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PREPARATION AND EVALUATION OF INACTIVATED SALMONELLA TYPHIMURIUM OIL ADJUVANTED VACCINE IN PIGEON

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A B S T R A C T

The present work was designated to prepare a whole-cell formaldehyde inactivated *Salmonella typhimurium* (*S. typhimurium*) vaccine using a local isolate obtained from naturally infected pigeons. The vaccine was prepared as double emulsified oil adjuvanted vaccine. Evaluation of such preparation following the quality control tests revealed that it is stable, free from foreign contaminants, safe and immunogenic. Fifty 4-weeks old pigeons were vaccinated twice subcutaneously with a dose of 0.3ml with 3 weeks intervals. Other twenty pigeons were kept as non vaccinated control. All birds were challenged orally against a virulent *S. typhimurium* organism using 10^9 colony forming units per bird. Clinical and necropsy examinations revealed that vaccinated birds showed 84% protection while non vaccinated birds were unable to withstand the challenge. *S. typhimurium* was detected in fecal shedding of challenged vaccinated birds at ratio of 13.3% on the first week post challenge but it could not be detected by the 4th week while this ratio was 64.3% and 20% from challenged non-vaccinated pigeons at the same periods. On the other hand, the organism was recovered from vaccinated challenged pigeons at ratio of 20% from the heart blood, liver and spleen and 25% from caecal junction on the 4thweek post challenge while these ratios were 66.6 and 83.3 % from non-vaccinated challenged birds. So, it could be clearly evident that the experimentally prepared vaccine is safe and potent vaccine which able to protect pigeons against *S. typhimurium* infection.

KEY WORDS: Challenge, Pigeons, Salmonella Typhimurium, Vaccine.

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1. I N T R O D U C T I O N

Salmonellosis is a major bacterial disease of pigeons resulting in gastroenteritis, arthritis, oophoritis or orchitis and granulomatous inflammation in all possible internal organs with high mortality rate [7]. The clinical signs included predominantly weight loss, diarrhea, polyuria, lameness and inability to fly.

The ability of *Salmonella* serovar *typhimurium* (*S. typhimurium*) to persist intracellular inside pigeon macrophages resulted in the development of chronic carriers which maintain the infection in the flock [11]. Treatment of infected flock is difficult since even long-term antibiotic therapy may leave subclinical carriers that keep salmonella infection in lofts going [18]. Recently, a newly developed salmonella bacterin vaccine has been marketed [6] demonstrating a significant decrease of mortality in vaccinated pigeons following intramuscular challenge with *S. typhimurium*. However the widespread of usage of antibiotics has led to emergence of multiple antibiotic-resistant bacteria. This problem directed the attention toward the increased demand

to effective vaccine that helps to control such important zoonotic infection [1]. The present work was planned to prepare and evaluate a specific inactivated oil vaccine able to protect pigeons against Salmonellosis.

2. MATERIAL AND METHODS

2.1. *Salmonella typhimurium*:
A local isolate was obtained from naturally infected pigeons was characterized as *S. typhimurium* depending on colonial characters on salmonella shigella medium, biochemical and serological properties [8, 9]. This isolate was used for vaccine preparation and challenge of vaccinated pigeons.

2.2. Pigeons:
Ninety of 4-weeks old pigeons were confirmed to be free from Salmonella infection and its antibodies. These birds were divided as follow: 20 pigeons were used in the safety test of the prepared vaccine. 70 pigeons were used in the potency test.

2.3. Mice:
A total of 250 Swiss albino mice of about 15-20g body weight supplied by Veterinary Serum and Vaccine Research Institute, were used for determination of LD_{50} of *S. typhimurium* [13] and to test the vaccine safety.

2.4. Vaccine preparation:
S. typhimurium vaccine was prepared using the isolated strain [19]. The final bacterial suspension was adjusted to contain 5×10^9 colony forming units/ml then inactivated by adding 0.3% formalin. The adjuvant used consisted of mineral oil, sorbitan monooleate and Tween 80 using double emulsification methods (water in oil in water)

2.5. Quality control testing of the prepared vaccine:

2.5.1. Emulsion stability:
Drop test was carried out according to according to the criterion defined by Becher [2], briefly 2 drops of the emulsion were placed separately on a clean glass slide and each drop was mixed well either with a drop of oil or water. Water in oil emulsion blends readily with oil but does not with water. In addition, vaccine storage at 4°C did not result in separation of the oil from the aqueous phase.

2.5.2. Sterility test:
Testing the freedom of the prepared vaccine from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma) was carried out following the directions of the British Veterinary Codex [3].

2.5.3. Safety test:
Safety of the prepared vaccine was tested according to British Veterinary Codex [3] through inoculation of the double dose subcutaneously in each of 20 pigeons which kept under daily observation for 14 days.

2.5.4. Potency test:
Seventy pigeons were divided as follow: 50 pigeons were vaccinated with the prepared vaccine with 2 doses of 0.3ml inoculated subcutaneously in the dorsal aspect of the neck with 3 weeks intervals depending on the recommended dose by Proux et al. [13]. 20 pigeons were kept without vaccination as control.
All birds were housed in separate isolates under hygienic measures receiving adequate ration and water. Serum samples were obtained regularly on week intervals to follow up the induced antibody levels up to 14 weeks post the first vaccination (11weeks post second vaccination).

