# Preparation and Evaluation of Local Polyvalent Inactivated Salmonella Enterica Serovar Enteritidis, Typhimurium and Kentucky Vaccine for Protection Against Salmonellosis in Chickens in Egypt.

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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 one of the best means for controlling salmonellosis in birds. In the present study the immunizing and protective efficacy of a newly locally prepared polyvalent inactivated oil adjuvanted *Salmonella* subspecies enterica serovar *Typhimurium*, *Enteritidis* and *Kentucky* vaccine had been studied. Evaluation of the prepared vaccine were adopted based on the standard regulations and comparison with the commercially available vaccine. This newly locally prepared vaccine was proved to be sterile and safe even if used in double field dose. Also the residues of both merthiolates and formaldhyde were under the standard permissible limit. Potency of the prepared vaccine was evaluated serologically using ELISA and by using challenge test. Challenge test showed 70 % protection when adopted post single dose vaccination raised to 82 % post booster dose vaccination when *Salmonella Typhimurium* virulent strain was used meanwhile it were 72 % and 84 % when virulent *Salmonella Enteritidis* was used and were 74 % and 86 % when *Salmonella Kentucky* virulent strain was used. From the obtained results, the locally prepared vaccine had the advantages over the commercial one allover the experimentation period and it be concluded that the locally prepared polyvalent *Salmonella* vaccine induced significant protection rates with higher antibody response in the vaccinated birds. Also it could be recommended that the production of local vaccine for usage in Egyptian farms is much better than importation of commercial vaccine.

## Introduction:

*Salmonellae* 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 as food born pathogen ***(Little et al., 2007)***. 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.

Avian salmonellosis is the term that designates a large group of acute or chronic bird diseases caused by one or more bacteria of the genus *Salmonella* ***(Gast, 1997)***. These bacteria are rods from the *Enterobacteriace* family, and most of them are motile and have a complex antigenic structure (somatic "O", flagellar "H", and capsular "K" antigens) ***(Barrow, 2000).***

Both *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most important serotypes for salmonellosis transmitted from animals to humans yet, ***(Foley and Lynne, 2008).*** Since in *Salmonella Kentucky* poultry products may be of particular interest because poultry is the main animal reservoir of *Salmonella Kentucky* ***(Molla et al., 2006).*** Successful control of *Salmonella* infections on poultry farms is reliant on good farming and husbandry practices (including all the aspects covering feed, birds, management, cleaning and disinfection, control of rodents, etc) as well as the testing and removal of positive flocks from production ***(EFSA, 2004).*** Vaccination likely to have a central role in the reduction of *Salmonella* in commercial operations and considered a potential option in poultry industry. So the aim of this work is to prepare and evaluate a polyvalent inactivated oil adjuvanted *Salmonella Typhimurium, Enteritidis* and *Kentucky* vaccine from locally isolated and identified strains.

-The aim of the present work is: preparation of the polyvalent inactivated *Salmonella* vaccine using local isolates of *Salmonella Enteritidis*, *Salmonella Typhimurium* and *Salmonella Kentucky* and evaluation of this prepared polyvalent inactivated vaccine.

## Material and Methods

### 1. Strains used:

*Salmonella Typhimurium, Salmonella Enteritidis* and *Salmonella Kentucky* were locally isolated and identified and were obtained from the reference strains bank at the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abassia, Cairo, Egypt.

### 2. Vaccine preparation: ***(Charles et al., 1994):***

Different *Salmonella* strains were grown separately on Salmonella Shigella agar for 24 hr at $37^0$C then

separate colonies were inoculated into tryptose soya broth in a gradual quantities and incubated for 24 hr at $37^0$C. Bacteria were concentrated by centrifugation and the separate final suspension from each was prepared and the count was adjusted $10^9$ CFU/final dose. Inactivation performed under stirring with formaldehyde solution 37% in a 0.2% of final concentration. The inactivated cultures was neutralized with sodium metabisulfite then stored at $4^0$C. The inactivated *Salmonella* strains were gently and thoroughly mixed with 4% tween80. This watery phase of the vaccine then emulsified oily phase (Mineral oil adjuvant (Extra white oil) + span80). Thiomersal was added as a preservative in a concentration of 0.05mg /liter.

