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EVALUATION OF DIFFERENT SEROLOGICAL AND IMMUNOLOGICAL METHODS USED FOR ASSESSMENT OF ANTI-P. MULTOCIDA ANTIBODIES IN SERA VACCINATED CHICKENS

(With 6 Figures)

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تقييم مختلف الاختبارات السيرولوجية والمناعية المستخدمة في تقدير الأجسام المناعية لميكروب الباستيريلا ملتوسيدا في سيرم الدجاج المحصن

وفاء على غنيمى ، سوزان فخرى جورجى ،
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تم في هذا البحث مقارنة مدى حساسية اختبارات التلازن الدموى غير المباشـــر، الالـيزا، اختبار الاليزا النقطى اللطعى في قياس الإستجابة المناعية السائلة بعد التحصين بلقاح كولـيرا طيور الدواجن. وبمقارنة متوسط مستوى الأجسام المناعية للأليزا واختبار التلازن الدمـــوى غير المباشر أتضح ان نتائج كلا الاختبارين كانت متوازنة في الجزء الأول من الأســـتجابة المناعية. ولكن في الجزء الثانى من الإستجابة المناعية قل مستوى الأجسام المناعيـــة فـــي اختبار الاليزا بصورة ضئيلة بينما قل مستوى الأجسام المناعية في اختبار التلازن الدمـــوى غير المباشر بسرعة. وقد كانت نتائج اختبار الاليزا النقطـــى اللطعـــى مرضيـــة بالنســـبة للحساسية (٩٩,٨%) والخصوصية (١٠٠%) والاستمرارية (١٠٠%). وكان هناك ارتبـــاط ضعيف (ر=٠,٥٧٩) بين اختبار التلازن الدموى ومستوى المناعة بعد اختبار تحدى المناعة. بينما كان هذا الارتباط أقوى (ر=٠,٦٨٥، ٠,٧٩٣) في اختبارى الاليزا، والالـــيزا النقطـــى اللطعى على التوالى.

SUMMARY

The sensitivity of indirect haemagglutination test, ELISA and dot immunobinding assay in measuring humoral immune response to avian cholera vaccine was determined. Comparison of mean antibody titres showed that ELISA titres were parallel to IHA titres in the first part of

the response. However, in the second part, ELISA titres decreased slightly while IHA titres decreased rapidly. Results obtained from dot blot ELISA were satisfactory for sensitivity (99.8%) specificity (100%) and consistency (100%). Weak correlation (r=0.579) was found between IHA titres and survivability after challenge. Meanwhile, it was stronger (r=0.685, 0.793) for ELISA and dot blot ELISA, respectively.

Key words: *P.multocida antibodies, sera, vaccinated chickens*

INTRODUCTION

Fowl cholera constitutes a major disease problem for avian species in Egypt. Vaccination is still considered one of the major tools for controlling the disease (Gergis et al., 1991). Extensive research has been conducted to develop an effective polyvalent vaccine against fowl cholera in Egypt.

At present, various serological procedures have been used to quantify humoral antibodies against *P. multocida*. Among the widely used methods in antibody detection and measurement are the microtitre agglutination test (Shlink and Olson, 1989); serum plate agglutination test (Heddleston et al., 1972); indirect haemagglutination test (Carter, 1972, Dua and Maheswaran, 1978) and the ELISA test (Avikian et al., 1986; Briggs and Skeels, 1983). The indirect haemagglutination and ELISA test have been found to be superior to the other serological tests in terms of convenience and sensitivity. It is possible that these two tests are measuring antibodies of different specificities and avialities. This may explain discrepancies in results obtained from different laboratories. The disadvantages of ELISA include the potential alteration of antigen confirmation upon binding to plastic, non-specific adherence of some antibodies to the plates and variable capacities of different antigens present in a complex mixture to bind to microtitre plate wells (Viadva et al., 1985).

