PREPARATION AND EVALUATION OF INACTIVATED SALMONELLA PULLORUM VACCINE IN TURKEY

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SUMMARY

An inactivated *Salmonella Pullourm* Vaccine was prepared from local strain of *Salmonella Pullourm* .The efficacy of a locally prepared inactivated *Salmonella Pullorum* vaccine was evaluated in turkey. Immune response of both vaccinated turkey breeder and hatched poults was monitored using ELISA which showed protective antibody titers in sera of breeders starting from the third week post vaccination, and at the first day of age in sera of hatched poults. Also good protection against experimental challenge by virulent strain of *Salmonella Pullourm* in turkey poults reached to 100 % in one day old and 70 % in 7 days old poults, but it was 15 % and 20 % in poults from non-vaccinated turkey breeders. In conclusion the prepared inactivated *Salmonella Pullorum* vaccine is potent and effective.

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INTRODACTION

Pullorum disease is a septicemic disease affecting primarily chickens and turkeys **(Saif, 2008)**. The disease was initially called fatal septicemia of young chicks **(Rettger, 1900)**, then it was designated as bacillary white diarrhea **(Rettger, 1909)** but later the term pullorum disease has gained universal acceptance. Pullorum disease is caused by Salmonella Pullorum and be transmitted vertically through the egg by trans-ovarian infection. It was first recognized in turkeys in 1928 **(Hewitt, 1928)** and by 1940 the disease was wide spread in turkey and responsible for severe economic losses. Both morbidity and mortality are highly variable in chickens and influenced by age, nutrition, flock management, concurrent diseases, route and dose of exposure. The greatest losses usually occur during the second week after hatching, with a rapid decline between the third and fourth week of age. Various sulfonamides or antibiotics have been found to be effective in

reducing mortality from pullorum disease, however no drug or combination of drugs has been found capable of eliminating infection from treated flock. Also using antimicrobials emerges the problem of antimicrobial resistance **(Zhang-Barber et al., 1999)**. Therefore, alternative safe strategies are emerged to overcome this important problem. **Priyantha (2009)** reported that vaccination is only alternative method to control salmonellosis in poultry and other precaution like biosecurity and good management practices must be taken to consideration first. Various investigators have evaluated killed and modified live vaccines **(Arora et al., 1998; Padmanaban et al., 1981; Zhang-Barber et al., 1998; Zhang-Barber et al., 1999)**. So the aim of this work was to prepare and evaluate Salmonella Pullorum inactivated vaccine in turkey breeders and their hatched poults via monitoring of the humeral immune response of both dams and their off springs by ELISA and challenge test of off springs.

MATERIALS AND METHODS

Bacterial Strain: *Salmonella Pullorum* local isolate was kindly obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. The strain was cultured on specific Salmonella media and re-identified according to (Collier et al. 1998 and Kauffmann et al. 1972).

Experimental Birds: Total number of 74 turkey breeders (60 breeder hens and 14 adult cooks fifteen weeks old) and 100 one day old poults; which hatched from the collected eggs of vaccinated and non - vaccinated turkey breeders; were used for evaluation of the prepared inactivated *Salmonella Pullorum* vaccine.

Vaccine preparation (Charles et al., 1997)

Salmonella pullorum was grown onto trypticase soya-agar in Roux bottle and incubated at 37°C for 48 hours. The colonies were harvested using normal saline and the bacterial suspension was adjusted to contain 10^{10} colony/ml. The live bacteria were killed by adding 0.3% formalin with agitation. Then 20% of aluminum hydroxide gel as an adjuvant.

Quality control of the prepared vaccine

1- **Purity test**: Testing of prepared vaccine to be free from any contaminants as aerobic , anaerobic bacteria and fungi. (**OIE Terrestrial Manual 2008**).
2- **Safety test:** (**OIE Terrestrial Manual 2008**): safety of the prepared vaccine was monitored through injection of double field dose of the vaccine subcutaneously in each of 20 poults and kept under daily observation for 2 weeks.
3- **Potency test:** The humeral immune response of the vaccinated turkey breeders and their off springs to the *Salmonella Pullorum* vaccine was evaluated using ELISA kit (Bio check UK Ltd Co.) following the manufacture instructions.
4- **Efficacy test:** via challenging of poults at 1^{st} and 7^{th} day of age. The challenge procedure were done on poults hatched from vaccinated and non-vaccinated turkey breeders, the challang dose/ poult was 1ml of *Salmonella Pullorum* broth culture containing $5x10^7$ virulent organism (**OIE terrestrial Manual 2012**).

Experimental Design

Two groups of turkey breeder each of 30 breeder hens and 7 breeder cooks were reared separately in two clean completely separated pens and were fed on balanced ration. Food and water were used adlibidum. The first group was injected with 0.5ml prepared formalized *Salmonella Pullorum* inactivated vaccine subcutaneously, then boostered with another dose after 3 weeks, the second group was left as un vaccinated control. The laied eggs of each group were collected separately and the egg production of a week were incubated together till hatching.