**3. Quality control of the prepared vaccine:**

**A. Physical properties:** ***(Stone et al., 1978)***

1. Emulsion type and emulsion stability were performed according to **(Becher, 1957)**.

**B. Purity test:** The test was done before preparation of vaccine according to **(OIE 2016)**

**C. Sterility tests:** These were carried out according to **OIE (2016)** and **Code Federal *Regulation (2013).***

**D. Safety test:** At least 25 SPF chickens, 7-14 days old chicks were inoculated intramuscularly with 1.0 ml (double field dose) of the vaccine under test then kept under observation for 14 days.

**4. Potency Test:** A total of 1080 H & N breed chickens were obtained from private farm, these birds were examined to ensure that they are free from bacterial pathogens and they had neither a history of infection nor vaccination with *Salmonella Enteritidis*, *Salmonella Typhimurium* or *Salmonella Kentucky*. Birds were divided into groups as shown in **Table (1)** to receive the intended regime of treatment according to the planned experimental design of this experiment where it will be vaccinated with either the locally prepared or commercial vaccine, single or booster vaccination then challenged with the virulent different salmonellae and or serologically tested at different intervals post vaccination.

**5. Monitoring the humoral immune response:** The humoral immune response against *Salmonella* antigens in the prepared vaccine was measured by ELISA using Salmonella antibody test kit (Jordan Bio-Industries Center - JOVAC) for *Salmonella Typhimurium* and *Salmonella Enteritidis* and using traditional ELISA for *Salmonella Kentucky* which performed according to ***Briggs and Skeels (1984).***

**6. Challenge test:**

Vaccinated birds were challenged intramuscularly with 0.1 ml containing $1x10^6$ CFU of virulent *Salmonella Typhimurium* and $1x10^7$CFU for each of virulent *Salmonella Enteritidis* and

*Salmonella Kentucky* 3 weeks post single vaccination and booster vaccination assays. Challenged birds were kept under observation for 2 weeks and examined daily for mortality, clinical signs and survived birds were necropsied and examined for the presence of grossly visible lesions. Challenge test is considered valid if 80% or more in the challenged non vaccinated control group showed either mortalities or lesions and the Protective indices (PIs) is calculated according to ***Timms and Marshall (1989)*** [PIs = % (M & PML) control - % (M& PML) vaccinated / (% (M& PML) control] where M is the mortality while PML is the post mortem lesions (PML).

**Table (1):** *Experimental design for the evaluation of locally prepared polyvalent inactivated salmonella vaccine against the commercial vaccine.*

| Groups | Birds No | Treatment |
|---|---|---|
| 1 | 150 | Vaccinated with single dose of locally prepared polyvalent inactivated Salmonella vaccine then challenged with the virulent strains of *S. Typhimurium, S. Enteritidis* and *S. Kentucky* 3 week post-vaccination. |
| 2 | 50 | Vaccinated with single dose of locally prepared polyvalent inactivated Salmonella vaccine then blood samples were collected weekly to follow and monitor humoral immune response post single dose vaccination. |
| 3 | 150 | Vaccinated with booster doses of locally prepared polyvalent inactivated Salmonella vaccine at 3 weeks post first vaccination then challenged with the virulent strains of *S. Typhimurium, S. Enteritidis* and *S. Kentucky* 3 week post boostering. |
| 4 | 50 | Vaccinated with booster dose of locally prepared polyvalent inactivated Salmonella vaccine at 3 weeks post first vaccination then blood samples were collected weekly post-vaccination up to 10 weeks post boostering. |
| 5 | 150 | Vaccinated with single dose of commercial imported inactivated Salmonella vaccine then challenged with the virulent strains of *S. Typhimurium, S. Enteritidis* and *S. Kentucky* 3 week post-vaccination. |
| 6 | 50 | Vaccinated with single dose of commercial imported inactivated Salmonella then blood samples were collected weekly to follow and monitor humoral immune response post single dose vaccination. |
| 7 | 150 | Vaccinated with booster dose of commercial imported inactivated Salmonella vaccine at 3 weeks post first vaccination then challenged with the virulent strains of *S. Typhimurium, S. Enteritidis* and *S. Kentucky* 3 week post boostering. |
| 8 | 50 | Vaccinated with booster dose of commercial imported inactivated Salmonella vaccine at 3 weeks post vaccination then blood samples were taken weekly post-vaccination up to 10 weeks post boostering. |
| 9 | 280 | Divided into groups acting as unvaccinated control group for each previous group. |

## Results

### 1- Quality control of the prepared vaccine:

The newly prepared polyvalent inactivated *Salmonella* vaccine was proved to be stable, pure and as no growth of any aerobic or anaerobic bacteria or fungal growth has been detected. The vaccine was safe and had no adverse side effects on inoculated chickens. The vaccine residue of formalin was less than 0.05 % and the thiomerthal residue was less than 0.02 mg/ml in the prepared vaccine.

