Assessment of Relative Potency of Inactivated Pasteurella multocida Vaccine in Poultry

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ABSTRACT

A total of 37 different inactivated *P. multocida* vaccines either locally prepared or imported from other countries were tested for potency using both vaccination challenge test and ELISA assay. Correlation between both tests were evaluated and the minimum requirement for protection (70%) were matched with 2.25 X or more the cut off value of the both used kits. At the same time, ELISA antibody titer less than the cut off value was always associated with unsatisfactory protection rate. So, in conclusion, ELISA could be valuable in the evaluation of inactivated *P. multocida* vaccine through determination of the humoral immune response depending on the finding achieved in this study.

INTRODUCTION

Fowl cholera is a wide and commonly distributed disease of poultry and of major economic importance ***(1)***. The disease can express itself in an acute or a chronic form. In the acute form, the clinical signs are seen only in the few hours before death as fever, have ruffled , have mucus discharge from mouth, suffer diarrhea and show an increased breathing rate.

The chronic form of the disease can follow an acute stage or may be the only form of the disease present in the flock. Signs of this form generally linked to localized infection at wattles, sinuses, leg or wing joints, swollen eyes, twisted neck, rales and pin headed necrotic foci in the live with a septicemic picture ***(1)***.

Fowl cholera can be prevented by eliminating all reservoirs of infections and then preventing the re-entry of the organism into the property. Implementation of standard good management practices, effective sanitation regime and good biosecurity program will help prevention of fowl cholera ***(2)***.

P. multocida vaccines are used to help control of Fowl cholera. *P. multocida* exists in 16 different serovars and the most common serovars associated with Fowl cholera outbreaks are serovars 1, 3 and 4. *P. multocida* vaccines based mainly on inactivated cells of *P. multocida* ***(1)***. Evaluation and quality control of the efficacy of this vaccine based mainly on vaccination challenge test by which the protective indices are estimated ***(3)***.

The immune system defends the organisms against infectious diseases and one of the major immunological defense mechanism is the humoral immune response, which is mediated by serum antibodies secreted by B cell ***(4)***. Serological testing is a useful tool in explanation of immune status of the birds and as the Enzyme Linked Immuosorbent Assay (ELISA) is a useful tool for determination of antibody response against certain pathogen infection of vaccine inoculated, the objective of the present work was to study the availability of using ELISA in the evaluation of potency of *P. multocida* vaccine in comparison with challenge test.

MATERIAL AND METHODS

Pasteurella multocida vaccines

Total of 37 different inactivated *p. multocida* vaccine batches from different manufacturers sources either locally prepared or imported from abroad were tested by vaccination challenge test using virulent *P. multocida* and serologically by ELISA yearling 2011 up to 2014.

Pasteurella multocida strains

Virulent *Pasteurella multocida* serovars 1, 3 and 4 were used to perform challenge test. These serovars were kindly supplied from the strain bank of CLEVB (Central Laboratory for The Evaluation of Veterinary Biologics).

Laboratory animals

A total of 96 Specific Pathogen Free (SPF) chickens aging 6 - 8 weeks were used per batch to perform this study starting from 2011 up to 2014. This birds were vaccinated with the corresponding Fowl cholera vaccine batch (0.5 ml subcutaneously) and revaccinated 3 weeks later. Three weeks post-boostering, blood samples were collected and challenge test was performed.

Swiss mice

Six Swiss mice weighed about 20- 25 gm were used to receive the stock culture of *P. multocida,* 2 for each *P. multocida* serovar. This done before every challenge test. Virulence of *P. multocida* serovars were regained through the inoculation of bacterial culture into Swiss mice (Two mice per each *P. multocida* serovar either 1, 3 or 4) in a dose of 100 – 50 CFU intraperitoneally.

Blood samples

Fifteen blood samples were collected per each tested batch of vaccines under testing 3 weeks post second vaccination then sera were separated to be tested using ELISA.

Challenge test

The vaccinated birds were challenged with 2 x 10^2 to 3 x 10^2 CFU/ challenge dose from the different regained virulent *P. multocida* strains (20 birds / each serovar) 3 weeks post second vaccination. Mortalities were observed, recorded and re-isolation of the challenge strain were done from the internal organs (Liver and heart blood) of dead cases and the protective indices (PI) were calculated using the following formula described by ***(5)***.

$$PI = \frac{\%\ (M\ \&\ PML)\ controls - \%\ vaccinated}{\%\ controls} \times 100$$

Where PI is the protective indices, M is the mortality and PM is the post-mortem lesions.

ELISA

ELISA was conducted according to standard procedures of the two different commercial kits. The first one is *Pasteurella multocida* antibody test kit (IDEXX Laboratories. Inc., Cat. No. 99-09251) while the second kit is *Pasteurella multocida* antibody test kit (Synbiotics Corporation, Cat. No.96-6527). ELISA were performed and interpretated as directed by the manufacturers.

RESULTS

Generally, Fowl cholera vaccines are evaluated by sterility, safety and potency tests. Potency testing depends mainly on challenge test as showen in Table (1). A total of 32 out of 37 Fowl cholera vaccine batches were tested and get a satisfactory results for approval to be used in the poultry farms. Six batches out of these 32 induced a protection of 70% and three batches induced a protection 72%. 75 % protection were obtained by 11 batches out of the satisfactory 32 and this was the higher count between the group batches that get a satisfactory results. In the same time 76% protection were obtained by only two batches.

