Efficacy of locally prepared *Salmonella* Kentucky vaccine in chicken

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A B S T R A C T

In the present study, efficacy of a locally prepared *Salmonella* Kentucky killed vaccine had been studied. A total of 120, two weeks old specific pathogen free (SPF) chicks were divided into two groups; 60 chicks each. First group was vaccinated with the prepared vaccine at the age of two weeks and boostered at four weeks, the second group was kept unvaccinated as a control group. The two groups were challenged orally with 1 ml of *Salmonella* Kentucky (5x10^7 CFU/ml), 3 weeks post boostering of the vaccine. 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 after first vaccination till fourth week after challenge and humoral immune response was measured against *Salmonella* strains using ELISA and microagglutination test. The prepared vaccine induced 80% protection rate in challenge test with reduced fecal shedding.

KEYWORDS: Aluminum hydroxide gel, vaccine, *Salmonella* Kentucky, chicken.

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1. INTRODUCTION

Salmonella is a member of Enterobacteriaceae consists of two species – Salmonella enterica and Salmonella bongori. Salmonella enterica consists of six subspecies (ssp.) under which there are 2500 serovars (Grimont and Weill, 2007) that can produce diseases in mammals including animals and humans, and a good number of them can be harboured by poultry without showing any clinical signs (Gast, 2007). Salmonellae are among the most important agents of food-borne infections. Poultry and poultry products are the major sources of *salmonella* contaminated food products that cause human salmonellosis (Tietjen and Fungdy, 1995). The United States centers for disease control and prevention (CDC) reported that human cases of salmonellosis were found to be caused by some sero types of which *S.* Enteritidis, *S.* Typhimurium and *S.* Kentucky were included in the report and reported to be wild spread (Task Force on Zoonoses Data Collection, TFZDC, 2007).

Since 2002, S. enterica serovar Kentucky has shown an increase in several countries with the concurrent emergence of multidrug-resistant isolates. The spread of such strains in the environment poses a major public health problem (Turki et al., 2012). *Salmonella* Kentucky isolated in Egypt showed high degree of antibiotic resistance especially toward ciprofloxacin which considered the drug of choice for *Salmonella* treatment. (Nourhan et al., 2014). 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* that poultry harbour can act as potential pathogens for man (Van Duijkeren et al., 2004). Many researchers all over the world have been trying to control and eradicate salmonellosis in poultry by vaccination. Live attenuated *Salmonella* vaccines may be hazardous because the residual virulence

due to insufficient attenuation (Arnon et al., 1983). Inactivated vaccines for the prevention of avian Salmonellosis have been reported by several authors (Barbour et al., 1993; Liu et al., 2001 and El-Enbaawy et al., 2013) which provided good protection with decrease or absence the residual virulence. So that, the following study was conducted to prepare and evaluate a killed vaccine of a locally isolated *Salmonella* Kentucky in specific pathogen free chicks by ELISA, Microagglutination test and challenge test.

2. MATERIAL AND METHODS

2.1. Bacterial strain

Local field isolate of *Salmonella* Kentucky (*S.*K.) was kindly obtained from Animal Health Research Institute, Dokki, Giza and then identified according to Hofstad et al., 1997.

2.2. Experimental birds:

A total number of 140 specific pathogens free (SPF) chicks of 1-day old was obtained from SPF poultry farm at Koom Osheem Fayuom province, Egypt. They were housed in batteries with the network floor. All birds were ascertained first to be free from *Salmonella*. They were fed on free balanced ration.

2.3. Vaccine preparation (Charles et al., 1994):

Salmonella Kentucky was grown on soya agar in Roux bottle at 37°Cfor 48 hours. The colonies were harvested with normal saline and the bacterial suspension was prepared and adjusted to contain 10^{10} colony forming unit / ml using total colony count technique. The inactivating agent (formalin solution 37%) was added to bacterial suspension in final concentration of 0.3%. The inactivation was carried out under stirring for 24 hrs at 24 ºC to complete the inactivation process. The inactivated cultures were neutralized with sodium meta-bisulfite then stored at temperature of 5-7ºC, and then 20% of aluminum hydroxide gel was added as an adjuvant.

2.4. Quality control on the prepared vaccine

2.4.1. Purity Test

Testing of the prepared vaccine to ensure that it is free from any contamination as aerobic, anaerobic bacteria and fungi (OIE Terrestrial Manual 2008).

2.4.2. Safety Test (OIE Terrestrial Manual 2008).

Safety of the prepared vaccine was monitored through injection of double field dose (1 ml) of the vaccine subcutaneously in each of 20 SPF chicks. The chicks were observed daily for two weeks for any signs of local reactions, clinical signs or deaths.

2.4.3. Potency test:

The humeral immune response of the vaccinated chicks against *Salmonella* Kentucky was evaluated by ELISA test and Microagglutination test.

2.4.4. Efficacy test:

2.4.4.1.Challenge test:

Via challenging of the vaccinated chicks 3 weeks post boostering dose by a dose of 1 ml *Salmonella* Kentucky broth culture containing $5x10^7$ virulent organisms (OIE, 2012).

