Vet. Med. J., Giza. Vol. 59, No. 3 (2011): 73 - 81

The use of Swiss mice in comparison with the specific host for the evaluation of live attenuated *Salmonella* Typhimurium and *Salmonella* Enteritidis vaccine for chicken

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Received: 14/03/2011
Accepted: 20/03/2011

SUMMARY

In this study, a total of 600 SPF chicks and 1200 Swiss mice were used to compare between both in the evaluation of live *Salmonella* Enteritidis and *Salmonella* Typhimurium vaccines. Results revealed that, 81% and 78% protection were obtained after vaccination and challenge with *Salmonella* Enteritidis and *Salmonella* Typhimurium respectively. At the same time, the Swiss mice gave protection reached to 74% and 71% for both organisms when vaccinated and challenged orally, while gave 80% and 82% when vaccinated and challenged intraperitoneally, respectively. The results showed that, the Swiss mice could be used as a model in the evaluation of live salmonella vaccines specially when the intraperitoneal route was used in the vaccination and evaluation programs.

INTRODUCTION

Many attempts have been made to control salmonellosis in animals and poultry with killed vaccines, but the obtained protection was short lived and heavy infection may occur. Specific live vaccines should be produced to protect chickens and animals against salmonellosis *(Knivett and Stevens, 1971)*. In developed countries, poultry and poultry products are considered as a major source of Salmonella which is one of the leading causes of human gastrointestinal disorders. Vaccination of poultry against salmonellosis can be used to decrease its incidence in poultry flocks *(Anonymous, 2006)*.

Attenuated Salmonella strains have been studied intensively as live carriers of heterologous vaccine antigens delivered by mucosal or parenteral routs (*Brey et al., 1991*). Orally delivered attenuated Salmonella strains induce both systemic and secretory immune response against the carrier strain as well as

the heterologous passenger antigen *(Garmory et al., 2002)*.

Recently, the need to use live Salmonella vaccine in poultry farms highly increased and strictly recommended by WHO. Also the evaluation of these types of vaccines are applied in the specific host so, the present study was planned to study the usage of the Swiss mice as a model in the evaluation of live Salmonella vaccine for chicken in comparison with the use of specific host.

MATERIALS AND METHODS

1-Vaccines:

a) Live *Salmonella.* Enteritidis vaccine was supplied by the CLEVB.

b) Live *Salmonella.* Typhimurium vaccine was supplied by the CLEVB.

2-Bacterial strains:

a) Salmonella. Enteritidis (K482/91) virulent strain were supplied by CLEVB strain bank.

b) Salmonella. Typhimurium (K284/93) virulent strain were supplied by CLEVB strain bank.

3-Chicks:

A total of 600 one day old SPF chicks were supplied by the CLEVB, reared in specific isolators up to 4 weeks of age and subdivided into 6 groups. The first group contained 100 chicks to be used in the determination of LD_{50} of *S.* Enteritidis virulent strain. The 2nd group was the same as the first one but used for determination of LD_{50} of virulent *S.* Typhimurium strain. The third and fourth groups were 100 chicks of each and were vaccinated with *S.* Enteritidis and *S.* Typhimurium live vaccines, respectively. The last 2 groups were comprised 100 chicks each and were used as unvaccinated control groups.

4-Swiss mice:

A total of 1200 Swiss mice (20-25 g) were subdivided into 12 groups each comprises 100 mice. The first and second groups were used for determination of the LD_{50} of *S.* Enteritidis virulent strain when infected either orally or intraperitoneally, respectively. The 3rd and 4th groups were used for the determination of the LD_{50} of *S.* Typhimurium virulent strain. The 5th and 6th groups of mice were vaccinated with the live *S.* Enteritidis vaccine either orally or intraperitoneally. Meanwhile, the 7th and 8th groups were used for the oral and intraperitoneal vaccination of live *S.* Typhimurium vaccine. The rest four mice groups were used as unvaccinated control groups.