### 2. Humoral immune response developed against *Salmonella Typhimurium:*

Humoral immune response in the vaccinated chickens against *Salmonella Typhimurium* was increased gradually and reached the peak at the 7$^{th}$ week post vaccination as shown in table (2). At this point the antibody titers were 467 compared with 453 for both locally prepared and commercial poly valent inactivated vaccines respectively while the immune status of chicken was decreased gradually up to 398 and 402 at 10$^{th}$ weeks post vaccination in the same aforementioned groups.

Regarding the immune response showed post booster vaccination, from table (3), it can clearly be noticed that the maximum level of antibody titers were 612 and 631 and were observed at 6$^{th}$ weeks post boostering respectively.

From 6 up to 11 weeks post boostering, the antibody titer continue at the plateau level showing a slow declining in titers up to 559 and 569 in the same vaccinated chicken groups respectively. The same immune picture was noted when antibody titers against *Salmonella Enteritidis* were measured. As shown in **Table (4)**, the peak of antibody titer was 545 and 557 which achieved at 7$^{th}$ weeks post vaccination with commercial and locally prepared polyvalent *Salmonella* vaccines respectively. The immune status of chicken was decreased gradually up to 470 and 485at 10$^{th}$ weeks post vaccination in the same aforementioned groups. As regards to the immune status corresponding to booster dose vaccination assay, the maximum level 629 and 645 were observed at 6$^{th}$ weeks post boostering, respectively. Also from 6 up to 11 weeks post boostering, the antibody titer continue at the plateau level showing a slow declining in the antibody up to 585 and 590 in the same vaccinated chicken groups respectively as shown in **Table (5)**.

Concerning the humoral immune response obtained against *Salmonella Kentucky*, the peak of antibody titer was 1119 and 1204 which achieved at 7$^{th}$ weeks post vaccination with commercial vaccine and locally prepared polyvalent *Salmonella* vaccine respectively as shown in **Table (6)**. At the same time, after booster vaccination the maximum antibody titers was 1432 and 1449 and were

observed at 7th weeks post boostering, respectively. These titers showing a slow declining in the antibody up to 1312 and 1336 in the vaccinated chicken sera in the vaccinated chicken with *Salmonella* commercial vaccine and the chicken which vaccinated with locally prepared polyvalent *Salmonella* vaccine, respectively. All these results were compared with the negative titers obtained from the unvaccinated chicken group all over the experiment.

**3. Results of vaccination challenge assay:**

Protection and efficacy of the locally prepared vaccine in comparison with the commercial one depend on the challenge test with different virulent *Salmonella* strains. Regarding protection obtained post challenge with virulent *Salmonella Enteritidis* strain in a dose of 1 x $10^7$ CFU/ bird were demonstrated in table (8).

The locally prepared polyvalent *Salmonella* vaccine showed marked and significant protection level more than the control unvaccinated chicken group calculated within two weeks post challenge. The overall protection of 70 with commercial and was 72% for the locally prepared vaccines when challenged 3 weeks post single vaccination while it was increased up to 80 and 84% in chicken vaccinated with commercial and locally prepared vaccines respectively when challenged with *Salmonella Enteritidis* virulent strain 3 weeks post boostering. These results were in parallel to the re-isolation of the challenge strain from the affected birds. As regards to the protection obtained in the vaccinated chickens after challenge with virulent *Salmonella Typhimurium* strain in a dose of 1 x $10^6$, **Table (9)** protection obtained post challenge 3 weeks after single dose vaccination were 70% for both commercial and locally prepared vaccines. These percent raised up to 78 and 82% in chicken vaccinated with commercial and locally prepared vaccines respectively when challenged 3 weeks post boostering. Re-isolation of virulent strains were occurred from all affected birds.

Concerning protection against virulent 1 x $10^7$ CFU/ bird *Salmonella Kentucky*, as shown in **Table (10)**, protection were 72 % and 74% for commercial and locally prepared inactivated vaccines, respectively 3 weeks post single vaccination. While in case of challenge 3 weeks post boosting the protections obtained were increased up to 82 and 86% in chicken vaccinated with commercial and locally prepared inactivated vaccines respectively. Re-isolation of challenge virulent *Salmonella Kentucky* strain were occurred from all affected birds.