The dot immunobinding (dot blot) assay, an ELISA based assay in which an antigen is directly applied onto a nitrocellulose membrane for antibody detection, has been introduced by (Hawkes et al., 1982 and Huet et al., 1982). Dot blot ELISA serves as an alternative to plate ELISA and possesses distinct advantages. The dot blot assay is reported to be of equal or greater sensitivity than plate ELISA and can function over a wide range of antigen antibody ratios. An additional advantage is that the results are easily interpreted without ELISA reader (Muneer et

al., 1988).

In the present study, an adaptation of dot blot ELISA for the detection of antibodies against avian cholera vaccine is described.

Our goals from the present study were: (a) to compare the sensitivity of indirect haemagglutination, ELISA and dot immunobinding assay in measuring humoral antibody response to avian cholera vaccine (b) to study the relationship between the anti-*P. multocida* antibody titres after vaccination and survival after challenge with virulent organism (c) to compare the dot immunobinding assay and ELISA for specificity and simplicity.

MATERIALS and METHODS

Experimental chicken:

A total of 80 broiler chicken, 8 weeks of age were vaccinated twice (6 weeks apart) with 0.5ml of monovalent fowl cholera vaccine prepared from serotype A:1 prepared in Veterinary Serum and Vaccine Research Institute.

Twenty chickens were kept as unvaccinated control.

Serum samples:

Blood samples (approximately 5 ml/chicken) were drawn every week from brachial vein for 9 weeks, extending from pre-vaccination to immediately before challenge. Serum was separated and frozen at -20°C until further use (Solano et al., 1983).

Indirect haemagglutination:

This test was conducted as described by Cho et al. (1976). Geometric mean titres were calculated for the serum samples.

ELISA procedure:

The technique of Solano et al. (1983) was followed. A checkerboard titration was performed to determine the optimum antigen concentration and conjugate dilution.

Dot immunobinding assay:

The procedure of Hawkes et al. (1982) was adopted. Initially the sensitivity and specificity of the assay were studied by using known *P. multocida* positive and negative chicken sera previously tested using IHA test and ELISA. The sensitivity of the test was calculated as the proportion of samples testing positive out of all known *P. multocida* positive samples. The specificity of the test was the proportion of samples testing negative out of all known negative samples tested. To determine reproducability, some samples were tested three times for consistency in the results.

The optimum dilution stock of *P. multocida* antigen used was established in a preliminary experiment using checkerboard titration, as described above two-fold serial dilutions of sera were made in dilution buffer, starting with 1:10. A single antigen dilution (1:100) was used in coating all of the wells. Results were expressed as either positive or negative based on colour development.

Statistical data analysis:

A regression analysis was performed between the titres of sera collected at the various bleedings and the survival rate after challenge as recommended by Snedecor and Cochran (1976). Three equations were used:

Equation 1 : $Y = bx + a$

Equation 2:

$$\frac{\sum XY - n(\sum X)(\sum Y)}{\sum X^2 - 2\ (\sum X)^2}$$

Equation 3: $a = Y - b(x)$

where, **Y :** value of the dependent variable.

a : value of Y intercept.

b : slope of the line.

x : value of the independent variable.

Correlation coefficient (r)

$$r = \frac{\sum XY - \frac{(\sum X)(\sum Y)}{n}}{\sqrt{\sum X^2 - \frac{(\sum X)^2}{n}\left(\sum Y^2 - \frac{(\sum Y)^2}{n}\right)}}$$

Challenge procedure:

Vaccinated chicken were challenged by intramuscular inoculation with 100 LD_{50} of virulent 24 hour broth culture of serotype A:1.

RESULTS and DISCUSSION

Antibody response:

The results of antibody response of chicken vaccinated with inactivated fowl cholera vaccine as measured by IHA and ELISA are summarized in Fig. (1) and Fig. (2). The results reveal that the immune

response was divided into two parts. The first, up to 10 days post immunization and the second after day 16 post immunization. Comparisons between IHA antibody titres and ELISA titres were made on a group and individual basis.

