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Preparation and Evaluation of Combined Inactivated Vaccine Against Salmonellosis and Colibacillosis in Chickens

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Abstract: Colibacillosis and Salmonellosis are responsible for great economic losses in the poultry industry worldwide and they are considered the most common avian diseases that are communicable to humans so the poultry researchers continuing to make progress in reducing and eliminating avian colibacillosis and Salmonellosis from the poultry flocks, there by reducing potential hazards to the public health posed by these bacterial diseases and many researchers all over the world have been trying to control and eradicate Colibacillosis and Salmonellosis in poultry by vaccination. The present investigation aimed to prepare a potent combiend inactivated vaccine from *Escherichia* coli(*E. coli)* and *salmonella* serogroups O1, O2, O78, SE and ST to control Colibacillosis and Salmonellosis in chickens. The *E. coli* and *Salmonella* strains were collected from diseased, apparently healthy and freshly dead chickens from different poultry farm and serotyped. In the present investigation preparation of inactivated combined inactivated *E. coli Salmonella* vaccine, inactivated *E. coli* vaccine and inactivated *Salmonella* vaccine and compared their purity, safety and potency to induce immune response against Colibacillosis and Salmonellosis using Micro Agglutination Test (MAT) and Enzyme Linked Immuno Sorbent Assay (ELISA). The prepared vaccine found to be sterile, safe, potent and protecting chickens against E. coli and salmonella serogroups O1, O2, O78, SE and ST and give high protection level 85%.

Key words: Salmonella · *E. coli* · MAT · ELISA

INTRODUCTION

Salmonella enterica (S. enterica), the most pathogenic species of the genus Salmonella, includes more than 2,500 serovars, many of which are of great veterinary and medical significance and The majority (99.5%) of the isolated serovars belong to *S. enterica* subsp. *enterica* cause acute, localized gastroenteritis rather than systematic disease [1] and Avian Pathogenic *Escherichia* coli (APEC) strains harbor a number of virulence genes and cause extra intestinal diseases, such as septicemia, swollen-head syndrome, salpingitis and omphalitis in poultry [2]. vaccination is recommended for protection against them proved by Nourhan *et al.* [3] and by Hanan *et al.* [4].

The objective of the present work is to evaluate the efficacy of the combined inactivated *E. coli Salmonella* vaccine compared with another inactivated *E. coli* vaccine and inactivated *Salmonella* vaccine in immunizing and protecting chickens against experimental challenge with colibacillosis and Salmonellosis. It also describes the immune response of chickens evoked by the vaccine.

MATERIALS AND METHODS

***E. coli* and *Salmonella* Strains:** Isolated from different poultry farm and Serotyped.

Laboratory Animals:

- Experimental chickens for the evaluation of the vaccine for the vaccine potency by Chaffer *et al.* [6]: A total of 240 (60/ group), two weeks old SPF Lohmman chicks that had the network floor and batteries were used for vaccination. They were fed on pelleting feed free from antibiotics. Water was

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clean and kept at libitum. All birds were tested first to be free from *E. coli* and *Salmonella* antibodies by slide agglutination test specific antigen for each strain.

- The vaccines safety according to OIE [5] At least 40 SPF chicks obtained from (poultry farm at Koom Osheem- Fayuom province, Egypt) (10 for each vaccine and 10 used as control), 7-14 days of age were injected subcutaneously with double field dose of the prepared vaccines and the chicks were observed for 14 sucessive days to detect any signs of local reaction, clinical symptoms or death.

Preparation, Inoculation and Evaluation of the Inactivated Vaccines:

- Colony count technique according to Read and Muench [7]. A separate final suspension from each of S.E. and S.T. was adjusted for each type to 10^{10} CFU/0.5ml were prepared according to Bachmeier [8]. *E.coli* O1, O2 and O78 were prepared for each type to 4 X 10^9 CFU/ml according to Camguilhem and Milon [9]. Then killed by adding 0.3% formalin and Mentonide 206 was used as an organic immunostimulant for chickens.
- Sterility test: was carried out according to OIE [5].
- Vaccine inoculation: The chicks in each group wereinoculated twice subcutaneously at 2 and 5 weeks of age with 0.5 ml of each vaccine. The inoculation was made in the middle part of the back neck and the three groups then challenged with virulent *Salmonella* and *E. coli* strains.
- Blood samples: Were collected before immunization and after the first dose of vaccination for three weeks (one / week), the second dose of vaccination for three weeks (one / week) and post challenge for three weeks (one / week) to measure and evaluate the developed immune response against *Salmonella* and *E. coli* strains was measured using, the micro agglutination test (MAT) and ELISA according to European pharmacopeia [10].

