally prepared vaccine, the 2nd group was vaccinated with the imported bivalent vaccine and the 3rd group was kept unvaccinated as a control group. The three groups were challenged with virulent S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis strains (10^6 CFU/ml of each) 1ml orally, 3 weeks post boosting of the vaccines. The degree of protection was assessed according to the severity of the clinical signs, the mortality and fecal shedding of the challenged organisms. Blood samples were collected weekly and humoral immune response was measured against Salmonella strains using micro-agglutination test (MAT) and ELISA. In Conclusion: the locally prepared polyvalent vaccine induced higher protection rates in challenge test with reduced fecal shedding and higher antibody response compared with the imported bivalent one.

Key words: Salmonella Typhimurium · Salmonella Infantis · Salmonella Enteritidis · Salmonella Meleagridis · Vaccines · Layers

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. A total of 51 strains of salmonella were isolated from four broiler chicken flocks in Kalubia governorate, Egypt. The serotypes were 19 S. Enteritidis (37.25%); 10 S. Infantis (19.60%); 4 S. Kentucky (7.84%); 1 S. Chiredzi (1.96%); 15 S. Typhimurium (29.41%) and 2 S. Tsevie (3.92%) [3].

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 harbored by poultry 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
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 [5-7].

The present work aimed to evaluate the immunizing and protective efficacy of *Salmonella* formalin inactivated oil adjuvant vaccines that was prepared from local strains and imported ones. Evaluation was conducted by monitoring the humoral immune response in sera and eggs by micro agglutination test and ELISA. In addition, determination of the fecal shedding of virulent *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* from the immunized layer chickens following challenge was studied.

**MATERIALS AND METHODS**

**Salmonella Local Field Strains:** *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* are four local field strains that represent groups B, C, D and E, respectively. These four strains were kindly obtained from department of Bacterial Sera and Antigens Research, Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. These four strains were used for preparation of vaccine under test.

**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 of *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* were kindly supplied by the Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. These antigens were used for evaluation of the immunizing and protective efficacy of *Salmonella* formalin inactivated oil adjuvant vaccines that was prepared from local strains and imported ones.

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

**Preparation of the Local Vaccine:** Bulk cultures from *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* were prepared according to Charles *et al.* [8]. A separate final suspension from each of the selected strains was prepared and adjusted to 10⁹ CFU/0.5ml (vaccinal dose) of each according to Read and Muench [9]. Inactivation of vaccine strains was performed by addition of formaldehyde solution 37% to the bacterial suspension to obtain a final concentration of 0.3%. The inactivation was carried out under stirring for 24hs at 37°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 vaccinal doses was calculated by total colony count technique before inactivation and centrifuged at 5000 r.p.m. for 20 minutes at 4°C. Then, the supernatant was discarded and the bacterial cell pellets were collected. The 50 ml of the final content containing 500 doses of the 4 inactivated *Salmonella* strains was gently and thoroughly mixed with 4% tween80. This watery phase of the vaccine was emulsified with 200 ml of the oily phase (Mineral oil adjuvant+span80) according to Stone *et al.* [10] to form a total of 250 ml containing 500 doses from each of the vaccinal immunogens (1 vaccinal dose / 0.5 ml). Thiomersal was added as a preservative in a concentration of 0.05mg /liter.

**Quality Control Tests of the Prepared Vaccine:**
The prepared *Salmonella* vaccine was tested for purity, complete inactivation, sterility and safety according to the Standard International Protocols as described by the British Veterinary Codes [11] as follows:

**Purity Test:** The test was done before formalin inactivation of *Salmonella* strains. It was applied to confirm that the broth culture of *Salmonella* strains did not contain any contamination by other organisms before inactivation. Such purity was detected by inoculation of the broth culture onto Salmonella Shigella (S. S.) agar and incubated at 37°C for 24 hr. Appearance of pure colonies of *Salmonella* and pure *Salmonella* organism after Gram staining of the organism indicated culture purity.

**Completion of Salmonella Strains Inactivation:**
In assurance that the used *Salmonella* organisms were completely inactivated, S. S agar media was inoculated with formalin inactivated bacteria. After 24-48 hrs of incubation at 37°C, no visible growth of *Salmonella* indicated complete inactivation of the organism.

