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IMMUNOLOGICAL STUDY ON CHICKENS VACCINATED WITH SALMONELLA ENTERITIDIS INACTIVATED VACCINE USING DIFFERENT ADJUVANTS

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SUMMARY

This study was carried out to investigate the efficacy of the locally prepared **Salmonella enteritidis (S.enteritidis)** bacterin which adjuvanted with three different adjuvants (mineral oil, aluminum hydroxide gel and Monatanide ISA 70). A number of 80 SPF chickens were divided into four groups. Groups I, II and III were vaccinated with different vaccines while group IV kept as unvaccinated control. Other 30 SPF chickens were used for safey test of vaccines. Specific IgG_1 antibodies detected by ELISA in sera of vaccinated birds showed higher antibody titers in chickens vaccinated with bacterin adjuvanted with Montanide ISA 70. Both bacterins emulsified in mineral oil (group I) and Montanide ISA 70 (group III) gave 100% protection against challenge with virulent **S.enteritidis** strain as well as provide protection of internal organs including, liver, spleen and prevent intestinal colonization following challenge. While birds vaccinated with **S.enteritidis** bacterin adjuvanted with aluminum hydroxide gel (group II) gave 85 % protection.The faecal shedding of the organism at 2^{nd} week post challenge as well as reisolation from internal organs was of 10 % and 20 % respectively. Therefore, the prepared inactivated **S.enteritidis** vaccine adjuvanted with Montanide ISA70 is recommended for field application as better and effective control for **S.enteritidis** in chickens.

INTRODACTION

Salmonella enteritidis remains one of the main cause of food borne illness considered to be the most important pandemic zoonosis produced under natural condition **(Javier Ochoa et al., 2007).** The potential for **S. enteritidis** to be transmitted to humans by contaminated eggs has been a significant public health issue

(Gast and Steven, 1996). S. enteritidis has been associated with unusually high mortality and morbidity in commercial chickens **(Barrow, 1991).** It has the ability to spread throughout and between flocks and to be deposited in the contents of eggs laid by infected hens, this horizontal transmission is directly related to their ability to colonize intestinal tracts of infected chickens. Similary the potential for **S.enteritidis** to be deposited in egg contents depends on the ability to disseminate to reach internal tissues of infected hens **(Nagraja and Rajashekara 1999)**, The infectivity of **S. enteritidis** for chickens assessed in both intestinal colonization and organ invasion. So vaccination is a practical approach to control **S. enteritidis** in chickens.

S. enteritidis bacterin has been reported by various workers **(Gast et al., 1992, Hussain 1994, Nakamura et al., 1994 and Timmes et al., 1990).** It is documented to protect against experimental Salmonella infection. There is a need for continual improvement of immunizing agents through the use of safe and effective immunomodulants (**Hussain , 1994).** There was an attempt to the use of inactivated **S. enteritidis** vaccines in breeder chickens included a comparison between alhydrogel and oil emulsion adjuvant **(Timmes et al., 1990).**

The objective of this study was to prepare formalized inactivated **S. enteritidis** vaccines adjuvanted with three different adjuvants mineral oil, aluminum hydroxide gel and Montanide ISA70, and to evaluate immunizing efficacy of these preparations through seroconversion and challenge test . Detection of faecal shedding of the organism in challenged birds and reisolation of it from internal organs.

MATERIALS AND METHODS

1. **Bacterial strain**

Local strain of **Salmonella enteritidis**, isolated from chickens, was obtained from Veterinary Serum and Vaccine Research Institute, Abbassia , Cairo was used for vaccine preparation.

2. Laboratory animals

2.1 Chickens

A total of 80 specific pathogen free (SPF) 8 weeks old chickens were supplied by the central laboratory for quality control of veterinary biologics, Abbassia , Cairo. These chickens were used in potency test. Other 30 SPF chickens were used for safety test of prepared vaccines.

2.2 Swiss mice

A total of 25 Swiss Webster mice 20-25g weight were used for passage of bacterial strain.

3. Vaccine preparation

Inactivated **S. enteritidis** vaccine was prepared according to **Nagaraja et al.(1991).** The final concentration of bacterial suspension was adjusted to contain 10^{11} organism/dose (0.5ml). Finally bacterial culture was inactivated by adding 0.3 % formalin. The prepared bacterin was divided into 3 portions. The first one was adjuvanted with mineral oil in a ratio of 1:1 according to **(Nagraja et al., 1991)**. The second one was adjuvanted with Montanide (ISA70 Sepic France) in a ratio of 1:1 as described by **Eman et al., (2010)** and the third one was adjuvanted with aluminum hydroxide gel in concentration of 25 % according to **Nahed et al., (1999)**.