2.4.4.2. Fecal shedding:

Shedding of *Salmonella* Kentucky was detected in fecal samples collected from challenged vaccinated and non-vaccinated chicks up to 4 weeks post challenge.

2.5.Experimental design:

Two groups of SPF chicks each of 60 chicks were reared separately; the first group of chicks was injected with 0.5 ml of the prepared vaccine subcutaneously at two weeks of age then boostered with another same dose after two weeks. The second group was used as a control (non-vaccinated). The two groups were challenged three weeks after the booster dose by oral administration of 1ml from *Salmonella* Kentucky virulent strain suspension containing $5x10^7$ CFU/ml (OIE,

2012). The inoculated chickens were observed for one month. 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, weekly after each vaccination and post challenge for three weeks (once/week) to measure and evaluate the developed immune response to *Salmonella* Kentucky. 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. 1997 and Cruickshank et al. 1975.

2.6. Evaluation of humoral immune response against Salmonella Kentucky in the vaccinated chicks:

The developed humoral immune response against *Salmonella* Kentucky in the vaccinated chickens was measured in the sera using ELISA according to Haider et al. 2007 and micro-agglutination test according to Brown et al. (1981)
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:

$$\text{S/P ratio} = \frac{Sample\ mean - Negative\ control}{\text{Positive control} - \text{Negative control}}$$

Calculation of Antibody Titer: Log^{10} Titer=1.13Log $^{(SP)}$ +3.156.
AntiLog= Antibody titer
The antibody titer in MAT was expressed as Geometric Mean Titer (GMT).

3. RESULTS

3.1. Results of quality control on the prepared vaccines:

The prepared vaccine proved to be pure, sterile, safe and free from adverse side effects on chicken's productivity and body weight gain.

3.2. Protective Efficacy of the vaccines:

The protection rate of the prepared vaccine was 80% after 4 weeks post challenge (Table 1).

3.3. Fecal Shedding of salmonellae from challenged broiler chickens:

The re-isolation rates of salmonellae from chickens vaccinated with the inactivated *Salmonella* Kentucky vaccine in the 1st, 2nd and 3rd weeks post challenge were 20.75%, 12.5% and 8.33%, respectively while in the 4th week the fecal shedding disappeared. Regarding the control unvaccinated birds, the re-isolation rates were 70.8%, 50%, 25% and 16.66% in the 1st, 2nd, 3rd and 4th weeks post challenge, respectively (Table 2). Chickens in vaccinated group 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, swollen liver, spleen and gallbladder with small necrotic foci in the liver, in some cases the pericardium was turbid and covered with yellowish white materials.

3.4. Re-isolation of Salmonella Kentucky from survived chickens after challenge:

Data presented in Table (3) showed that *Salmonella* Kentucky could only be re-isolated from ceca (18.75%) of the vaccinated group while it was re-isolated from heart blood, liver, spleen and ceca in 75%, 58.33%, 58.33% and 75%, respectively for control non vaccinated group.

3.5. Evaluation of humoral immune responses in the vaccinated chickens:

3.5.1. ELISA Test:

The ELISA antibody titer in sera of vaccinated chicks was increased from 162.8 pre-vaccination level to 189.2 at the 1st week and 659.5 at the 2nd week after the primary immunization while post boostering it increased to1085.2 at the 1st week, 1130.2 at the 2nd week and 2234.1 at

the 3rd week post boostering. After challenge a decrease occurred at the 1st week as it reached 1630 then increased to 2222.3 at the 2nd week and 2249.6 at the 3rd week and 2272.1 at the 4th week post challenge (Table 4). On the other hand, The ELISA antibody titer in sera of unvaccinated chicks was 155.3. Moreover, an abrupt increase of antibody titer was recorded, where the antibody titer was 891.2 4th week of challenge (Table 4).

3.5.2. Microagglutination Test:

The antibody titer in sera of vaccinated chicks was increased from zero pre-vaccination level to 43 at the 1st week and 64 at the 2nd week after the primary immunization while post boostering it increased to132 at the 1st week, 141 at the 2nd week and 178 at the 3rd week post boostering. After challenge, a decrease occurred at the 1st week as it reached 125 then increased to 170 at the 2nd week and 180 at the 3rd week and 185 at the 4th week post challenge (Table 5). On the other hand, the antibody titer in sera of unvaccinated chicks was zero. Moreover, an abrupt increase of antibody titer was recorded, where the antibody titer was 65 at the 4th week of challenge (Table 5).

4. 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 (O'Brien, 1988). In terms of the loss to producers annually, it is difficult to estimate, however any strategies which reduce the incidence of salmonellosis in

Table (1): Protective Efficacy of *Salmonella* Kentucky inactivated vaccine in SPF chicks challenged with virulent *Salmonella* Kentucky strain.