The serum samples were collected weekly from breeders starting from 0 week pre vaccination up to 10 week post vaccination and from hatched poults for estimation of humeral immune response using ELISA. The hatched poults from the eggs collected after 3 weeks post boostering; from each group, were grouped into 4 groups each of 25, groups I and II from vaccinated turkey breeder while group III and IV from non- vaccinated turkey breeder.Twenty poults from group I & III were challenged orally with the virulent strain of *Salmonella Pullorum*

at the 1st day of age. Twenty poults from group II & IV were challenged orally with virulent strain of *Salmonella Pullorum* at the 7th day of age. The challenged poults were observed daily for 10 days post challenge. Serum samples were collected from the non- challenged poults from each group weekly for determination of the maternal derived antibodies by ELISA.

RESULTS

Purity test: The prepared inactivated vaccine proved to be free from aerobic bacterial, anaerobic bacterial and fungal contamination.

Safety test: The prepared vaccine proved to be safe as there were neither local nor systemic reaction, also no clinical signs or mortalities in the injected poults.

Potency test: Concerning turkey breeders, the mean ELISA antibody titers were 195.8 as pre-vaccination level then reached 611.5, 1050, 1390 at 1st, 2nd and 3rd week after the first dose of vaccination which represent the 16th, 17th and 18th week of age respectively. After boostering at the 18th week of age the ELISA titers increased to reach 1890, 2310, 3150, 3140, 3080, 3050 and 3000 at the 19th, 20th, 21st, 22nd, 23rd, 24th and 25th week of age respectively (table 1).

Table (1): Mean antibody titers measured by ELISA in vaccinated and non- vaccinated turkey breeders

Weeks post Vaccination	**Weeks of Age**	**Antibody titers**	
		Vaccinated Group	**Non vaccinated Group**
0 (pre vaccination)	15	195.8	200
1	16	611.5	205
2	17	1050	200
3*	18*	1390	199
4	19	1890	210
5	20	2310	195
6	21	3150	208
7	22	3140	210
8	23	3080	206
9	24	3050	198
10	25	3000	200

* booster dose was given.
*cut off value = 654.

Concerning hatched poults from vaccinated turkey breeders, the mean antibody titers were 1850, 1110, 765 and 615 at the first day, 7^{th} day , 14^{th} day and 21^{st} day of age respectively, other while in the control group the titers were 20.89, 18.62, 16.21 and 16 at the same intervals as mentioned in table (2).

Table (2): Mean antibody titers measured by ELISA in poults hatched from vaccinated and non-vaccinated turkey breeders.

	Day (0)	Day (7)	Day (14)	Day (21)
Poults from vaccinated turkey breeders	1850±751	1110±501	765±239	615±152
Poults from non-vaccinated turkey breeders	20.89±2.1	18.62±1.89	16.21±1.78	16±1.21

Efficacy test

The results revealed that the mortalities were 0% in group I, while in group III the total mortalities were 17 out of 20 poults (85%) representing protection percentage of 100% and 15% ; respectively (Table 3). On the other hand the protection percentages were 70% and 20% in groups II and IV; respectively (table 4).Reisolation of the virulent *Salmonella Pullorum* strain from the internal organs of the died poults post challenge was positive.

Table (3): Efficacy of prepared *Salmonella pullorum* vaccine in day old turkey poults from vaccinated and non- vaccinated turkey breeders.

Group of poults	**Challenge dose**	**No. of poults**	**No. of poults/ died**	**Protection %**
From vaccinated breeders (group I)	5 x 10^7 CFu	20	0	100%
From non vaccinated breeders (group III)	5 x 10^7 CFu	20	17	15%

Table (4): Efficacy of prepared *Salmonella pullorum* vaccine in 7 days old turkey poults from vaccinated and non-vaccinated turkey breeders.

Group of poults	**Challenge dose**	**No. of poults**	**No. of poults/ died**	**Protection %**
From vaccinated breeders GII	5 x 10^7 CFu	20	6	70%
From non-vaccinated breeders GIV	5 x 10^7 CFu	20	16	20%

DISCUSSION

Vaccination against *Salmonella Pullorum* disease is not widely used all over the world due to many reasons, one of them is the interference of vaccination with the detection of positive birds of the herd using pullorum test in test and slaughter programme.

In Egypt, and from an economical point of view this program of salmonella control is not applied. So it is necessary to apply a vaccination programme against *Salmonella Pullorum* in turkey flocks.

Sero conversion in vaccinated turkey breeder group were found to be increased gradually from first week post vaccination as in table (1) till the time of boostering at the third week, where the titer sharply increased from the first week post-boostering (1890) reaching its peak at the fourth week post boostering (3140 ELISA antibody titer), and still around this value till the end of the experiment, same results were obtained by **Uddin etal., (2009).**

For table (2) it was clearly that the vaccinated turkey breeders achieved a protective ELISA titer level at the second week post primary vaccination according to the cut of value of the used ELISA kit (654) Biocheck UK ltd Co.