**Sterility Test:** The prepared *Salmonella* vaccine was confirmed to be free from any fungal contaminants by inoculation onto Sabouraud Dextrose Agar (SDA) plates.
and incubated at 25°C for 7 days. Also the vaccine was inoculated on Pleuropneumonia Like Organism (PPLO) broth tubes and agar plates and incubated at 37°C for 72 hrs and 14 days, respectively in CO2 incubator to ensure the freedom of the vaccine from mycoplasma organisms.

**Safety Test:** Ten, day old broiler chicks were inoculated intramuscularly (I/M) with a large dose of the prepared bacterin (ten fold the normal vaccine dose). The chicks were observed daily for 7 successive days for any signs of local reactions, clinical signs or deaths.

**Experimental Design:** A total of 150, six-weeks old SPF Lohmann layer chickens were divided into 3 groups; 50 chickens each. The first group of chickens was vaccinated with the local polyvalent oil adjuvant vaccine and the second group was vaccinated with the imported commerical oil adjuvant vaccine, while the third group was used as a control (non-vaccinated). The chicks 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 were challenged three weeks after the booster dose by oral administration of 1ml from each S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis virulent strains suspension containing 10^4 CFU/ml. The inoculated chickens were observed for one month [12]. 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 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 materials. Chickens in both vaccinated groups suffered from mild white diarrhea, with slight lesions of 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 Humoral Immune Response Against the Local Vaccinal Strains in the Vaccinated Layers:** The developed humoral immune response against S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis in the vaccinated chickens was measured in the sera using the micro-agglutination test according to Brown et al [15] and ELISA according to Haider et al. [16].

The antibody titer in MAT was expressed as Geometric Mean Titer (GMT). Calculation of the antibody titers was performed In ELISA; the antibody titer was calculated in relation to S/P ratio according to the following formulae:

\[
S/P \text{ratio} = \frac{\text{samplemean} - \text{Negativecontrol}}{\text{positivecontrol} - \text{Negativecontrol}}
\]

**Calculation of Antibody Titer:** \(\log^{10} \text{Titer} = 1.13 \log 10 (S/P) + 3.156.\)  
AntiLog = Antibody titer

**RESULTS**

**Protective Efficacy of the Local and Imported Oil Adjuvant Vaccines:** The protection rate of the locally prepared polyvalent vaccine was 86% in comparison with the imported vaccine was 76% after 4 weeks post challenge (Table 1).

**Fecal Shedding of Salmonellae from Vaccinated Challenged Layers:** The re-isolation rates of salmonellae from chickens vaccinated with the polyvalent locally prepared vaccine in the 1st, 2nd and 3rd weeks post challenge were 12.76, 9.3 and 4.6%, respectively compared to 28.5, 21 and 10.5%, respectively in those vaccinated with the imported vaccine. In the 4th week the fecal shedding disappeared in both groups (Table 2).

**Evaluation of Humoral Immune Responses in the Vaccinated Layers:**

**Micro-Agglutination Test:** The GMT in sera of chickens vaccinated with local and imported vaccine increased from (0), pre-vaccination level, to 64 and 60, against Salmonella Typhimurium, to 63 and 0, against Salmonella Infantis, to 66 and 67, against Salmonella Enteritidis and to 68 and 0, against Salmonella Meleagridis, respectively.
Table 1: Protective efficacy of local polyvalent and imported vaccines in layers challenged with virulent \textit{S. Typhimurium}, \textit{S. Infantis}, \textit{S. Enteritidis} and \textit{S. Meleagridis} strains

<table>
<thead>
<tr>
<th>Chicken groups</th>
<th>Total No. of birds</th>
<th>1\textsuperscript{st} week</th>
<th>2\textsuperscript{nd} week</th>
<th>3\textsuperscript{rd} week</th>
<th>4\textsuperscript{th} week</th>
<th>Dead/Total</th>
<th>Survive/Total</th>
<th>Mortality rate</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local vaccinated group (A)</td>
<td>50</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>7/50</td>
<td>43/50</td>
<td>14%</td>
<td>86%</td>
</tr>
<tr>
<td>Imported vaccinated group (B)</td>
<td>50</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>12/50</td>
<td>38/50</td>
<td>24%</td>
<td>76%</td>
</tr>
<tr>
<td>Control non vaccinated group (C)</td>
<td>50</td>
<td>15</td>
<td>18</td>
<td>7</td>
<td>0</td>
<td>40/50</td>
<td>10/50</td>
<td>80%</td>
<td>20%</td>
</tr>
</tbody>
</table>