2.5.5. Challenge test:
Vaccinated and non-vaccinated pigeons were challenged with the virulent *S. typhimurium* using 0.1ml of 10^9 colony

forming units/ml inoculated intramuscular according to Vereecken et al. [19].

2.6. Serological evaluation of pigeon immune response to the prepared vaccine:
2.6.1. Tube agglutination test:
Tube agglutination test was carried out for monitoring salmonella following the method described by Cruickshank et al. [5].

2.6.2. Micro-agglutination test:
Micro-agglutination test was carried out as described by Brown et al. [4] and Thaxton et al. [14].

2.7. Shedding of Salmonella typhimurium from experimental pigeons:
Shedding of *S. typhimurium* was detected in fecal samples collected from challenged vaccinated and non-vaccinated pigeons weekly up to 4 weeks post challenge using salmonella shegella medium.

2.8. Recovery of Salmonella typhimurium from experimental pigeons:
On the fourth week post challenge, samples were collected from the heart blood, liver, spleen and caecal junction from 20 vaccinated and 6 non- vaccinated challenged pigeons for recovery of the organism.

3. RESULTS AND DISCUSSION

Paratyphoid caused by *S. typhimurium* is the main bacterial disease affecting pigeons. The organism ability to persist intracellularly inside pigeon macrophages, results in the development of chronic carriers maintaining the infection in a flock [11].

Table 1 Geometric mean of *Salmonella typhimurium* antibody titers in pigeons using tube agglutination test

	antibody titers													
		Post-vaccination											Post-challenge	
Pigeon group	0	1 W	2 W	3 W	2nd dose	4 W	6 W	8 W	10 W	12 W	14 W	Ch*	1 W	2 W
Vaccinated	8	46	86	106		394	597	755	905	1114	1194		597	1280
Non-Vaccinated	6	8	7	8		10	11	8	6	8	10		92	121

Ch* i.e. challenge.

Table 2 Geometric mean of *Salmonella typhimurium* antibody titers in pigeons using micro-agglutination test

	antibody titers													
		Post-vaccination											Post-challenge	
Pigeon group	0	1 W	2 W	3 W	2nd dose	4 W	6 W	8 W	10 W	12 W	14 W	Ch*	1 W	2 W
Vaccinated	7	49	80	113		343	557	735	970	1040	1194		640	1280
Non-Vaccinated	8	10	10	8		9	8	10	8	7	8		86	106

Ch* i.e. challenge

Table 3 Protective efficacy of the prepared inactivated *Salmonella typhimurium* vaccine

Pigeon group	Number of challenged Pigeons	Number of dead birds post challenge 1W	2W	3W	4W	Dead/ survived	Mortality Rate	Protection rate
Vaccinated	50	6	2	0	0	8/50	16%	84%
Non- vaccinated	20	6	4	3	1	14/20	70%	30%

Table 4 Fecal shedding of *Salmonella typhimurium* from challenged pigeons

Pigeon group	Number of positive shedding/ survived birds post challenge			
	1WPC*	2WPC	3WPC	4WPV
Vaccinated	2/15 (13.3%)	2/16 (12.5%)	1/15 (6.6%)	0/15 (0%)
Non- vaccinated	9/14 (64.3%)	6/10 (60%)	2/6 (33.3%)	1/5 (20%)

*WPC= week post challenge

Table 5 Recovery of *Salmonella typhimurium* from challenged pigeons

Pigeon group	Number of positive samples for *Salmonella typhimurium* recovery			
	Heart blood	Liver	Spleen	Caecal junction
Vaccinated	4/20 (20%)	4/20 (20%)	4/20 (20%)	5/20 (25%)
Non- vaccinated	4/6 (66.6%)	4/6 (66.6%)	4/6 (66.6%)	5/6 (83.3%)

Vaccination appears to be the most important and economical means for control of infectious diseases and has contributed in the eradication of some of them. For these considerable reasons, efforts have been done to develop *S. typhimurium* vaccines which induce protective immunity for vaccinated birds [20].

An inactivated oil double emulsion *S. typhimurium* vaccine was prepared from a locally isolated strain from naturally infected pigeons. Application of quality control tests on such vaccine revealed that it is free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma), stable and safe showing no post vaccinal reactions in vaccinated birds in agreement with the directions of USA-CFR [16].

