TRIALS FOR APPLICATION OF SOME UNFAMILIAR TECHNIQUES TO EVALUATE THE IMMUNE RESPONSE OF CHICKENS TO FOWL CHOLERA VACCINE COMPARED WITH CHALLENGE

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ABSTRACT

During the present work a group of Knuckles chickens was vaccinated with the locally produced fowl cholera vaccine. Another chicken group was kept unvaccinated as a test control. Serum samples were obtained from all birds weekly four times where the challenge test was carried out at the 4th week post vaccination using the virulent strain of pasteurella multocida (A:1). The familiar tests used for estimation of fowl cholera antibodies as the indirect haemagglutinatin test (IHAT) and ELISA were carried out. Some unfamiliar tests used for the same purpose as the indirect fluorescent antibody technique (IFAT); serum neutralization test (SNT) in mice and agar gel precipitation test (AGPT). There was a agreement of the obtained results by these tests where the challenge and SNT showed the same protection percent (80). However IFAT showed rapid and sensitive and accurate results followed by SNT and IHAT while AGPT was the less sensitive and less accurate one. So, it could be said that the IFAT and SNT in mice could be used for estimation of fowl cholera antibodies in vaccinated chickens and to evaluate the potency of fowl cholera vaccine.

INTRODUCTION

Fowl cholera (FC) is a bacterial disease caused by Pasteurella multocida affecting chicken and turkeys. The disease is characterized by septicemia with high morbidity and mortality rates **(Briggs and Skeels, 1984)**. The disease causes high economic losses not only due to high mortality but also due to drop in egg production.Immunization against fowl cholera dates back over 90 years to the experiments of Pasteur and many successful vaccines were used to this purpose

(Heddleston, et. al., 1975; Schlink and Olson, 1987 and Saif Eldin et.al., 1992).

Evaluation of the humeral immune status of vaccinated chickens was carried out using classical tests as the indirect haemagglutination test (IHAT) as done by **Alexander and Soltys (1973); Dua and Maheswaram) 1978a); Nabed (1993) and Eman (1995)**; Enzyme linked immunosorbant assay (ELISA) as carried out by **Solano, et.al., (1983); Brigss and Skeels (1984); Dick and Johnson (1985) and Sacco et. al. (1994)**; and challenge test as reported by **Heddleston et.al. (1970); Wichmann and Stoner (1974); Chong (1984) and Ficken et.al. (1996)**.

Other unfamiliar tests could be used for the same purpose and such tests may be more accurate, sensitive and rapid. Among these tests serum neutralizatin test in mice (SNT) was used to classify Pasteurella multocida **(Roberts, 1947)** and to evaluate the immune response of vaccinated chickens **(Roberts et. al., 1947 and Boljar et. al., 1982)**. Agar gel precipitation test (AGPT) was used to a less extent by **Yusef (1935) and Heddleston (1971)** while the fluorescent antibody technique (FAT) was used as a rapid test to detect Pasteurella multocid antigen and to estimate the induced antibodies in vaccinated chickens by **Lu et. al. (1978); Chengappa et.al. (1982) and Hanan el.al. (2003)**.

The present work is aimed to detect an accurate, sensitive and rapid test other than the present used tests for evaluation of the immune response of vaccinated chickens with fowl cholera vaccine. These tests include MPT; AGPT and FAT in a comparison with IHAT; ELISA and challenge test.

MATERIAL AND METHODS

1- Fowl cholera vaccine :

The local produced inactivated fowl cholera vaccine was supplied by Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo. The vaccine was used to vaccinate the experimental chickens at the dose of 0.5ml for each bird inoculated subcutaneous in the neck according to the producer directions.

2- Virulent strain of Pasteurella multocida :

Virulent field isolate of Pasteurella multocida (A: 1) was supplied by the Central Laboratory for Quality Control of Veterinary Biologics (CLQCVB), Abbassia, Cairo. It was used for challenge of vaccinated birds using 24 hours culture adjusting its concentration to the McFarland density tube number 1and diluted as 1:9 for swabbing of the nasal cleft according to Heddleston and **Watko (1965)**.

3- Pasteurella multocida antigen :

The antigen of Pasteurella multocida (A:1) was supplied by the CLQCVB and used in ELISA: AGPT and FAT.

4- Antichiken seum conjugated with flourescine isothlocyanate :

It was supplied by the CLQCVB and used in FAT.