*Protection % = (Survival birds/ total number of birds) X100

Table 2: Results of \textit{salmonellae} fecal shedding from vaccinated chickens by local and imported after the challenge with virulent \textit{Salmonella} strains

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>1\textsuperscript{st} week</th>
<th>2\textsuperscript{nd} week</th>
<th>3\textsuperscript{rd} week</th>
<th>4\textsuperscript{th} week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local vaccinated group (A)</td>
<td>6/47 (12.76%)</td>
<td>4/43 (9.3%)</td>
<td>2/43 (4.6%)</td>
<td>0/43 (0%)</td>
</tr>
<tr>
<td>Imported vaccinated group (B)</td>
<td>12/42 (28.5%)</td>
<td>8/38 (21%)</td>
<td>4/38 (10.5%)</td>
<td>0/38 (0%)</td>
</tr>
<tr>
<td>Control non vaccinated group (C)</td>
<td>19/35 (54.2%)</td>
<td>6/17 (35.29%)</td>
<td>2/10 (20%)</td>
<td>1/10 (10%)</td>
</tr>
</tbody>
</table>

Table 3: Results of Micro-agglutination test for measurement of antibody against \textit{Salmonella} in sera of layers vaccinated with local and imported vaccines

<table>
<thead>
<tr>
<th>Intervals</th>
<th>3\textsuperscript{rd} week post 1\textsuperscript{st} vaccination</th>
<th>3\textsuperscript{rd} week post boosting</th>
<th>3\textsuperscript{rd} week post challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>S. \textit{T}</td>
<td>S. \textit{I}</td>
<td>S. \textit{E}</td>
</tr>
<tr>
<td>Local vaccinated group (A)</td>
<td>0</td>
<td>64</td>
<td>63</td>
</tr>
<tr>
<td>Imported vaccinated group (B)</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Control non vaccinated group (C)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\textit{a=} \textit{Salmonella Typhimurium} \textit{b=} \textit{Salmonella Infantis} \textit{c=} \textit{Salmonella Enteritidis} \textit{d=} \textit{Salmonella Meleagridis}

at the 3\textsuperscript{rd} week after the primary immunization. Moreover, a gradual increase was shown post boosting till reached to 178 and 165, against \textit{Salmonella Typhimurium}, 176 and 0, against \textit{Salmonella Infantis}, 177 and 163, against \textit{Salmonella Enteritidis} and 175 and 0, against \textit{Salmonella Meleagridis}, at the 3\textsuperscript{rd} week post boosting, respectively.

After challenge, the antibody titer had increased in both groups vaccinated with local and imported vaccines reaching 275 and 225, against \textit{Salmonella Typhimurium}, 275 and 0, against \textit{Salmonella Infantis}, 271 and 228, against \textit{Salmonella Enteritidis} and 270 and 65, against \textit{Salmonella Meleagridis}, at the 3\textsuperscript{rd} week after challenge, respectively (Table 3).

On the other hand, an abrupt increase of GMT was recorded in the control non-vaccinated group, where the titer against \textit{Salmonella Typhimurium}, \textit{Salmonella Infantis}, \textit{Salmonella Enteritidis} and \textit{Salmonella Meleagridis} from (0) to (65), (65), (65) and (65) at 3\textsuperscript{rd} week post challenge, respectively (Table 3).

\textbf{ELISA Test}: The ELISA antibody titer in sera of chickens vaccinated with local and imported vaccine was 839.5 and 595.5, against \textit{Salmonella Typhimurium}, 843.6 and 152.3, against \textit{Salmonella Infantis}, 847.2 and 599.2, against \textit{Salmonella Enteritidis} and to 847.5 and 155.2, against \textit{Salmonella Meleagridis}, at the 3\textsuperscript{rd} week after the primary immunization, respectively. Moreover, a gradual increasing was shown post boosting till reach to 2249.2 and 1611.4, against \textit{Salmonella Typhimurium}, to 2252.5 and 209.1, against \textit{Salmonella Infantis}, to 2255.6 and 1617.1, against \textit{Salmonella Enteritidis} and to 2259.3 and 201.5, against \textit{Salmonella Meleagridis}, at the 3\textsuperscript{rd} week post boosting in both groups vaccinated with local and imported vaccines, respectively. After the 3\textsuperscript{rd} week of challenge, the antibody titer had increased in both vaccinated groups reaching to 2265.5 and 1439, against \textit{Salmonella Typhimurium}, to 2269.7 and 895.5, against \textit{Salmonella Infantis}, to 2269.1 and 1440, against \textit{Salmonella Enteritidis} and to 2269.1 and 847.5.
Table 4: Results of ELISA for measurement of antibody against *Salmonella* in sera of layers vaccinated with local and imported vaccines