Tube agglutination test was performed to follow up the induced immune response of vaccinated pigeons with the prepared *S. typhimurium* vaccine. The results demonstrated in table (1) showed that there was a detectable increase in the geometric mean of *S. typhimurium* antibody titers in vaccinated pigeons, from 8 before vaccination to 106 by the third week post the first vaccination. Such rising in antibody titer was continues to reach 1194 by the 11th week post the second vaccination. Following the challenge of vaccinated and non-vaccinated pigeons, there was a decline in antibody titers then to 579 by the first week followed by an abrupt increase to 1280 by the 2nd week post challenge of vaccinated birds while these titers, in non-vaccinated birds were 92 and 121 by the 1st and 2nd week post challenge respectively in survived birds. In addition the micro-agglutination test revealed results parallel to those of the tube agglutination test as demonstrated in table (2) where there was an increased in antibody titers in vaccinated pigeons from 7 pre-vaccination to 113 by the 3rd week after administration of the first dose then increased gradually to reach 1194 by the 11th week after the second dose (14 week after the first week). Challenge of vaccinated birds against the virulent organism resulted in reduction of such antibody titers to 640 by the first week post challenge then re-increased to 1280 on the second week post challenge. Non-vaccinated challenged survived pigeons showed antibody titers of 86 and 106 by the first and second week post challenge respectively. These findings come in agreement with Nakamura et al. [10] and Uyttebroek et al. [17] who recommended the use of formalized inactivated oil emulsion *S. typhimurium* vaccine for protection of chicken against infection of *S. typhimurium* and Pritchard et al. [12] who used the micro-agglutination test for estimation of *S. typhimurium* antibody titers.

Concerning the protection efficacy of the prepared *S. typhimurium* vaccine; table (3) showed that it was evident that 84% of

vaccinated pigeons were protected against challenge with the virulent organism, meanwhile 70% of challenged non-vaccinated pigeons were unable to withstand the experimental infection confirming that the prepared vaccine is a potent vaccine able to protect pigeons against infection through the induction of sufficient antibody titers in agreement with Vogel et al. [20]. However, Barrow [1] suggested that the degree of immune responses to *Salmonella* depend on host species and *Salmonella* serotype infection.

The tabulated results in table (4) revealed that *S. typhimurium* could be detected in fecal shedding of challenged vaccinated birds with the ratio of 13.3% on the first week post challenge but it could not be detected by the 4th week while this ratio was 64.3% and 20% from challenged non-vaccinated pigeons on the first and fourth week post challenge respectively. On the other hand, table (5) showed that the organism could be recovered from vaccinated challenged pigeons with the ration of 20% from the heart blood, liver and spleen and 25% from caecal junction on the 4th week post challenge while these ratios were 66.6 and 83.3 % from non-vaccinated challenged birds. These results came confirming by those of Timms et al. [15] and Uyttebroek et al. [18] who found that formalized *S. typhimurium* vaccine protected pigeons against experimental challenge with shedding of the organism on the same period with declined rate post challenge which indicated that the highest incidence of the organism was that in the caecal junction. In addition, Barrow [1] concluded that the efficacy of vaccine preparation is judged by the level of intestinal and systemic colonization, morbidity and mortality rates after vaccination and experimental infection using oral or parental routes of administration.

So, depending on the present obtained results, it could be concluded that the prepared inactivated oil emulsion S. typhimurium vaccine is a safe potent vaccine able to protect pigeons against salmonellosis.

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تحضير وتقييم لقاح السالمونيلا تيفيميوريوم المثبط الزيتى للحمام

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الملخص العربى

تم خلال هذا العمل تحضير لقاح مثبط زيتى مزدوج الاستحلاب من ميكروب السلمونيلا تيفيميوريوم المعزول محليا من حمام مصاب طبيعيا هذا وقد تم استخدام الميكروب بكامله وتثبيطه بالفورمالين وعند إخضاع هذا اللقاح لاختبارت الجودة تبين أنه آمن حيث لم تظهر أية أعراض مرضية على الحمام المحصن كما أنه فعال حيث احدث ارتفاعا فى الأجسام المناعية النوعية ضد المرض باستخدام جرعتين كل جرعة عبارة عن 0.3 مل بينهما فترة زمنية ثلاثة أسابيع تحقن تحت الجلد كما أظهرت نتائج اختبارى التلزن الأنبوبى والتلزن الدقيق أن الأجسام المناعية تصل أقصاها بعد الأسبوع الرابع عشر من الجرعة الأولى (11 أسبوع بعد الجرعة الثانية) بمعايير 1280 وعند إجراء أختبار التحدى للطيور المحصنة وغير المحصنة كانت نسبة الحماية لهذه الطيور 84، 30% على التوالى وقد أظهرت النتائج العملية أن الميكروب يتواجد فى زرأ الطيور المحصنة بعد اختبار التحدى بنسب 13.3، 0% عند الأسبوع الأول والرابع على التوالى بعد التحدى بينما كانت هذه النسب 64.3 ، 20% فى الطيور غير المحصنة بعد التحدى على نفس الفترات كذلك تبين أن أعلى نسبة لتواجد الميكروب فى الحمام بعد تعرضه لاختبار التحدى يكون فى اتصال الزائدة الدودية من الأمعاء. مما سبق يتضح أن اللقاح التجريبى المحضر للسلمونيلا تيفيميوريوم المثبط الزيتى مزدوج الاستحلاب هو لقاح آمن وفعال ذو قدرة على وقاية للحمام ضد عدوى السلمونيلا تيفيميوريوم.