The ELISA antibody titers of hatched poults revealed that the poults had a protective ELISA antibody titers through the first 2 weeks of life and this was confirmed by the results of challenge which revealed that the vaccination of turkey breeders induced a protection level of 100% at 1st day of age and 70% at the 7th day of age of the hatched poult, against challenge with virulent strain of *Salmonella Pullorum* (as in table 3, 4)

McCapes et al., 1967; **Truscott & Friars, 1972** supported earlier contentions that maternal vaccination with bacterins doesn't reduce excreaction of salmonella in progeny significantly although mortalities can be reduced.

Although the economic impact of pullorum disease in turkey, it's the first time to study the efficacy of inactivated *Salmonella Pullorum* vaccine in turkey. The vaccine induced good protection in turkey breeders and hatched poults till the 7th day of age which declined will the 21st day of age.

REFERENCES

Arora, A.K., Sandhu, K.S. and Sodhi, S.S. (1998): Comparative studies on efficacy of different vaccines against fowl typhoid in chickens. Ind. J. Ani. Sci., 68: 297-299.

Charles, S.D.; Hussein I.; Nagraja, K.V. and Sivanandan, V. (1997): Adjuvant subunit vaccine for control of *Salmonella Enteritidis* infection in turkeys. Amer. J. Vet Res., 55. (5): 336-342.

Collier, L.; Balows, A. and Sussman, M. (1998): Topely and Wilson's Microbiology and Microbiol., Infections Ninth ed., Vol.2, Arnold, Oxford. University Press InC. New York, USA, PP. 969-997.

Hewitt, E.A. (1928): Bacillary white diarrhea in baby turkeys. Cornell Vet., 18: 272-276.

Kauffmann, F. (1972): Serological diagnosis of Salmonella species. Kauffman white Sceme Minkagaard Copenhagen, Denmark.

M.Z. Uddin, M.S.R. Khan, F. Begum, M.A. Mannan (2009): Efficacy study of bio-typhoid vaccine against fowl typhoid in back yard layer chicken. J. Bangladesh Agril. Univ. 7(2): 295-299, 2009.

Mc capes, R.H., Coffland, R.T. & L.E. (1967): Challenge of turkey poults originating from hens vaccinated with *Salmonella-Typhimurium* bacterin. Avian Diseases, 11, 15-24.

Padmanaban, V.D., Mittal, K.R. and Gupta B.R. (1981): Cross protection against fowl typhoid: Immunizayion trails and humoral immune response. Dev. Comp. Immunol., 5: 301-312.

Priyantha, M.A.R. (2009): An overview: vaccination to control fowl typhoid in commercial layers.Wild J. Anim. Sci., 52: 53-56.

Rettger, L.F. (1900): Septicemia among young chickens.NY. Med, J., 71: 803-805.

Rettger, L.F. (1909): Further studies on fatal septicemia in young chickens or "white diarrhea". J. Med. Res., 21: 115-123.
Truscott, R.B. and Friars, G.W. (1972): The transfer of endotoxin induced immunity from hens to poults. Canadian Journal of comparative Medical and Veterinary Science, 36, 64-68.
Zhang-Barber, L., Turner, A.k. and Barraw, P.A. (1999): Vaccination for control of Salmonella in poultry. Vaccine, 17: 2538-2545.
Zhang-Barber, L., Turner, A.k., Dougan, G. and Barraw, P.A. (1998): Protection of chickens against experimental fowl typhoid using anuoG mutant of Salmonella serotype Gallinarum.Vaccine, 16: 8899-903.

تحضيروتقييم لقاح السالمونيلا بلورم في الرومي

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الملخص العربي

تم تحضير لقاح سالمونيلا بلورم مثبط ثم تقييم الكفاءه المناعيه للقاح المحضر في امهات الرومي .وقد تم تتبع الاستجابه المناعيه لكل من الامهات وكتاكيت الرومي باستخدام اختبار الاليزا والذي تبين منه ارتفاع المستوي المناعي في مصل الامهات بدايه من الاسبوع الثالث للتحصين حتي الاسبوع العاشر وكذلك في اليوم الاول بعد الفقس لكتاكيت الرومي. وكذلك لوحظ مستوي جيد من الحمايه عند اجراء اختبار التحدي في كتاكيت الرومي حيث كانت نسبه الحمايه ١٠٠% في الكتاكيت عمر يوم و ٧٠% في الكتاكيت عمر ٧ ايام. بالمقارنه مع فقس كتاكيت الرومي الغير محصنه فقد كانت نسبه الحمايه للكتاكيت الغير محصنه ١٥% و ٢٠% علي التوالي. ومن هذا يمكن الاستنتاج ان هذا اللقاح المثبط ذو كفاءه مناعيه وحمايه عاليه ضد عدوي السالمونيلا بللورم في الرومي وفي فقس كتاكيت الرومي من امهات محصنه كمناعه اميه وخاصه في الاسبوع الاول من الفقس .