All challenge assays were done in unvaccinated control groups parallel to the all challenged vaccinated groups and the protections in these unvaccinated control groups were 20% or less.

**Table (2)** *Salmonella Typhimurium ELISA mean titer of the serum samples of vaccinated chickens with single dose of commercial and locally prepared polyvalent inactivated Salmonella vaccines:*

| Weeks post vaccination | Commercial polyvalent Salmonella vaccine | | Locally prepared polyvalent Salmonella vaccine | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 185 | 50 | 196 | 10 | 80 |
| **2** | 50 | 340 | 50 | 347 | 10 | 85 |
| **3** | 50 | 366 | 50 | 378 | 10 | 78 |
| **4** | 50 | 385 | 50 | 408 | 10 | 90 |
| **5** | 50 | 409 | 50 | 419 | 10 | 67 |
| **6** | 50 | 422 | 50 | 441 | 10 | 72 |
| **7** | 50 | 453 | 50 | 467 | 10 | 95 |
| **8** | 50 | 435 | 50 | 451 | 10 | 68 |
| **9** | 50 | 410 | 50 | 425 | 10 | 84 |
| **10** | 50 | 398 | 50 | 402 | 10 | 70 |

**Table (3)** Salmonella Typhimurium ELISA mean titer of the serum samples of vaccinated chickens with booster dose of commercial and locally prepared polyvalent inactivated Salmonella vaccines:

| Weeks post boostering | commercial polyvalent *Salmonella* vaccine | | Locally prepared polyvalent *Salmonella* | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 460 | 50 | 480 | 10 | 75 |
| **2** | 50 | 485 | 50 | 508 | 10 | 70 |
| **3** | 50 | 512 | 50 | 524 | 10 | 85 |
| **4** | 50 | 535 | 50 | 549 | 10 | 83 |
| **5** | 50 | 558 | 50 | 606 | 10 | 90 |
| **6** | 50 | 612 | 50 | 631 | 10 | 72 |
| **7** | 50 | 608 | 50 | 622 | 10 | 80 |
| **8** | 50 | 590 | 50 | 607 | 10 | 83 |
| **9** | 50 | 582 | 50 | 595 | 10 | 85 |
| **10** | 50 | 575 | 50 | 582 | 10 | 87 |
| **11** | 50 | 559 | 50 | 569 | 10 | 81 |

***Table (4)*** *Salmonella Enteritidis ELISA mean titer of the serum samples of vaccinated chickens with single dose of commercial and locally prepared polyvalent Salmonella vaccines:*

| Weeks post vaccination | commercial polyvalent *Salmonella* vaccine | | Locally prepared polyvalent *Salmonella* vaccine | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 302 | 50 | 315 | 10 | 86 |
| **2** | 50 | 405 | 50 | 420 | 10 | 91 |
| **3** | 50 | 425 | 50 | 431 | 10 | 87 |
| **4** | 50 | 446 | 50 | 462 | 10 | 66 |
| **5** | 50 | 472 | 50 | 492 | 10 | 91 |
| **6** | 50 | 518 | 50 | 540 | 10 | 82 |
| **7** | 50 | 545 | 50 | 557 | 10 | 74 |
| **8** | 50 | 520 | 50 | 542 | 10 | 90 |
| **9** | 50 | 495 | 50 | 503 | 10 | 59 |
| **10** | 50 | 470 | 50 | 485 | 10 | 82 |

**Table (5)** *Salmonella Enteritidis ELISA mean titer of the serum samples of vaccinated chickens with booster dose of commercial and locally prepared polyvalent Salmonella vaccines:*

| Weeks post boostering | commercial polyvalent *Salmonella* vaccine | | Locally prepared polyvalent *Salmonella* vaccine | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 552 | 50 | 561 | 10 | 60 |
| **2** | 50 | 561 | 50 | 582 | 10 | 75 |
| **3** | 50 | 585 | 50 | 599 | 10 | 83 |
| **4** | 50 | 598 | 50 | 614 | 10 | 90 |
| **5** | 50 | 616 | 50 | 623 | 10 | 77 |
| **6** | 50 | 629 | 50 | 645 | 10 | 68 |
| **7** | 50 | 627 | 50 | 640 | 10 | 72 |
| **8** | 50 | 621 | 50 | 636 | 10 | 88 |
| **9** | 50 | 608 | 50 | 622 | 10 | 79 |
| **10** | 50 | 597 | 50 | 608 | 10 | 64 |
| **11** | 50 | 585 | 50 | 590 | 10 | 80 |