4. Vaccine evaluation:

It was done according to the following:

4.1 Sterility test

It was done according to the regulation of **OIE (2012) Mannal** .

4.2 Safety testing

It was carried out by subcutaneous injection of chicken with a double field dose (1ml) of the prepared vaccines and observed for two weeks post inoculation.

5- Experimental design

Eighty chickens were divided into 4 groups each of 20 chickens. Three groups of chickens were vaccinated subcutaneously with 0.5 ml of adjuvanted **S.enteritidis** vaccine at 8 weeks of age and revaccinated at 11 weeks of age. Group IV was left as control non vaccinated chickens.

Group (I): vaccinated with **S.enteritidis** vaccine adjuvanted with mineral oil.

Group (II): vaccinated with **S.enteritidis** vaccine adjuvanted with aluminum hydroxide gel.

Group (III): vaccinated with **S.enteritidis** vaccine adjuvanted with Monatanide ISA 70.

Group (IV): Non vaccinated control.

All groups were challenged with virulent **S.enteritidis** strain at 14 weeks of age according to **Adriaesen et al., (2007)**. Chickens were kept under strict hygienic measure and supplied with adequate ration and water. Serum samples were aseptically collected from each group of chicken just before vaccination at weekly intervals after first and second vaccination.

6- Enzyme linked immunosorbent assay (ELISA)

Serum samples were assayed for the presence of specific antibodies using ELISA kit CX-OVO Flock Screen SE antibody ELISA kit. Cat # Vol15).

7- **Challenge test**

Faecal samples were collected from challenged vaccinated and non-vaccinated birds weekly up to 4 weeks post challenge to detect **S. enteritidis** was performed on Salmonella Shigella (S.S.) agar medium.

8-**Re-isolation of Salmonella enteritidis from challenged chickens**

Four weeks post challenge, samples of heart, blood' liver, spleen and caecal junction were collected from vaccinated and non-vaccinated challenged chickens for recovery of the organism.

RESULTS AND DISCUSSION

Vaccination used primarily for public health reasons as the vaccines are targeted for the most often reported serovars of human infection **(Salmonella enteritidis and Salmonella typhimurium)**.The greatest interest has been directed towards vaccination against **S. enteritidis** infection in chickens **Gast and Bear (1993) and Nagaraja and Rajashekara (1999).** Different preparations of classical killed **Salmonella enteritidis** have been reported by **Barrow (1991), Barbour et. al., (1993) and Gast et al., (1993).**In this study a locally prepared **S. enteritidis** bacterin was adjuvanted into three different adjuvants (mineral oil, aluminum hydroxide gel and Montanide ISA 70). The prepared vaccine formulations proved to be pure, sterile, safe for chickens following the **OIE manual (2012)**.

The comparative efficacy for sero-conversion were illustrated in table (1) by Enzyme linked immunosorbent assay (ELISA) which have been developed for detection of Salmonella antibody titers, **Christopher et al.,(1996).** The three formulations of the vaccine gave significant levels of antibodies in vaccinated birds in comparing to the non-vaccinated ones. It is clearly that group III which was vaccinated with **S. enteritidis** bacterin adjuvanted with Montanide ISA 70 has higher antibody response than other groups (group I vaccinated with mineral oil adjuvanted vaccine and group II vaccinated with aluminum hydroxide adjuvanted vaccine) all over the vaccination intervals. The injection of killed Salmonella vaccines or purified porins give rise to IL4 dominated The type response and low levels of DTH and high levels of specific IgG1 isotype **Thatte et al.**, **(1993) Pitro and Nathalie (2003)**.

From the data presented in table (2) it is clearly that both group I and III gave 100% protection against virulent challenge while group II gave 85 % protection in comparison with control group. These findings were in agreement with that of **Eman et. al., (2010)** who used Montanide ISA 70 as an adjuvant for fowl cholera vaccine in chickens and found that its superiority in protection

against virulent challenge in comparing with mineral oil vaccine. Similar observation reported by **Nahed et al., (1999)** who recorded 90% protective ability in turkeys immunized with Salmonella vaccine adjuvanted with aluminum hydroxide.