Table 1. Comparison between ELISA mean titer and protection percent of the satisfactory tested fowl cholera vaccine

No. of Tested Vaccine batches	ELISA Mean Titer		Protection % as a result of challenge with P. multocida serovar			
	Kit 1	Kit 2	1	3	4	Protection Mean
6	341	896	70	71	70	70
3	362	922	70	73	74	72
11	373	958	77	73	75	75
2	379	1104	78	74	74	76
7	406	1143	78	81	81	80
2	412	1157	81	83	82	82
1	435	1192	86	85	84	85
Total 32						

80% protection or more were obtained from 10 out of 32 batches from which 7 get 80%, 2 get 82% and only one get 85% protection.

Table (1) showed a comparison between the protection results and the humoral immune response expressed ELISA mean titre for the same batch group at the same protection level. It was noticed that , the minimum requirement of protection (70%) are paralell to 341 and 896 ELISA antibody titre on using ELISA kit (1) and ELISA kit (2) respectively. Also, the antibody titre is increased when protection rate increased in a harmonious manner, at the all levels of protection.

By the same manner, Table (2) illustrated that 5 fowl cholera vaccine batches out of 37 are evaluated as unsatisfactory where it get a protection level lower than the minimum requirement for protection starting with 60% protection in 2 vaccine batches, 48% protection with one vaccine batch, 47% protection with one vaccine batch and 45% protection with other one batch.

Table 2. Comparison between ELISA mean titer and protection percent of the unsatisfactory tested fowl cholera vaccine

No. of Tested Vaccine batches	ELISA Mean Titer		Protection % as a result of challenge with P. multocida serovar			
	Kit 1	Kit 2	1	3	4	Protection Mean
2	292	767	58	62	60	60
1	241	621	50	44	50	48
1	219	589	45	43	53	47
1	217	499	44	43	48	45
Total 5						

As regards to the ELISA antibody titre of such unsatisfactory resulted batches, the corresponding antibody titres were 292 and 767 with the protection rate 60%. Also antibody titre decreased as the protection percent decreased in a paralell manner matched the immune status of the tested vaccine and birds.

DISCUSSION

It is extremely important for poultry producers to be able to get a good vaccine against all poultry pathogens especially that they have great effect on this industry like Fowl cholera.

Evaluation of the efficacy of inactivated P. multocida or Fowl cholera vaccine depends mainly on testing of its potency using vaccination- challenge test prior to sale and distribution *(3)*.

Results of this study compare between two ways for the evaluation of inactivated Fowl cholera vaccine which were vaccination – challenge test and monitoring the immune response through determining the antibody titre against the inoculated vaccine using ELISA.

Because of the minimum requirement of protection is 70% , this study focused on the correlated antibody titre at this protection level which was and using kit (1) and (2) respectively. Analytical view of these titre revealed that, it is equal to or more than the 2.25 x the calculated cut off value of such kit. Also, these titres are increased when the protection rate increased and are decreased when the protection rate decreased as shown in Table (1) and Table (2). The same criteria was obtained by *(6)* who stated that ELISA assay showed a considerable increase in antibody titre after twice vaccination of 6 - 8 weeks aged chicken.

Also *(6)* reported that, the antibody measured with ELISA highly correlated with protection against challenge with virulent organisms. In the same direction, *(7)* reported that a blocking ELISA was developed and standardized for the detection of antibodies to *P. multocida* in vaccinated animal. Also *(8)* used a commercial ELISA kit in a study to detect both IgA and IgG in vaccinated laying hens.

On the other hand, *(9)* recorded that ELISA test did not appear to be adequate for the evaluation of the degree of protection induced in turkey flocks vaccinated at one day of age meanwhile it had acceptable degree of antibody titre resistant to virulent challenge with *P. multocida* on birds (like Turkey) vaccinated at 3 and or 6 weeks of age.

It may be concluded from the results that, the most important finding from the results of this study is that ELISA test could be valuable in the evaluation of the immune response of vaccinated chicken with killed vaccines especially after 6- 8 weeks of age. Also these findings are also consisted with those calling for the mercy of the animal and not to be used in the challenge tests.

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الملخص العربى

تقييم الكفاءة التقديرية للقاحات الباستيريللا مالتوسيدا المثبطة فى الدجاج

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فى هذه الدراسة تم اختبار ٣٧ تشغيلة مختلفة من لقاحات كوليرا الطيور المثبط سواء مصنعة محليا او مستورده من الخارج للتأكد من فعاليتها و قوتها العيارية باستخدام كل من اختبار التحدي و اختبار الإليزا. تم تقييم العلاقة بين الاختبارين من خلال النتائج و كانت متطلبات الحد الأدنى من الحماية (٧٠% حماية) متوافقة مع ما يعادل ٢,٢٥ X أو أكثر من القيمة القطعية لاختبار الاليزا باستخدام نوعين مختلفين من اطقم الإليزا على حد سواء. في الوقت نفسه ، كان مستوى المناعة الخلطية و المختبر باستخدام الاليزا يتناسب طرديا مع زياده او نقصان مستوى الحماية سواء فى التشغيلات ذات النتائج المرضية أو غير المرضية للإستخدام الحقلى. من النتائج المتحصل عليها و المتحققة فى هذه الدراسة يمكننا التوصية بأنه من الممكن استخدام اختبار الإليزا كوسيلة لتقييم لقاحات كوليرا الطيور المثبطة.