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Geometric mean antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3\textsuperscript{rd} week post 1\textsuperscript{st} vaccination</td>
</tr>
<tr>
<td>Local vaccinated group (A)</td>
<td>S. T\textsuperscript{t}</td>
</tr>
<tr>
<td>Imported vaccinated group (B)</td>
<td>839.5</td>
</tr>
<tr>
<td>Control non vaccinated group (C)</td>
<td>155.3</td>
</tr>
</tbody>
</table>

a= *Salmonella* Typhimurium b= *Salmonella* Infantis
c= *Salmonella* Enteritidis d= *Salmonella* Meleagridis

The GMT for Local formalized oil adjuvant and imported oil adjuvant vaccine at the 3\textsuperscript{rd} w post boosting by micro-agglutination test against *S. Typhimurium* were 173 and 165 respectively. While against *S. Infantis* were 173 and 0 respectively. While against *S. Enteritidis* were 173 and 163 respectively. While against *S. Meleagridis* were 173 and 0 respectively. While by ELISA test against *S. Typhimurium* were 2246.2 and 1614.4 respectively. While against *S. Infantis* were 2253.7 and 212.4 respectively. While against *S. Enteritidis* were 2257.4 and 1613.6 respectively. While against *S. Meleagridis* were 2249.3 and 203.5 respectively. The protection rates after 28 days post challenge orally with the virulent strains of *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* protection rates were 86% and 76% for Local formalized oil adjuvant vaccine and imported oil adjuvant vaccine respectively (Table 6).

899, against *Salmonella* Meleagridis, in both groups vaccinated with local and imported vaccines, respectively (Table 4). On the other hand, an abrupt increase of antibody titer was recorded in the control non-vaccinated group, where the antibody titer was 895.5, against *Salmonella* Typhimurium, 892.3, against *Salmonella* Infantis, 897.2, against *Salmonella* Enteritidis and 891.2, against *Salmonella* Meleagridis, at the 3\textsuperscript{rd} week of challenge (Table 4).

The ELISA antibody titer against *S. Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* of both local and imported vaccines in eggs of vaccinated layer chickens was 2244.3, 2250.1, 2269.1 & 2255.3 and 1769.1, 872, 1737.3 and 772, respectively. While in control non vaccinated group (C) it was 178.2, 188.9, 184.9 and 194.2, respectively (Table 5).

Table 5: Results of ELISA for measurement of antibody against *Salmonella* in eggs of layer chickens vaccinated with local and imported vaccines

<table>
<thead>
<tr>
<th>Strains</th>
<th>Geometric-mean antibody titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. T\textsuperscript{t}</td>
</tr>
<tr>
<td>Local vaccinated group (A)</td>
<td>2244.3</td>
</tr>
<tr>
<td>Imported vaccinated group (B)</td>
<td>1769.1</td>
</tr>
<tr>
<td>Control non vaccinated group (C)</td>
<td>178.2</td>
</tr>
</tbody>
</table>

a= *Salmonella* Typhimurium b= *Salmonella* Infantis
c= *Salmonella* Enteritidis d= *Salmonella* Meleagridis

Table 6: Comparative results of overall means of the different tests used for evaluation of both local and imported vaccines in layers

<table>
<thead>
<tr>
<th>Type of the vaccine</th>
<th>Protection %</th>
<th>Micro-agglutination Test</th>
<th>ELISA test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. T\textsuperscript{t}</td>
<td>S. P</td>
<td>S. E\textsuperscript{t}</td>
</tr>
<tr>
<td>Local vaccine</td>
<td>86%</td>
<td>178</td>
<td>176</td>
</tr>
<tr>
<td>Imported vaccine</td>
<td>76%</td>
<td>165</td>
<td>0</td>
</tr>
<tr>
<td>Control non vaccinated group</td>
<td>20%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a= *Salmonella* Typhimurium b= *Salmonella* Infantis
c= *Salmonella* Enteritidis d= *Salmonella* Meleagridis
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 [17, 18]. In terms of the loss to producers annually, it is difficult to estimate, however any strategies which reduce the incidence of salmonellosis in poultry are clearly important to all facts of the industry. Reducing Salmonella incidence has become monitored and regulated by Food Safety and Inspection Service [19]. Perales and Audicana [20] 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 [21].

