

## 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 Lohmman layer chickens were divided into 3 groups; 50 chickens each. The 1<sup>st</sup> group was vaccinated with the polyvalent locally prepared vaccine, the 2<sup>nd</sup> group was vaccinated with the imported bivalent vaccine and the 3<sup>rd</sup> 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 ( $10^8$  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

attenuation [4]. 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 (Wellcome, 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  $10^{10}$  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 24hrs 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 Co<sub>2</sub> 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 Lohmman 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 commercial oil adjuvant 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 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<sup>8</sup> 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 examined bacteriologically for shedding of *Salmonellae* according to Hofstad *et al.* [13] and Cruickshank *et al.* [14].

**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:

$$S/P\text{ratio} = \frac{\text{sample mean} - \text{Negative control}}{\text{positive control} - \text{Negative control}}$$

**Calculation of Antibody Titer:**  $\text{Log}^{10} \text{Titer} = 1.13 \text{Log}^{(SP)} + 3.156$ .

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 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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 4<sup>th</sup> week the fecal shedding disappeared in both groups (Table 2).

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 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 *S.Typhimurium*, *S. Infantis*, *S. Enteritidis* and *S. Meleagridis* strains

Chicken groups	Total No. of birds	No. of dead birds / Week post challenge				Dead/Total	Survive/Total	Mortality rate	Protection %
		1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week				
Local vaccinated group (A)	50	3	4	0	0	7/50	43/50	14%	86%
Imported vaccinated group (B)	50	8	4	0	0	12/50	38/50	24%	76%
Control non vaccinated group (C)	50	15	18	7	0	40/50	10/50	80%	20%

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

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

Type of vaccine	No. of birds positive for isolation / total No. of living birds			
	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
Local vaccinated group (A)	6/47 (12.76%)	4/43 (9.3%)	2/43 (4.6%)	0/43 (0%)
Imported vaccinated group (B)	12/42 (28.5%)	8/38 (21%)	4/38 (10.5%)	0/38 (0%)
Control non vaccinated group (C)	19/35 (54.2%)	6/17 (35.29%)	2/10 (20%)	1/10 (10%)

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

Groups	Pre-vaccination	Intervals											
		Geometric mean antibody titers											
		3 <sup>rd</sup> week post 1 <sup>st</sup> vaccination				3 <sup>rd</sup> week post boosting				3 <sup>rd</sup> week post challenge			
		S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>
Local vaccinated group (A)	0	64	63	66	68	178	176	177	175	275	275	271	270
Imported vaccinated group (B)	0	60	0	67	0	165	0	163	0	225	60	228	65
Control non vaccinated group (C)	0	0	0	0	0	0	0	0	0	65	65	65	65

a= *Salmonella Typhimurium* b= *Salmonella Infantis*

c= *Salmonella Enteritidis* d= *Salmonella Meleagridis*

at the 3<sup>rd</sup> week after the primary immunization. Moreover, a gradual increase was shown post boosting till reached 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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 *Salmonella Typhimurium*, *Salmonella Infantis*, *Salmonella Enteritidis* and *Salmonella Meleagridis* from (0) to (65), (65), (65) and (65) at 3<sup>rd</sup> week post challenge, respectively (Table 3).

**ELISA Test:** The ELISA antibody titer in sera of chickens vaccinated with local and imported vaccine was 839.5 and 595.5, against *Salmonella Typhimurium*, 843.6 and 153.2, against *Salmonella Infantis*, 847.2 and 599.2, against *Salmonella Enteritidis* and to 847.5 and 155.2, against *Salmonella Meleagridis*, at the 3<sup>rd</sup> 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 3<sup>rd</sup> week post boosting in both groups vaccinated with local and imported vaccines, respectively. After the 3<sup>rd</sup> week of 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

Table 4: Results of ELISA for measurement of antibody against *Salmonella* in sera of layers vaccinated with local and imported vaccines

Groups	Intervals											
	Geometric mean antibody titers											
	3 <sup>rd</sup> week post 1 <sup>st</sup> vaccination				3 <sup>rd</sup> week post boosting				3 <sup>rd</sup> week post challenge			
	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>
Local vaccinated group (A)	839.5	843.6	847.2	847.5	2249.2	2252.5	2255.6	2259.3	2265.5	2269.7	2267.3	2269.1
Imported vaccinated group (B)	595.5	153.2	599.2	155.2	1611.4	209.1	1617.1	201.5	1439	895.5	1440	899
Control non vaccinated group (C)	155.3	155.7	158.2	157.2	206.3	206.8	209	206.2	895.5	892.3	897.2	891.2

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

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

Groups	strains			
	Geometric-mean antibody titers			
	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>
Local vaccinated group (A)	2244.3	2250.1	2269.1	2255.3
Imported vaccinated group (B)	1769.1	872	1737.3	772
Control non vaccinated group (C)	178.2	188.9	184.9	194.2

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

Type of the vaccine	Protection %	Micro-agglutination Test				ELISA test			
		S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>	S. T <sup>a</sup>	S. I <sup>b</sup>	S. E <sup>c</sup>	S. M <sup>d</sup>
Local vaccine	86%	178	176	177	175	2249.2	2252.5	2255.6	2259.3
Imported vaccine	76%	165	0	163	0	1611.4	209.1	1617.1	201.5
Control non vaccinated group	20%	0	0	0	0	206.3	206.3	209	206.2

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

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<sup>rd</sup> 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).

The GMT for Local formalized oil adjuvant and imported oil adjuvant vaccine at the 3<sup>rd</sup> 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).

## 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 20<sup>th</sup> 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 1<sup>st</sup> group of chickens (vaccinated with locally prepared vaccine) reached 4.6 % which was lower than that in the 2<sup>nd</sup> 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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 595.5, 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 3<sup>rd</sup> 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 3<sup>rd</sup> 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 3<sup>rd</sup> week of challenge 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, For local formalized oil adjuvant vaccinated group (A) GMT 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.

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