**Table (6)** *Salmonella Kentucky ELISA mean titer of the serum samples of vaccinated chickens with single dose of commercial and locally prepared polyvalent inactivated inactivated Salmonella vaccines:*

| Weeks post vaccination | commercial polyvalent *Salmonella* vaccine | | Locally prepared polyvalent *Salmonella* vaccine | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 505 | 50 | 514 | 10 | 108 |
| **2** | 50 | 772 | 50 | 808 | 10 | 124 |
| **3** | 50 | 847 | 50 | 921 | 10 | 100 |
| **4** | 50 | 925 | 50 | 986 | 10 | 101 |
| **5** | 50 | 986 | 50 | 1055 | 10 | 96 |
| **6** | 50 | 1082 | 50 | 1112 | 10 | 107 |
| **7** | 50 | 1119 | 50 | 1204 | 10 | 104 |
| **8** | 50 | 1101 | 50 | 1189 | 10 | 102 |
| **9** | 50 | 1044 | 50 | 1126 | 10 | 106 |
| **10** | 50 | 972 | 50 | 1065 | 10 | 95 |

**Table (7)** *Salmonella Kentucky ELISA mean titer of the serum samples of vaccinated chickens with booster dose of commercial and locally prepared polyvalent inactivated Salmonella vaccines:*

| Weeks post boostering | commercial polyvalent *Salmonella* vaccine | | Locally prepared polyvalent *Salmonella vaccine* | | Control | |
|---|---|---|---|---|---|---|
| | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer | No. of tested birds | ELISA mean titer |
| **1** | 50 | 1185 | 50 | 1226 | 10 | 95 |
| **2** | 50 | 1244 | 50 | 1285 | 10 | 90 |
| **3** | 50 | 1279 | 50 | 1316 | 10 | 98 |
| **4** | 50 | 1318 | 50 | 1379 | 10 | 100 |
| **5** | 50 | 1382 | 50 | 1403 | 10 | 89 |
| **6** | 50 | 1411 | 50 | 1435 | 10 | 95 |
| **7** | 50 | 1432 | 50 | 1449 | 10 | 92 |
| **8** | 50 | 1418 | 50 | 1426 | 10 | 88 |
| **9** | 50 | 1368 | 50 | 1381 | 10 | 90 |
| **10** | 50 | 1312 | 50 | 1336 | 10 | 94 |

**Table (8).** *Results of challenge test against virulent Salmonella Enteritidis strain 3 weeks post single and booster vaccination:*

| Vaccine assay treatment | Single dose vaccine assay | | | Booster dose vaccine assay | | |
|---|---|---|---|---|---|---|
| **Type of vaccine** | **Commercial vaccine** | **Local prepared vaccine** | **control** | **Commercial vaccine** | **Local prepared vaccine** | **control** |
| **No. of tested birds** | 50 | 50 | 20 | 50 | 50 | 20 |
| **Mortalities** | 6 | 4 | 9 | 3 | 3 | 9 |
| **Symptoms** | 9 | 10 | 8 | 7 | 5 | 8 |
| **Total affected** | 15 | 14 | 17 | 10 | 8 | 17 |
| **Re-isolation** | 15 | 14 | 17 | 10 | 8 | 17 |
| **Protection%** | 70 | 72 | 15 | 80 | 84 | 15 |

**Table (9):** *Results of challenge test against virulent Salmonella Typhimurium strain 3 weeks post single and booster vaccination:*

| Vaccine assay treatment | Single dose vaccine assay | | | Booster dose vaccine assay | | |
|---|---|---|---|---|---|---|
| **Type of vaccine** | Commercial vaccine | Local vaccine | control | Commercial vaccine | Local vaccine | control |
| **No. of tested birds** | 50 | 50 | 20 | 50 | 50 | 20 |
| **Mortalities** | 7 | 6 | 10 | 4 | 3 | 10 |
| **Symptoms** | 8 | 9 | 8 | 7 | 6 | 8 |
| **Total affected** | 15 | 15 | 18 | 11 | 9 | 18 |
| **Re-isolation** | 15 | 15 | 18 | 11 | 9 | 18 |
| **Protection%** | 70 | 70 | 10 | 78 | 82 | 10 |