In the vaccinated group of chicken, there was a significant ($P < 0.05$) increase in both GMT and ELISA titres beginning from the second week post immunization. Comparison of mean antibody titres showed that ELISA titres were parallel to IHA titres along the first part of the response. However, in the second part, ELISA titres decreased slightly and IHA titres decreased rapidly. No individual correlation was found in either of the two parts. There was a wide range in the ELISA antibody titres for each IHA titre.

Similar results were reported by Mires et al. (1983) in a study comparing a modified ELISA and a haemagglutination inhibition test to detect antibody against Newcastle disease virus. These are explained by the fact that the mechanisms associated with the two tests measure the two main serum isotypes IgM and IgG differently. It should be emphasized that in IHA test, IgM agglutinate the sensitized sheep erythrocytes significantly better than IgG (Tizard, 1996).

Although anti-chicken IgG (H + L chain) was used in the ELISA in the present study, the mean antibody titres were parallel with IHA titre in the first part of the primary response, which was characterized by IgM isotype. Because this conjugate specifically recognizes an epitope on the IgG H+L chain, it may also be applied for detection of the IgM isotype sharing the light chain with IgG. The slight decrease of ELISA titres in the second part of the immune response indicate that IgG antibodies are detected more efficiently by the ELISA. The use of specific anti-chicken U-chain conjugate may improve the sensitivity of the ELISA in the first part of the immune response.

Comparison between ELISA and dot blot ELISA:

The results obtained from dot blot ELISA were satisfactory for sensitivity (99.8%) specificity (100%) and consistency (100%). The cut off level of absorbance for ELISA was 0.69, while it was 0.54 for dot blot ELISA. In the dot blot ELISA the reaction of the negative sera at a dilution of 1:20 resulted in a drastically reduced absorbance of less than 0.23. Thus a dilution of 1:40 was selected as the dilution factor.

Although ELISA has been extensively used in the past decade for screening either monoclonal or polyclonal antibodies, doubt has been raised about its reliability in other applications (Vaidva et al., 1985). More recently the disadvantage of plastic plates was circumvented by

dotting on nitrocellulose membranes. The efficiency of coating antigen onto the nitrocellulose membrane is more uniform than microtitre plates for ELISA.

The present study showed that the specificity of dot blot ELISA was significantly ($P < 0.05$) higher than that of ELISA by comparison of positive negative ratios of bacterin antisera. Another advantages of dot blot ELISA is that it can be used with more accuracy and specificity if the source of the serum is known whether it was from vaccinated or diseased chicken.

Relationship between levels of anti-P. multocida titres and survival rate after challenge:

Regarding the relationship between IHA titres and survivability after challenge it can be noted from Fig. (3) that there was significant ($P < 0.01$) positive correlation between these two variables. The dots representing the distribution of titres were scattered more widely in this diagram than they were in Fig. (4) and the correlation coefficient (r:0.579) was weak.

There was a highly significant ($P < 0.001$) positive correlation between the average antibody titres as measured by ELISA and dot blot ELISA. The correlation coefficient was (r: 0.685) (Fig. 4) and (r: 0.793) (Fig. 5) for the two tests respectively indicating higher and strong correlation between antibody titre and survivability after challenge. Fig. (6) display dot blot ELISA of fowl cholera in vaccinated chicken.