5-Determination of LD_{50}:

Using tenfold dilution of the original virulent salmonella cultures were used for the determination of the LD50 in both chicks and mice according to Reed and Muench method as described by Davis *et al* (1973) as follows:

Laboratory animals groups	Strains	Route
Chicks group (1)	*S.* Enteritidis virulent strain	Oral
Chicks group (2)	*S.* Typhimurium virulent strain	Oral
Mice group (1)	*S.*. Enteritidis virulent strain	Oral
Mice group (2)	*S.*. Enteritidis virulent strain	Intraperitoneal
Mice group (3)	*S.* Typhimurium virulent strain	Oral
Mice group (4)	*S.* Typhimurium virulent strain	Intraperitoneal

6-Vaccination programs:

The freeze dried live *Salmonella* Enteritidis vaccine was reconstituted in sterile water and 0.1 ml (containing 1 x 10^8 CFU/dose) were administered either orally or intraperitoneal in 5^{th} and 6^{th} mice groups, respectively. Also 0.1 ml was administered orally in the chicks group (3). The same procedure was applied on the mice groups (7) and (8) and so chicks group (4) using the freeze dried live *Salmonella* Typhimurium vaccine.

7-Challenge test:

Four weeks post vaccination, all vaccinated groups either chicken or mice were challenged with the corresponding LD_{50} as follows:

Groups	Treatment	Challenge Rout
Chicks group (3)	Vaccinated orally with live *S.* Enteritidis vaccine.	Oral
Chicks group (4)	Vaccinated orally with live *S.* Typhimurium vaccine.	Oral
Mice group (5)	Vaccinated orally with live *S.* Enteritidis vacoine.	Oral
Mice group (6)	Vaccinated Intraperitoneally with live *S.* Enteritidis vaccine.	Intraperitoneal
Mice group (7)	Vaccinated orally with live *S.* Typhimurium vaccine.	Orally
Mice group (8)	Vaccinated I Intraperitoneally with live *S.* Typhimurium vaccine.	Intraperitoneal

RESULTS AND DISCUSSION

Salmonellosis is one of the most common food-borne bacterial diseases in the world. The great majority of salmonella infection in humans is food born with *Salmonella* Enteritidis and *Salmonella* Typhimurium accounting for a major part of the problem *(OIE, 2010)*. Also the primary source of salmonella infections in poultry flocks is either through infected poultry or through vertical transmission in poultry farms. Thus introduction of these organisms in poultry flocks can be well controlled by standard biosecurity measures *(Shivaprasad, 2003)* and vaccination programs which is an additional effective control tool particularly in high field infection pressure farms, particularly multiage ones *(Barrow, 2007)*. Recently great attentions were undertaken for salmonella vaccination especially with the live type vaccines.

At the beginning of our experiment, it was very important to determine the LD_{50} of the challenge virulent strains of Salmonella species either in chicken or in mice. The

results as shown in Table (1) revealed that the LD_{50} determined in chicken was $7.5x10^3$ and $1.2x10^5$ CFU regarding *Salmonella* Enteritidis and *Salmonella* Typhimurium, respectively, while in mice the LD_{50} was determined as $3.2x10^4$ for *Salmonella* Enteritidis and $4.2x10^5$ for *Salmonella* Typhimurium when inoculated orally, meanwhile it was $1.3x10^3$ for *Salmonella* Enteritidis and $2.5x10^3$ for *Salmonella* Typhimurium when inoculated intraperitoneally.

Table (1): The LD_{50} of different Salmonella strains used in challenge tests in chicken and mice:

Lab Animals	Route	LD_{50}	
		S. Enteritidis	*S.* Typhimurium
Chicken	Oral	7.5×10^3 CFU	1.2×10^5 CFU
Mice	Oral	3.2×10^4 CFU	4.2×10^5 CFU
	Intra peritoneal	1.3×10^3 CFU	2.5×10^3 CFU

Young et al. (2007) used an overnight brain heart infusion *Salmonella* Typhimurium culture for the challenge in chicken. Also *Barrow et al. (1990)* used the oral route for the Salmonella challenge in chicken 38 days after initial immunization. In the same time *Knivett and Stevens (1971)* used a dose of 10^6 CFU for the challenge of *S.* Cholerasuis, *S.* Dublin and *S.* Typhimurium two weeks after the initial vaccination in mice.