**Table (10)** *Results of challenge test against virulent Salmonella Kentucky strain 3 weeks post single and booster vaccination:*

| Vaccine assay treatment | Single dose vaccine assay | | | Booster dose vaccine assay | | |
|---|---|---|---|---|---|---|
| **Type of vaccine** | **Commercial vaccine** | **Local vaccine** | **control** | **Commercial vaccine** | **Local vaccine** | **control** |
| **No. of tested birds** | 50 | 50 | 20 | 50 | 50 | 20 |
| **Mortalities** | 5 | 4 | 8 | 3 | 2 | 7 |
| **Symptoms** | 9 | 9 | 8 | 6 | 5 | 9 |
| **Total affected** | 14 | 13 | 10 | 9 | 7 | 10 |
| **Re-isolation** | 14 | 13 | 10 | 9 | 7 | 10 |
| **Protection%** | 72 | 74 | 20 | 82 | 86 | 20 |

**Discussion**

*Salmonella* infection in poultry is thought to be responsible for many of the food-borne infections in humans in addition to the economic and production losses. *Salmonella Enteritidis*, *Salmonella Typhimurium* and *Salmonella Kentucky* are presented separately from other serotypes of *Salmonella* because, on the one hand, these bacteria are often specifically cited in zoonosis control legislation, and, secondly, there are differences in the epidemiology as compared to other salmonellae ***(Radford, S.A.; and Board, R.G. 2004).*** Vaccination as part of a *Salmonella* control program contributes to the achievement of *Salmonella* free poultry meat and eggs. Mass poultry vaccination programs introduced to combat *Salmonella* infections have led to a dramatic fall in the number of cases since the late 1990s ***(APA, 2013)***.

The prepared polyvalent inactivated *Salmonella* vaccine adjuvanted with mineral oil was proved to be pure, sterile, safe and safe without adverse side effects on chicken productivity and body weight gain. The vaccine residue of formalin was less than 0.05 % and the thiomerthal residue was less than 0.02 mg/ml. These values are under the permissible limits accepted by **The *Egyptian standards for evaluation of veterinary biologics – CLEVB (2009)***.

Regarding to the vaccine efficacy, potency was evaluated through estimation of humoral immune response and challenge test. From data available in **Table (2)**, it can be seen clearly that the level of antibody titers in chickens vaccinated by *Salmonella Typhimurium* commercial vaccine started as early as the first week post vaccination and increased gradually till reached the peak at the 7$^{th}$ week post vaccination with marked higher titers all over the experiment with the locally prepared polyvalent inactivated vaccine than the commercial one as shown in **Table (2)**. Similar results about the enhancement of anti-salmonella antibody production by the use of mineral oil adjuvants have been reported by ***(Baily et al., 2007)*** who used trivalent autogenous bacterin for serogroups B, C and D and used ELISA to monitor immune response against *Salmonella Typhimurium* and *Salmonella Enteritidis* and concluded that the IgG titer against *Salmonella* was matched with the protections obtained post challenge with the virulent *Salmonella* species. Also ***(Suzette et al., 2014)*** reported that ELISA can demonstrated the specific antibody production after vaccination with two different *Salmonella Typhimurium* vaccines. Booster dose vaccination elevated the antibody titers for both vaccines under evaluation but with significant advantage for the locally prepared one as demonstrated in **Table (3)**. These finding indicated

that the antibody response to *Salmonella Typhimurium* is highly elevated post-boostering taken in consideration that the locally prepared combined vaccine of *Salmonella Typhimurium*, *Salmonella Enteritidis* and *Salmonella Kentucky* was more efficient with higher antibody titers which may provide higher protection rate than others especially under Egyptian environment. The same findings were reported by ***(Nourhan et al., 2015)*** where humoral immune response was measured by ELISA and micro agglutination test. In case of ELISA antibody titers were increased gradually up to the 3rd week and reached its maximum level at the 5th up to the 6th week post-boostering. Also ***(Mona et al., 2013)*** used ELISA with challenge test to study the protective efficacy of prepared polyvalent formalin inactivated oil adjuvant vaccine against *Salmonella Typhimurium*, *Salmonella Enteritidis*, *Salmonella Infantis*, and *Salmonella Meleagridis* in layer chickens.

The immune response developed against *Salmonella Enteritidis* was evaluated post single and booster doses vaccinations. The obtained data illustrated in **Table (4)**, revealed that the peak of antibody titer was 545 and 557 and achieved at 7th weeks post vaccination for commercial and locally prepared vaccines respectively.