5- Chikens :

Fifty Knuckles one day old chicks were reared under hygienic measures up to 6 weeks of age and screened using IHAT to be sure that they were free from Pasteurella multocida antibodies. 40 birds were vaccinated with the locally produced inactivated fowl cholera vaccine while the last 10 birds were kept unvaccinated as test control. Serum samples were obtained weekly from all birds for 4 weeks post vaccination to estimate the induced antibodies using the different serological tests.

6- Mice :

120 adult Swiss albino mice were used in serum neutralization test where each 10 mice were inoculated with a dilution of vaccinated chicken serum (using 2 fold dilution) up to 210 mixed with equal volumes of 1: 10 diluted 24 hours culture of virulent Pasteurella multocida (A:1).Each mouse was inoculated I/P with 1ml of such mixture and 10mice were kept as control inoculated with the virulent strain only.

7- Indirect haemagglutination test (IHAT) :

IHAG was carried out according to **Carter and Rappy (1962).**

8- Solid phase ELISA ;

This assay was carried out following that described by **Briggs and Skeels (1984).**

9- Serum neutralizatin test in mice :

It was applied according to **Bain (1963).** The survived mice in vaccinated groups indicate that

the inoculated serum dilution was immune while unvaccinated mice dead.

10- Agar gel precipitation test (AGPT) :

It was carried out according to **Heddleston (1971).**

11- Indirect Fluorescent antibody technique (IFAT) :

The IFAT was done following the method adopted by **Habel and Salznan (1969).**

12- Challenge test :

The challenge of vaccinated chickens against the virulent Pasteurella multocoda (A:1) was carried out 4weeks post vaccination by swabbing of the nasal cleft according to **Heddleston and Watko (1965).**

RESULTS AND DISCUSSION

Regarding the estimation of antibody titers induced in chickens by bacterial vaccine some classical tests were used to be the base in this respect whoever some of them may be of low accuracy; low sensitivity and need long time and may be of high cost. Some unfamiliar tests in the bacterial field may be of a benefit providing the accuracy, sensitivity, and time saving and have low cost. So, the present work was designed to use some of these tests as AGPT; IFAT; and serum neutralization test in mice in a comparison with each of IHAT; ELISA and the challenge test.

The obtained results showed that fowl cholera antibodies were detectable in the sera of vaccinated chickens by the first week and reached their peak by the fourth week post vaccination as demonstrated by IHAT (Table-1); ELISA (Table-2); IFAT (Table-3); SNT in mice (Table-4) and AGPT (Table-5). The fowl cholera antibody titers estimated by these different serological tests in the present work could be considered of protective values where **Dua and Panduranga (1978)** showed that IHA antibody titer 64 or over were satisfy to protect chickens against challenge with virulent Pasteurella multocida; **Hofacre,et.al. (1987)** concluded that ELISA titer greater than 1000 result at least 92% protection against virulent strain and similar results were obtained by-**Zeinab (1999).** IFAT showed antibody titer (256) higher than that obtained by IHA (128) revealing the high sensitivity of such technique in addition to its rapid results as stated by **Goldman (1968); Lu, et. al. (1978); Chengappa, et. al. (1982) and Hanan, et. al. (2003).** It was found that SNT in mice resulted in values similar to those of IHA showing the agreement of the two

tests in evaluation of fowl cholera antibodies and appear to be confirm each other and similar findings were obtained by **Eman; et. al. (2003)**. AGPT gave the lowest antibody titers showing a less sensitivity. There are no available data that discuses the use of AGPT to evaluate the immune response of chickens to fowl cholera vaccine. However; the challenge test revealed that the vaccinated birds were able to survive the virulent strain with a protection rate of 80% confirming that the obtained antibody titers by the applied serological tests are of protective values. Similar findings were obtained by **Choi;et.al. (1989) and Wang and Glisson (1994)**.

From the presented results it could be concluded that IFAT and SNT in mice can be used as IHAT and ELISA for evaluation of fowl cholera immune status in birds and accordingly the potency of fowl cholera vaccine where these tests reflect the protection % induced by the vaccine saving time and cost.