Regarding the protection percent two weeks post challenge with the virulent *S.* Enteritidis strain in chicken vaccinated with live *S.* Enteritidis vaccine, the results in Table (2) indicate that, the vaccinated chicken group had a 81% protection rate in comparison with 24% for the unvaccinated control group.

Table (2): Protection percent in chicken group vaccinated with *Salmonella* Enteritidis live vaccine and challenged orally with virulent *Salmonella* Enteritidis strain:

Days Post Challenge	Protection post challenge							
	vaccinated Chicken group				unvaccinated chicken Control group			
	No. used	Survival	Death	Protection%	No. used	Survival	Death	Protection%
2nd	100	97	3	97%	100	90	10	90%
4th		92	5	92%		78	12	78%
6th		87	5	87%		64	14	64%
8th		83	4	83%		49	15	49%
10th		82	1	82%		40	9	40%
12th		81	1	81%		30	10	30%
14th		81	0	81%		24	6	24%
Total		81	19	81%		24	76	24%

In comparison, the protection percent after two weeks post challenge in mice vaccinated with live *Salmonella* Enteritidis vaccine were 74% and 80% in the vaccinated groups, respectively and were 28% and 32% in the unvaccinated control group when vaccinated orally and intraperitoneally respectively as shown in Table (3).

Table (3): Protection percent in mice vaccinated with *S.* Enteritidis live vaccine and challenged with virulent *S.* Enteritidis strain:

Days post challenge	Protection post challenge in mice groups															
	Orally vaccinated group								Intraperitoneally vaccinated group							
	Vaccinated mice				Unvaccinated mice				Vaccinated mice				Unvaccinated mice			
	No.	S	D	%	No.	S	D	%	No.	S	D	%	No.	S	D	%
2nd	100	94	6	94%	100	85	15	85%	100	97	3	97%	100	86	14	86%
4th		85	9	85%		62	23	62%		93	4	93%		62	24	62%
6th		79	6	79%		48	14	48%		89	4	89%		47	15	47%
8th		75	4	75%		40	8	40%		82	7	82%		41	6	41%
10th		74	1	74%		35	5	35%		81	1	81%		37	4	37%
12th		74	-	74%		31	4	31%		80	1	80%		34	3	34%
14th		74	-	74%		28	3	28%		80	-	80%		32	2	32%
Total		74	26	74%		28	72	28%		80	20	80%		32	68	32%

No: Number of mice used S: Survival D: Death %: Protection Percentage

A more or less similar result was obtained corresponding to the chicken and mice groups vaccinated with live *Salmonella* Typhimurium vaccine and challenged with virulent *Salmonella* Typhimurium strain as shown in Table (4).

Table (4): Protection percent in chicken group vaccinated with *S.* Typhimurium live vaccine and challenged orally with virulent *S.* Typhimurium strain:

Day Post Challenge	Protection post challenge							
	Vaccinated Chicken group				Unvaccinated chicken Control group			
	No. used	Survival	Death	Protection %	No. used	Survival	Death	Protection %
2nd	100	97	3	97%	100	90	10	90%
4th		92	5	92%		78	12	78%
6th		86	6	86%		64	14	64%
8th		81	5	81%		48	16	48%
10th		79	2	79%		36	12	36%
12th		78	1	78%		28	8	28%
14th		78	0	78%		22	6	22%
Total		78	22	78%		22	78	22%

The chicken group vaccinated with live *Salmonella* Typhimurium vaccine gave protection up to 78% comparing with 22% for the unvaccinated control groups observed up to two week post challenge. In the same time, as mentioned in Table (5), mice group vaccinated orally with live *Salmonella* Typhimurium vaccine showed protection of 71% as compared to 25% for the unvaccinated control group, while the mice group vaccinated intraperitoneally with the same vaccine gave 82% protection in comparison with 34% for the unvaccinated control group two weeks post challenge with the virulent *Salmonella* Typhimurium strain.