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 more recently (5549 isolations in 1985 and 6952 in 1987) according to Barbour et al. [22]. 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 [23]. 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 [24]. European Food Safety Authority (EFSA, 2010) reported that the most frequently isolated salmonella serovars in broiler chickens were, respectively in decreasing order, S. Infantis (29.2% of the Salmonella positive broiler carcass samples), S. Enteritidis (13.6%), S. Kentucky (6.2%) and S. Typhimurium (4.4%) [24].

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. [12]. This test is considered the master test for determination of the protective value of a vaccine [5]. The protective value against virulent Salmonella strains; post oral challenge, in chickens vaccinated with the locally prepared vaccine reached to (86%) which was higher than the protection value in chickens vaccinated with imported vaccine (76%). The achieved protection values by both vaccine formulations are accepted to pass the vaccine for use according to Heddleston [25] and Egyptian Veterinary Codex- CLEVB [26]. In the present study, 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. [16]. Fecal shedding of Salmonella organisms in the 1st group of chickens (vaccinated with locally prepared vaccine) reached 4.6% which was lower than that in the 2nd group (vaccinated with the imported vaccine); 10.5% while the non-vaccinated control group at 3 week post challenge revealed fecal shedding of 20%. Similar fecal shedding rates were reported by Mohamed [6] and Sayed [27]. Concerning the locally polyvalent prepared oil adjuvant vaccine, The GMT titer against S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis of both local and imported vaccines increased from (0), pre-vaccination level, to 64 and 60, against Salmonella Typhimurium, to 63 and (0), against Salmonella Infantis, to 66 and 67, against Salmonella Enteritidis and to 68 and (0), against Salmonella Meleagridis at the 3rd week after the primary immunization in local and imported vaccines, respectively. Moreover, a gradual increase was shown post boosting till reach to 178 and 165, against Salmonella Typhimurium, 176 and (0), against Salmonella Infantis, 177 and 163, against Salmonella Enteritidis and 175 and (0), against Salmonella Meleagridis, at the 3rd week post boosting, respectively. After challenge, the antibody titer had increased in both groups vaccinated with local and imported vaccines reaching 275 and 225, against Salmonella Typhimurium, 275 and 60, against Salmonella Infantis, 271 and 228, against Salmonella Enteritidis and 270 and 65, against Salmonella Meleagridis, at the 3rd week of challenge, respectively. These results coincide with that proved by Nagraja et al. [21], Mohamed [6] and Gast and Beard [28]. The ELISA antibody titer against S. Typhimurium, S. Infantis, S. Enteritidis and S. Meleagridis of both local and imported vaccines was 839.5 and 599.5, against Salmonella Typhimurium, 843.6 and 153.2, against Salmonella Infantis, 847.2 and 599.2, against Salmonella Enteritidis and 847.5 and 155.2, against Salmonella Meleagridis, at the 3rd week after the primary immunization, respectively. Moreover, a gradual increasing was shown post boosting till reach to 2249.2 and 1611.4, against Salmonella Typhimurium, to 2252.5 and 209.1, against Salmonella Infantis, to 2255.6 and
1617.1, against *Salmonella* Enteritidis and to 2259.3 and 201.5, against *Salmonella* Meleagridis, 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 to 2265.5 and 1439, against *Salmonella* Typhimurium, to 2269.7 and 895.5, against *Salmonella* Infantis, to 2267.3 and 1440, against *Salmonella* Enteritidis and to 2269.1 and 899, against *Salmonella* Meleagridis, at the 3rd week of post boosting in both groups vaccinated with local and imported vaccines respectively. These results coincide with those obtained by several authors [6, 7 and 29]. The ELISA antibody titer against *S*. Typhimurium, *S*. Infantis, *S*. Enteritidis and *S*. Meleagridis of both local and imported vaccines in eggs of vaccinated layer chickens was 2244.3, 2250.1, 2269.1 and 2255.3, respectively. While it was 1769.1, 872, 1737.3 and 772, for imported oil adjuvant vaccinated group (B) respectively. While in control non vaccinated group (C) it was 178.2, 188.9, 184.9 and 194.2, respectively.

In conclusion, it is deduced that the difference in the effect of the local and the imported 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* strains and this point and other points need more investigations.

REFERENCES