Measurement of Post Vaccination Humoral Immune Response Developed Against the Vaccinal Strains

Micro-agglutination test for *E.coli* and *Salmonella* Strains:

- Micro-agglutination test technique [MAT] according to Thaxton *et al.* [11] and Brown *et al.* [12].
- Preparation of stained antigens for Salmonella and E. coli:
  were prepared according to Eissa [13].
- Safranin O-stained microtest antigen: The antigen was prepared as described by Brown *et al.* [12].

Determination of Enzyme Linked Immuno Sorbent Assay:

- Testing serum samples using ELISA technique according to Vollar *et al.* [14].
- Preparation of ELISA antigen: according to Barrow *et al.* [15].

Challenge Test

Challenge for Salmonella: was done orally using 10^8 CFU/ml of each strain. The degree of protection was assessed according to Clifton-Hadley *et al.* [16] and Paiva *et al.* [17].

Cultures from cloacal swabs and from internal organs after culling of birds one month post challenge were cultured in tetrathionate broth at 37°C for 18-24h were done according to Hofstad [18].

Challenge For *E. coli*: was done by intramascular injection using 10^9 CFU/ml of virulent *E. coli* organism according to Susantha Gomis *et al.* [19]. Cultures from internal organs after culling of birds one month post challenge were cultured MacConkey medium according to Whiteman and Bickford [20].

RESULTS AND DISCUSSION

Fecal shedding of Salmonella organisms in the chickens vaccinated with Salmonella vaccine and combined vaccine reached to 0 after 4th week post challenge (Table 1). The protective value against virulent *E. coli and Salmonella* strains reached to (83.75%), (88.75%) and (86.25%) in *E. coli* vaccine, *Salmonella* vaccine and combined vaccine, respectively, post challenge while the control reach to 48.75% (Table 2). The detection of humoral immune response in the *E. coli* vaccine, the Geometric mean titer (GMT) of micro-agglutination test against O1, O2 and O78 reached to (49), (43) and (140) after the 3rd week from primary vaccination, respectively and to (320), (211) and (184) after 3rd week from boostring dose, respectively. While in combined vaccine, the GMT of micro-agglutination test against O1, O2 and O78 reached to (70), (98) and (113) after 3rd week from primary vaccination, respectively and to (299), (211) and (184) after 3rd week from boostring dose, respectively.

Table 1: Results of fecal shedding from vaccinated chickens by the different vaccines after the challenge with virulent *Salmonella* strains

		No. of birds positive for isolation / total No. of living birds			
Type of vaccine		1st week	2nd week	3rd week	4th week
Salmonella vaccine	SE	11/37(29.7%)	8/37(21.7%)	6/37(16.2%)	0/37(0%)
	ST	9/36(25%)	7/34(20.5%)	5/34(14.7%)	0/34(0%)
Combined	SE	5/15(33.3%)	2/15(13.3%)	1/15(6.7%)	0/15(0%)
Vaccine	ST	5/15(33.3%)	3/14(21.4%)	1/14(7%)	0/14(0%)
Control	SE	9/11(81.8%)	5/8(62.5%)	3/8(37.5%)	2/8(25%)
	ST	11/12(92%)	8/9(89%)	5/7(71.4%)	2/7(28.5%)

Table 2: Comparative results of overall mean of the different tests used for evaluation of both E.coli, Salmonella and Combined vaccines

			Results of antibody titer at 3rd week post 2nd vaccination				
			MAT			ELISA	
Type of the vaccine	Protection %		3rd WP1stV		3rd WP2ndV	3rd WP1stV	3rdWP2ndV
E. Coli Vaccine	83.75%	81%	O1	49	320	1380	4936
		85%	O2	43	211	752	1417
		85%	O78	140	184	2789	6620
Salmonellavaccine	88.75%	92.5%	S.E	49	149	1642	4695
		87.5%	S.T	106	211	3995	5307
combined vaccine	86.25%	81.25%	O1	70	299	1905	3851
		87.5%	O2	98	211	1471	1843
		81.25%	O78	113	184	1193	6255
		93.75%	S.E	40	139	1120	4397
		87.5%	S.T	98	226	3072	5297
Control	48.75%	50%	O1	0	0	338	277
		56.25%	O2	0	0	127	279
		43.75%	O78	0	0	164	301
		50%	S.E	0	0	515	200
		43.75%	S.T	0	0	274	167

-ELIZA antibody titer of prevaccinated E.coli O1 = 182

- ELIZA antibody titer of prevaccinated E.coli O2 = 57

- ELIZA antibody titer of prevaccinated E.coli O78 = 102

- ELIZA antibody titer of prevaccinated S.E = 142

- ELIZA antibody titer of prevaccinated S.T = 54

-3rd WP1stV:Third week post first vaccine. -GMT of prevaccinated = 0

-3rd WP2ndV:Third week post second vaccine.