Table (5): Protection percent in mice vaccinated with *live S.* Typhimurium vaccine and challenged with virulent *S.* Typhimurium strain:

Day post challenge	Protection post challenge in mice groups															
	Oral route								Intraperitoneal route							
	Vaccinated mice				Unvaccinated mice				Vaccinated mice				Unvaccinated mice			
	No.	S	D	%	No.	S	D	%	No.	S	D	%	No.	S	D	%
2nd	100	93	7	93%	100	85	15	85%	100	97	3	97%	100	87	13	87%
4th		84	9	84%		61	24	61%		93	4	93%		62	25	62%
6th		78	6	78%		47	14	47%		90	3	90%		48	14	48%
8th		74	4	74%		38	9	38%		84	6	84%		43	5	43%
10th		72	2	72%		32	6	32%		83	1	83%		38	5	38%
12th		71	1	71%		28	4	28%		82	1	82%		36	2	36%
14th		71	-	71%		25	3	25%		82	-	82%		34	2	34%
Total		71	29	71%		25	75	25%		82	18	82%		34	66	34%

No: Number of mice is used S: Survival D: Death %: Protection Percentage

The results in this experiment are in accordance with the findings of other investigation using various other experimental systems. *Knivett and Stevens (1971)* used mice and chicken in the evaluation of live Salmonella vaccines and reported that oral and subcutaneous vaccinations were equally effective. Also *Diena et al. (1977)* showed that, the best protection against death from challenge organism in mice was afforded by the live and acetone treated salmonella vaccine when administrated intraperitoneally.

In the same time *Xiao-Feng et al. (2005)* stated that after oral immunization of mice with the attenuated *Salmonella* Typhimurium vaccine significant systemic immune response was induced and the serum specific IgG antibodies were much higher than the control ones. In the same direction *Kevin et al. (1995)* used BALB/c mice to study the antigen specific immunity by *Salmonella* Typhimurium after single mucosal or intravenous immunization and concluded that such vaccine could induce both humoral and

cellular immunity after oral immunization. Also *Massis et al. (2008)* used the BALB/c mice to investigate the flagellin specific serum (IgG) and fecal (IgA) antibody responses elicited in BALB/c mice immunized with attenuated *Salmonella enterica* serovar Typhimurium orally.

So from the results of these experiments and by the comparison of its finding, it could be concluded that, firstly the results obtained in chicken experiments were greatly matched with that obtained in mice experiments specially those groups vaccinated and challenge intraperitoneally. Secondary, mice could be used as an alternative model for the evaluation of live Salmonella vaccine either *Salmonella* Enteritidis or *Salmonella* Typhimurium specially when the intraperitoneal route was used for the vaccination program.

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مقارنة إستخدام الفئران السويسرية و العائل الأساسى فى تقييم لقاحات السالمونيللا الحية المستضعفة للدجاج

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المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية – العباسية – القاهرة

في هذه الدراسة تم استخدام عدد ٦٠٠ كتكوت خالي من المسببات المرضية وكذلك عدد ١٢٠٠ فار سويسري للمقارنة بين كليهما في تقييم لقاحى السالمونيلا انتريتيدس والسالمونيلا تيفيميوريم الحية. و قد أوضحت التجارب ان هناك تقارب لدرجة عالية في النتائج المتحصل عليها عند استخدام كليهما في التقييم حيث اعطت مجموعات الدجاج نسبة حماية وصلت الي ٨١% وكذلك ٧٨% عند اجراء اختبار التحدي لكل من السالمونيلا انتريتديس والسالمونيلا تيفيميوريم علي التوالي بينما اعطت مجموعات الفئران السويسرية نسبة حماية وصلت الي ٧٤% و ٧١% لميكروبين علي التوالي عند التحصين عن طريق الفم ووصلت الي ٨٠% و ٨٢% عند التحصين عن طريق الحقن داخل الغشاء البريتوني . واكدت النتائج انه يمكن استخدام الفئران السويسرية كنموذج جيد لتقييم اللقاحات الحية لميكروب السالمونيلا خاصة عند استخدامها عن طريق الحقن في الغشاء البريتوني.