As regards to the *Salmonella Enteritidis* antibody responses with booster dose vaccination program, As mentioned in **Table (5)**, there were rapid rise of antibody titers post booster dose and the maximum level 629 and 645 were observed at 6th weeks post boostering, respectively. The period from 6 up to 10 weeks post boostering showed slow declining in the antibody titers up to 585and 590 respectively at the 11th week post boostering. More or less ***(Mona et al., 2013)*** stated that ELISA antibody titer against *Salmonella Enteritidis* was 599.2 with gradual increase at the 3rd week post-boostering in all groups vaccinated with polyvalent formalin inactivated oil adjuvant vaccine in layer chickens. On the other hand, ***(Bailey et al., 2007)*** used the indirect ELISA to assess the immunological response generated by three different *Salmonella* vaccination protocols and concluded that the killed vaccine is vital in eliciting adequate IgG in serum.

Concerning *Salmonella Kentucky* humoral immune response, as illustrated in **Table (6)**, the antibody titers post single dose vaccination was at the peak titer at the level of 1119 and 1204 at 7th weeks post vaccination with commercial vaccine and locally prepared polyvalent *Salmonella* vaccine respectively. At the same time, data in **Table (7)** showed that was rapid rise of antibody titer post boostering in both chicken groups and the maximum antibody titers 1432 and 1449 were observed at 7th

weeks post-boostering, respectively. The same picture was recorded by ***(Nourhan et al., 2015)*** who stated that ELISA antibody titers were raised up to 659 after the 2nd week post primary vaccination while it increased sharply up to 1136 post-boostering. Also these results in agreement with that reported by ***(Okamura et al., 2007)*** as an abrupt increase of antibody titers were recorded post-boostering. In the same concern, ***(Berghaus et al., 2014)*** reported that the vaccinated breeder flocks had significantly higher *Salmonella* specific antibody titers and the broiler flocks originated had significantly lower *Salmonella* prevalence and loads than the broiler flocks that were the progeny of unvaccinated breeder flocks.

As regards to the protection obtained in the vaccinated chickens after challenge with virulent salmonellae post single or booster doses, this test is considered the master test for determination of the protective value of a vaccine ***(Timms et al., 1990).*** The obtained data showed that the vaccinated chickens gave protection rate of 70 and 72% for both commercial and locally prepared vaccines chicken groups when challenged 3 weeks post single dose vaccination while this level of protection raised to 80 and 84% in the same groups respectively 3 weeks post booster vaccination when challenged with the virulent *Salmonella Enteritidis* strain as shown in **Table (8)**. Also challenge with virulent *Salmonella Typhimurium* faced with a protection of 70 and 70% when challenged 3 weeks post single dose vaccination for both vaccines which increased up to 78 and 82% respectively when challenged 3 weeks post booster vaccination as shown in **Table (9)**. At the same time as shown in **Table (10)**, challenging with virulent *Salmonella Kentucky* strain showed over all protection of 72 and 74% for both commercial and locally prepared vaccines 3 weeks post single vaccination raised to 82 and 86% in the same chicken groups respectively 3 weeks post boostering.

The achieved protection values by both vaccine formulations are accepted to pass the vaccine for use according to ***The Egyptian standards for evaluation of veterinary biologics – CLEVB (2009)***. These results are in agreement with that obtained by ***Wendy I et al., (1998)*** who immunized chickens with *Salmonella Typhimurium* vaccine then subsequently challenged them with the virulent strain and concluded to the vaccinated birds showed resistance to infection with virulent *Salmonella Typhimurium* and this was reflected in bacterial infection and shedding. Also ***Meenakshi et al. (1999)*** confirmed that vaccinated birds with killed *Salmonella Enteritidis* vaccine can protect them from challenge with virulent organisms at the end of 2nd

week post booster dose. ***(Timms et al., 1990)*** stated that challenge test is the master test for the determination of *Salmonella Kentucky* vaccine and ***(Nourhan et al., 2015)*** reported that the protective value against virulent *Salmonella Kentucky* post challenge in chickens vaccinated with *Salmonella kentucky* vaccine was 80 % and the achieved protection value by the used vaccine was accepted to pass the vaccine for use according to ***Heddleston (1975)** and **The Egyptian standards for evaluation of veterinary biologics – CLEVB (2009)***. At the same concern, ***(Baily et al., 2007)*** used ELISA to monitor the efficacy of trivalent autogenous bacterin containing *Salmonella Typhimurium* and *Salmonella Enteritidis* and concluded that the IgG titer against *Salmonella* was matched with the protections obtained post challenge with the virulent *Salmonella* species in the corresponding vaccine.