Table (1): IHA titers of fowl cholera antibodies in vaccinated chickens

Chicken groups	Prevaccination	Log $_2$IHA titer/ weeks post vaccination			
		1WPV*	2WPV	3WPV	4WPV
Vaccinated	0	16	32	64	128
Unvaccinated	0	0	2	0	0

*WPV= Week post vaccination

Table (2): ELISA titers of fowl cholera antibodies in vaccinated chickens

Chicken groups	Prevaccination	ELIZA titer/ weeks post vaccination			
		1WPV*	2WPV	3WPV	4WPV
Vaccinated	50	320	788	905	1194
Unvaccinated	80	75	80	139	139

*WPV= Week post vaccination

Table (3): Titers of fowl cholera antibodies in vaccinated chickens as estimated by the indirect fluorescent antibody technique (IFAT) using 2 fold serum dilutions.

Chicken groups	Prevaccination	Weeks post vaccination			
		1WPV*	2WPV	3WPV	4WPV
Vaccinated	0	32	64	128	256
Unvaccinated	0	0	0	0	0

*WPV= Week post vaccination.

Table (4): **Serum neutralizing antibody titers of fowl cholera antibodies in vaccinated chickens as measured by serum neutralization test (SNT) in mice**

Chicken groups	Prevaccination	SNT titer*/ weeks post vaccination			
		1WPV**	2WPV	3WPV	4WPV
Vaccinated	2	16	32	64	128
Unvaccinated	0	2	2	4	4

*SNT titer= The reciprocal of serum dilution which neutralized 1: 10 dilution of 24 hours culture of virulent Pasteurella multocida (A:1).

*WPV= Week post vaccination

Table (5): Titers of fowl cholera antibodies in vaccinated chickens as estimated by agar gel precipitation test (AGPT) using 2 fold serum dilutions.

Chicken groups	Prevaccination	Weeks post vaccination			
		1WPV*	2WPV	3WPV	4WPV
Vaccinated	0	8	16	32	64
Unvaccinated	0	0	0	0	0

*WPV= Week post vaccination

Table (6): Results of the challenge of vaccinated chickens against virulent Pasteurella multocida (A: 1)

Chicken groups	Number of birds in the group	Number of challenged birds	Number of survived birds	Protection percent
Vaccinated	40	40	32	80
Unvaccinated	10	10	2	20

Table (7): A collective table for evaluation of the obtained results.

	The used tests					
	IHA	ELIZA	AGPT	IFAT	SNT in mice	Challenge test
Results	128	1194	64	256	128	80% protection

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الملخص العربى

محاولات تطبيق بعض الطرق السيرولوچية الغير معتادة لتقييم إستجابة الطيور للقاح كوليرا الطيور مقارنة باختبار التحدى

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المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية*

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فى هذا العمل تم تحصين مجموعة من الدجاج بلقاح كوليرا الطيور المحلى مع ترك مجموعة طيور أخرى غير محصنة كضابط للتجربة، وقد تم جمع عينات من أمصال كل الطيور على فترات إسبوعية لمدة أربعة أسابيع بعد التحصين تم بعدها إجراء إختبار التحدى بالعترة الضارية من ميكروب الباستيرلا ملتوسيدا عترة (A:1). كذلك أجريت إختبارات قياس المستوى المناعى فى الطيور قيد التجربة باستخدام الاختبارات المعتادة مثل إختبار التلزن الدموى الغير مباشر والأليزا والاختبارات الغير معتادة مثل إختبار المصل المتعادل فى الفئران والوميض الفلوريسنتى المناعى الغير مباشر والترسيب فى الأجار.

وقد أظهرت نتائج هذه الاختبارات توافقاً إيجابياً من حيث معيار الأجسام المناعية المتكونة فى الطيور نتيجة التحصين ونسبة الحماية والتى بلغت ٨٠٪ بعد التحدى إلا أن إختبار الوميض الفلوريسنتى المناعى الغير مباشر أظهر نتائج أسرع وأكثر حساسية يليه إختبار المصل المتعادل فى الفئران (حيث كانت نسبة الحماية فيه هى نفس النسبة فى الطيور) ثم إختبار التلزن الدموى الغير مباشر والاليزا وأخيراً إختبار الترسيب فى الأجار.

وعلى ذلك يمكن القول بأن إختبار الوميض الفلوريسنتى المناعى الغير مباشر واختبار المصل المتعادل فى الفئران يمكن إستخدامها فى تقييم إستجابة الطيور المناعية للقاح كوليرا الطيور وتقييم قوة اللقاح المناعية.