Faecal shedding is completely reduced in group I and III (table 3) along the 4 weeks post challenge. **S. enteritidis** were isolated from group II which vaccinated with aluminum hydroxide adjuvanted vaccine at 2nd week post challenge. Aluminum hydroxide gels is used in vaccines where a humoral immune response against a protein antigen is required, especially for the stimulation of IgG and IgE, in addition it is doubtful whether a cell mediated response or delayed-type hypersensitivity can be elicited with adsorbed antigen **Stewart-Tull (1996).**

The protective effect against **S. enteritidis** invasiveness into vesral organs (liver, spleen and Caecal Junction) 4 weeks post challenge were shown in table (4) . This is due to vaccination with two doses of inactivated vaccine show high level of serum antibody response result in protection against **S. enteritidis** invasiveness in liver, spleen as well as caecal junction in group I and III in comparison with control group that showing 60 % invasiveness. This observation are in agreement with **Zeina et al. (2008)** who stated that hens vaccinated twice with inactivated vaccine result in protection against invasiveness as bacterins help in neutralizing the invasive **S. enteritidis** organism which used in challenge of chickens thus leading to clearance of infection from spleen and liver. Control group could not produce level of serum antibodies as a result of infection caused by challenge organism which could not protect bird against invasiveness of **S. enteritidis**, this is due to deprivation of vaccination and absence of memory cells **Barrow et al. (1991) and finally et al., (1989).**

It is clearly that Montanide **S. enteritidis** oil adjuvanted bacterin conferred sufficient protection at the Gut level to reduce inavasion of the tissues and intestinal shedding after oral challenge. The colonization of spleen, liver and caecal junction reduced in oil

vaccine in comparison with aluminum hydroxide gel **Cooper et al., (1992).** Both oil vaccine able to confer protective effect against **S. enteritidis** demonstrated by a reduction of colonization of the liver, caecal mucosa with S. enteritidis challenge strain these results corresponds with the results of **Hanan et al., (2012)** who stated that a protective effect against **S. enteritidis** after vaccination with inactivated combined vaccine of S. **typhimurium and S. enteritidis**.

So vaccination against **S.enteritidis** can reduce public health risk that caused by Salmonella in poultry or its products by reducing colonization in internal organs as well as reducing faecal shedding. Vaccinated bird result in reduced contamination of table egg and environment. It could be recommended to use **S. Enteritidis** bacterin adjuvanted to Montanide ISA 70 as it has the privilege of ease in injection and giving a good level IgG1 (a higher detectable level of IgG1) together with good farming and hygienic practice for successful control of Salmonella infection in poultry farms.

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دراسة مناعية علي الدجاج المحصن بلقاح السالمونيلا انتريتيديس المثبط بإستخدام محفرات مختلفة.

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**** المعمل المركزي للرقابة علي المستحضرات الحيوية.**

أجريت هذه الدراسة لتحضير و تقييم لقاح السالمونيلا إنتريتيديس المثبط لقاح السالمونيلا المحفز بثلاث أنواع من المحفزات المناعية و هي الزيت المعدني و الألومنيوم هيدروكسيد جيل و المونتانيد و ذلك لحماية الدواجن من عدوي السالمونيلا إنتريديس. تم إجراء التجربة بإستخدام ٨٠ دجاجة خالية من الممسببات المرضية حيث ثم تقسيمها الي أربعة مجموعات. تم تحصين المجموعات الأولي و الثانية و الثالثة بالأنواع المختلفة من اللقاح بينما تركت المجموعة الرابعة غير محصنة كضابط ، بالإضافي الي عدد ٣٠ دجاجة لإجراء إختبار سلامة اللقاحات . بإستخدام إختبار الإليزا لوحظ ارتفاع معدل مستوي الأجسام المناعية في دواجن المجموعة الثالثة التي تم تحصينها باللقاح المحضر بالمونتانيد ISA 70 . وكانت نسبة الحماية لإختبار التحدي بإستخدام العترة الضارية ١٠٠% للدجاج في كلا المجموعتين الأولي و الثالثة و المحصن باللقاح المحفز بالزيت و المونتانيد ISA70 علي التوالي. كما لوحظ خلو الكبد و الطحال و الأمعاء من مستعمرات الميكروب فى تلك المجموعتين بينما كانت نسبة الحماية ٨٥% في المجموعة الثانية المحصنة باللقاح المحفز بالألومنيوم هيدروكسيد جيل كما نم عزل الميكروب من الأعضاء الداخلية للدواجن في هذه المجموعة بنسبة ٢٠%. و من هذه النتائج البحثية ينصح بإستخدام لقاح السالمونيلا إنتريتيديس المثبط و المحفز بالمونتانيد ISA70 لحماية الدواجن من عدوي السالمونيلا إنتريتيديس.