Chicken groups	Total No. of birds	No. of dead & or diseased birds / Week post challenge				Dead & or diseased/ Total	Survive/ Total	Mortality rate	Protection %
		1st week	2nd week	3rd week	4th week				
vaccinated group	60	7	5	0	0	12/60	48/60	20%	80%
Control non vaccinated group	60	24	10	8	6	48/60	12/60	80%	20%

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

Table (2): Results of fecal shedding of *Salmonella* Kentucky from chicks after challenge

Chicken groups	No. of birds positive for isolation / total No. of living birds			
	1st week	2nd week	3rd week	4th week
vaccinated group	11/53 (20.75%)	6/48 (12.5%)	4/48 (8.33%)	0/48 (0%)
Control non vaccinated group	17/24 (70.8%)	5/10 (50%)	2/8 (25%)	1/6 (16.66%)

Table (3): Re-isolation of *Salmonella* Kentucky from vaccinated chickens survived following challenge

Chickens groups	No. of birds positive for isolation / Total No. of survived birds				Higher % of re-isolation
	Heart blood	Liver	spleen	ceca	
vaccinated group	0/48 (0%)	0/48 (0%)	0/48 (0%)	9/48 (18.75%)	18.75%
Control non vaccinated group	9/12 (75%)	7/12 (58.33%)	7/12 (58.33%)	9/12 (75%)	75%

Table (4): Results of ELISA for measurement of antibody against *Salmonella* Kentucky in sera of vaccinated chicks

Groups	Pre-vaccination level	Weeks post 1st vaccination		Weeks post boostering			Weeks post challenge			
		1st week	2nd Week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	4th week
Vaccinated group	162.8	189.2	659.5	1085.2	1130.2	2234.1	1630	2222.3	2249.6	2272.1
Control non vaccinated group	155.3	167	176	180.6	193.4	206.3	774	1066	895.5	891.2

Table (5): Results of Microagglutination for measurement of antibody against *Salmonella* Kentucky in sera of vaccinated chicks

Groups	Pre-vaccination level	Weeks post 1st vaccination		Weeks post boostering			Weeks post challenge			
		1st week	2nd Week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	4th week
Vaccinated group	0	43	64	132	141	178	125	170	180	185
Control non vaccinated group	0	0	0	0	0	0	35	70	65	65

poultry are clearly important to all facts of the industry. Reducing *Salmonella* incidence has become monitored and regulated by Food Safety and Inspection Service (Helmick et al., 1994). Perales and Audicana (1988) 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 (Nagraja et al., 1991). 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 (EFSA, 2006). 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%).
Evaluation of the protective value of a locally prepared inactivated *Salmonella* Kentucky vaccine was performed by applying the challenge test according to Paiva et al. (2009). This test is considered the master test for determination of the protective value of a vaccine (Timms et al., 1990). The protective value against virulent *Salmonella* Kentucky; post oral challenge, in chickens vaccinated with the prepared vaccine was 80%. The achieved protection value by the prepared vaccine is accepted to pass the vaccine for use according to Heddleston (1975) and Egyptian Veterinary Codex- CLEVB (2009). Fecal shedding of *Salmonella* organisms in the vaccinated group of chickens reached 8.33% while the unvaccinated control group at 3-week post challenge revealed fecal shedding of 25 %. No shedding detected at the fourth week post challenge in vaccinated group, while there was 16.6% shedding in control unvaccinated group. Similar fecal shedding rates were reported by Sayed (2010) and Ibrahim (2014). *Salmonella* Kentucky was only isolated from ceca of the vaccinated group (18.75%), on the other hand in the control non vaccinated group *Salmonella* Kentucky was re-isolated from heart blood, liver, spleen and ceca with the higher percent isolated from ceca and heart blood (75%). The ELISA antibody titer in sera of vaccinated chicks was increased from 162.8 pre-vaccination level to 189.2 at the 1st week and 659.5 at the 2nd week after the primary immunization while post boostering it increased to1085.2 at the 1st week, 1130.2 at the 2nd week and 2234.1 at the 3rd week post boostering. After challenge a decrease occurred at the 1st week as it reached 1630 then increased to 2222.3 at the 2nd week and 2249.6 at the 3rd week post challenge (Table 4). On the other hand, The ELISA antibody titer in sera of unvaccinated chicks was 155.3. Moreover, an abrupt increase of antibody titer was recorded, where the antibody titer was 895.5 3rd week of challenge (Table 4). These results agree with those obtained by Okamura et al. (2007) and El-Enbaawy et al., (2013). The antibody titer in sera of vaccinated chicks by microagglutination test was increased from zero pre-vaccination level to 43 at the 1st week and 64 at the 2nd week after the primary immunization while post boostering it increased to132 at the 1st week, 141 at the 2nd week and 178 at the 3rd week post boostering. After challenge an decrease occurred at the 1st week as it reached 125 then increased to 170 at the 2nd week and 180 at the 3rd week and 185 at the 4th week post challenge (Table 5). On the other hand, the antibody titer in sera of unvaccinated chicks was zero. Moreover, an abrupt increase of antibody titer was recorded, where the antibody titer was 65 at the 4th week of challenge (Table 5). These results agree with those obtained by Abd El-Ghany et al. (2012) and Ibrahim (2014). In Conclusion: killed vaccine of a locally isolated *Salmonella* Kentucky gave 80% protection by challenge test in SPF chicks with decreased fecal shedding rate.

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