In the E. coli vaccine the ELISA antibodies titers against O1, O2 and O78 reached to (1380), (752) and (2789) after the 3rd week from primary vaccination, respectively and to (4936), (1417) and (6620) after 3rd week from boostring dose, respectively. While in combined vaccine, the ELISA antibodies titers against O1, O2 and O78 reached to (1905), (1471) and (1193) after 3rd week from primary vaccination, respectively and to (3851), (1843) and (6255) after 3rd week from boostring dose, respectively and In the *Salmonella* vaccine the Geometric mean titer (GMT) of micro-agglutination test against S.E and S.T reached to (49) and (106) after the 3rd week from primary vaccination, respectively and to (149) and (211) after 3rd week from boostring dose, respectively. While in combined vaccine, the GMT of micro-agglutination test against S.E and S.T reached to (40) and (98) after the 3rd week from primary vaccination, respectively and to (139) and (226) after 3rd week from boostring dose, respectively and the ELISA antibodies titers against S.E and S.T reached to (1642) and (3995) after the 3rd week from primary vaccination, respectively and to (4695) and (5307) after 3rd week from boostring dose, respectively. While in combined E. coli Salmonella vaccine, the ELISA antibodies titers against S.E and S.T reached to (1120) and (3072) after the 3rd week from primary vaccination, respectively and to (4397) and (5297) after 3rd week from boostring dose, respectively, (Table 2). Reisolation of O1, O2 and O78 organisms in challenged chickens vaccinated by E. coli vaccine and combined vaccine is reached to (24%), (17.4%), (22%), (31%), (21.4%) and (23%), respectively, (Table 3).

Table 3: Re-isolation of *E. coli* from vaccinated chickens with the E. coli vaccine and the combined vaccine which survived following challenge

Chickens groups		No. of birds positive for isolation / Total No. of live birds				Higher No of isolation	Total no. of birds positive for isolation
		Heart blood	Liver	Spleen	Bone marrow		
E. coli vaccine	O1	5/21(24%)	2/21(9.5%)	3/21(14.2%)	2/21(9.5%)	5	24%
	O2	4/23(17.4 %)	2/23(8.7 %)	4/23(17.4 %)	2/23(8.7 %)	4	17.4 %
	O78	5/23(22 %)	3/23(13 %)	5/23(22 %)	2/23(8.7 %)	5	22%
Combined vaccine	O1	4/13(31 %)	2/13(15.4 %)	4/13(31 %)	2/13(15.4 %)	4	31%
	O2	3/14(21.4 %)	1/14(7 %)	2/14(14.2 %)	1/14(7 %)	3	21.4%
	O78	3/13(23 %)	1/13(7.7 %)	2/13(15.3 %)	1/13(7.7 %)	3	23%
Control	O1	5/8(62.5 %)	4/8(50 %)	5/8(62.5 %)	6/8(75 %)	6	75%
	O2	8/9(89 %)	7/9(78 %)	7/9(78 %)	7/9(78 %)	8	89%
	O78	5/7(71.5 %)	5/7(71.5 %)	6/7(85.7 %)	5/7(71.5 %)	6	85.7%

Table 4: Re-isolation of *Salmonellae* from vaccinated chickens with Salmonella vaccine and combined vaccine which survived following *Salmonella* challenge

Chickens groups		No. of birds positive for isolation / Total No. of live birds				Higher No of isolation	% of re-isolation
		Heart blood	Liver	Spleen	Caecal junction		
Salmonella vaccine group	SE	6/37(16.2 %)	3/37(8%)	3/37(8%)	3/37(8%)	6	16.2%
	ST	4/34(11.7 %)	2/34(5.8 %)	4/34(11.7 %)	2/34(5.8 %)	4	11.7 %
Combined vaccine group	SE	1/15(6.7 %)	1/15(6.7 %)	0/15(0 %)	2/15(13.3 %)	2	13.3%
	ST	2/14(14.2 %)	1/14(7 %)	2/14(14.2 %)	1/14(7 %)	2	14.2%
Control non vaccinated group	SE	6/8(75%)	6/8(75%)	6/8(75%)	4/8(50%)	6	75%
	ST	4/7(57 %)	4/7(57%)	5/7(71.4%)	5/7(71.4%)	5	71.4%

Reisolation of S.E and S.T organisms in challenged chickens vaccinated by Salmonella vaccine and combined vaccine is reached to (16.2%), (11.7%), (13.3%) and (14.2%), respectively, (Table 4).