The obtained findings in these study either related to antibody titers or related to protection post challenges, showed a significant advantages for the polyvalent inactivated Salmonella vaccine that prepared from local strains than that commercial one allover the experimentation period with all Salmonellae components in the vaccine either *Salmonella Typhimurium*, *Salmonella Enteritidis* or *Salmonella Kentucky* which prove that the locally prepared vaccine was more efficient and more protective for birds under Egyptian field conditions.

SO, it could be concluded that:

- The locally prepared polyvalent *Salmonella* inactivated vaccine is vital in eliciting adequate antibody titers which aiding in conferring adequate immunity enable birds to pass experimental and so natural infections.
- The locally prepared combination of *Salmonella Typhimurium, Salmonella Enteritidis* and *Salmonella Kentucky* vaccine proved to be of great value because of its high efficacy with a good and significant result in safety and protection when compared with the commercially imported one.
- Vaccination with the locally prepared polyvalent *Salmonella* inactivated vaccine could contribute protection and lower *Salmonella* prevalence in layers, breeders and in turn in broiler chickens.

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## الملخص العربى

# تحضير وتقييم لقاح متعدد العترات للحماية ضد ميكروبات السالمونيلا وكفاءته فى برامج التحصين فى الدواجن

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-الغرض من هذه الدراسة هو تطوير وانتاج لقاح مثبط ومركب من عترات محلية لميكروبات السالمونيلا انتريتيدس و تيفيميوريم و كنتاكى فى الدجاج و التى تؤدى الى خسائر اقتصادية تتمثل فى حالات النفوق وحالات الاصابات وتكلفة العلاج و الاعدامات فى مجازر الدواجن و كذلك تاثيرها على الصحة العامة للانسان.
تم تحضير اللقاح و تقييمة من حيث النقاوة البكتيرية و وجد انه خالى تماما من الملوثات البكتيرية و الفطرية و الميكوبلازما و كذلك عند اختباره للامان وجد انه امن تماما حتى عند استخدامه بجرعة حقلية مضاعفة و كذلك أن نسبة مادة الميرثيولات و الفورمالين باللقاح تحت الحد المسموح بة عالميا
.
تم اختبار اللقاح المحضر للكفاءة المناعية سيرولوجيا و باجراء اختبار التحدى اوضحت النتائج باستخدام اختبار الاليزا ان اعلى مستوى مناعى كان فى الاسبوع السابع بعد التحصين بجرعة واحدة وكان فى الاسبوع السادس بعد التحصين بالجرعة التانية وذلك ضد كل مكونات القاح.
عند تقييم اللقاح المحضر باستخدام اختبار التحدى بواسطة العترات الضارية للميكروبات المكونة له وجد ان نسبة الحماية الناتجة هى 70% فى حالة التحصين بجرعة واحدة ارتفعت الى 82% عند استخدام جرعتين متتاليتين من اللقاح وذلك باستخدام العترة الضارية لميكروب السالمونيلا تيفميوريم. اما عند استخدام العترات الضارية لميكروب السالمونيلا انترتيديس وجد ان نسبة الحماية كان 72% مع الجرعة الواحدة ارتفعت الى 84% عند استخدام جرعتين من اللقاح. كذلك كانت نسبة الحماية 74% مع الجرعة الواحدة ارتفعت الى 86%عند التحصين بجرعتين متتاليين و ذلك عند استخدام العترة الضارية لميكروب السالمونيلا كنتاكى.
ثبت بشكل معنوى تفوق اللقاح المحضر محليا من العترات المحلية على نظيرة التجارى و المستورد فى جميع الاختبارات و طوال فترة التجربة. من النتائج يمكننا ان نلخص الى ان اللقاح المحضر محليا والمركب من ميكروبات السالمونيلا يعطى مستوى مناعى معنوى وحماية اعلى للدجاج المحصن ضد الاصابة بميكروبات السالمونيلا وذلك مقارنة بنظيره المستورد. واعتمادا على هذه الدراسة ينصح بانتاج و استخدام هذا اللقاح حقليا للحماية ضد العدوى بالميكروبات المكونة خاصة لكفائته عن اللقاح المستورد.