The incidence of foodborne pathogens Salmonella and E. coli in meat and dairy products was determined in a large-scale survey in Africa [21] and lead to mortality [22]. Several experiments have been performed to prevent Salmonellosis by vaccination reported by Hanan *et al.* [23] and by Berghaus *et al.* [24] and for E. coli by Gina [25] which prepared a potent vaccine from *E*. coli serogroups O2 and O78 to control *Colibacillosis* in chickens. The protective value against virulent *E. coli and* Salmonella strains reached to (83.75%), (88.75%) and (86.25%) in E. coli vaccine, Salmonella vaccine and combined vaccine, respectively, post challenge while the control reach to 48.75% and The achieved protection values are accepted to pass the vaccine for use according to Egyptian veterinary codex– CLEVB [26]. Reisolation of O1, O2 and O78 organisms in challenged chickens vaccinated by E. coli vaccine and combined vaccine is reached to (24%), (17.4%), (22%), (31%), (21.4%) and (23%) and Similar re-isolation rates and organs from immunized chickens were reported by Whiteman and Bickford [20] and by Hanan *et al.* [4]. Reisolation of S.E (S.E) and S.T (S.T) organisms in challenged chickens vaccinated by Salmonella vaccine and combined vaccine is reached (16.2%), (11.7%), (13.3%) and (14.2%), respectively and Similar re-isolation rates and organs from immunized chickens were reported by Barbour *et al.* [27], Hanan *et al.* [23] and Mohmed [28]. The detection of humoral immune response in the E. coli vaccine, the Geometric mean titer (GMT) of micro-agglutination test against O1, O2 and O78 reached to (49), (43) and (140) after the 3rd week from primary vaccination, respectively and to (320), (211) and (184) after 3rd week from boostring dose, respectively. While in combined vaccine, the GMT of micro-agglutination test against O1, O2 and O78 reached to (70), (98) and (113) after 3rd week from primary vaccination, respectively and to (299), (211) and (184) after 3rd week from boostring dose, respectively. In the E. coli vaccine the ELISA antibodies titers against O1, O2 and O78 reached to (1380), (752) and (2789) after the 3rd week from primary vaccination, respectively and to (4936), (1417) and (6620) after 3rd week from boostring dose, respectively. While in combined vaccine, the ELISA antibodies titers against O1, O2 and O78 reached to (1905), (1471) and (1193) after 3rd week from primary vaccination, respectively and to (3851), (1843) and (6255) after 3rd week from boostring dose, respectively. This results similar to the result proved by Gina [25] and by Hanan *et al*. [4] and In the Salmonella vaccine the Geometric mean titer (GMT) of micro-agglutination test against Salmonella Enteritidies (S.E) and Salmonella Typhimurium (S.T) reached to (49) and (106) after the 3rd week from primary vaccination, respectively and to (149) and (211) after 3rd week from

boostring dose, respectively. While in combined vaccine, the GMT of micro-agglutination test against S.E and S.T reached to (40) and (98) after the 3rd week from primary vaccination, respectively and to (139) and (226) after 3rd week from boostring dose, respectively and the ELISA antibodies titers against S.E and S.T reached to (1642) and (3995) after the 3rd week from primary vaccination, respectively and to (4695) and (5307) after 3rd week from boostring dose, respectively. While in combined E. coli Salmonella vaccine, the ELISA antibodies titers against S.E and S.T reached to (1120) and (3072) after the 3rd week from primary vaccination, respectively and to (4397) and (5297) after 3rd week from boostring dose, respectively. This results similar to the result proved by Assadian *et al.* [29], Nourhan *et al.* [3] and Hanan *et al.* [23]. Fecal shedding of Salmonella organisms in the chickens vaccinated with Salmonella vaccine and combined vaccine reached to 0 after 4th week post challenge similar fecal shedding rates were reported by Nourhan *et al.* [3]. During the present work the prepared vaccines were successfully protected against E. coli and Salmonella strains found to be stable, free from foreign contaminants (aerobic and anaerobic bacteria and fungi) and safe in vaccinated birds where such birds remained healthy all over the experimental period with slight local reaction at the site of inoculation. These observations agree with the recommendation of OIE [5]. So, the vaccination studies performed here showed that chicks immunized with two doses of inactivated E. coli Salmonella vaccine are protected to a high degree 86.25% from challenge with the same pathogenic *E. coli* and *Salmonella* strains.

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