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Fig. (1) Geometric mean titres of P. multocida antibody titres as measured by IHA test

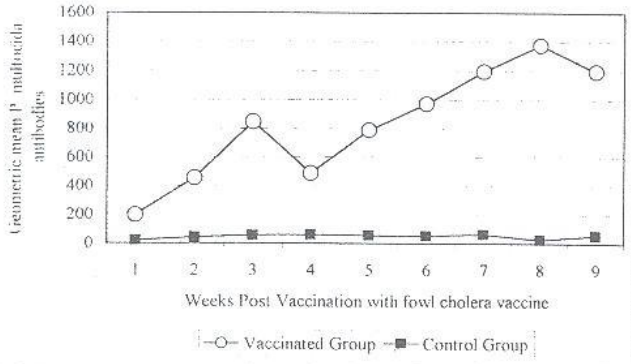


Fig. (2) P. multocida antibody titres as measured by ELISA. Bars represent standard deviation

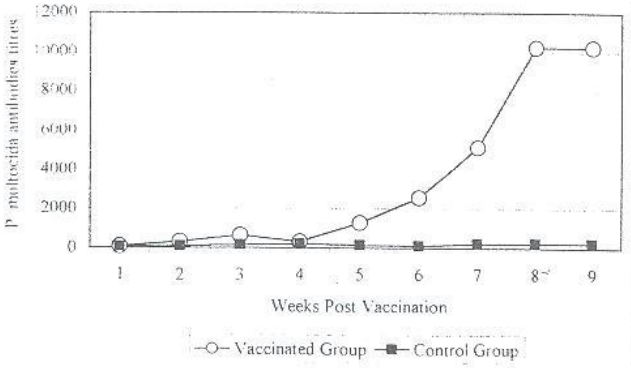


Fig. (3) Correlation between P. multocida antibody titres as measured by IHA observed before challenge and survivability after challenge. The dots represent the distribution of titres

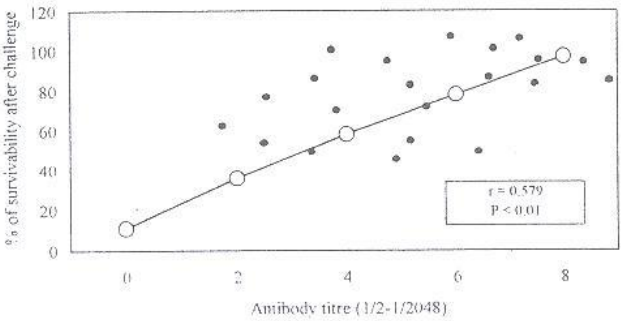


Fig. (4) Correlation between P. multocida antibody titres as measured by ELISA observed before challenge and survivability after challenge. The dots represent the distribution of titres

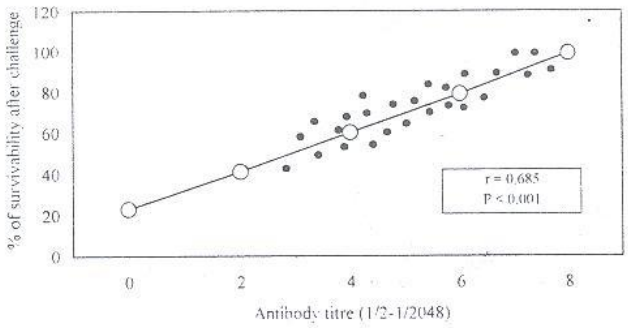
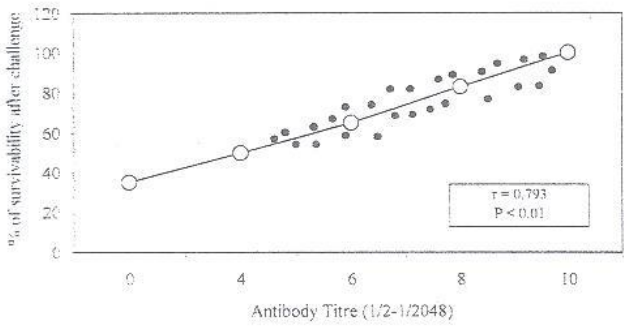


Fig. (5). Correlation between P. multocida antibody titres as measured by Dot-ELISA observed before challenge and survivability after challenge. The dots represent the distribution of titres



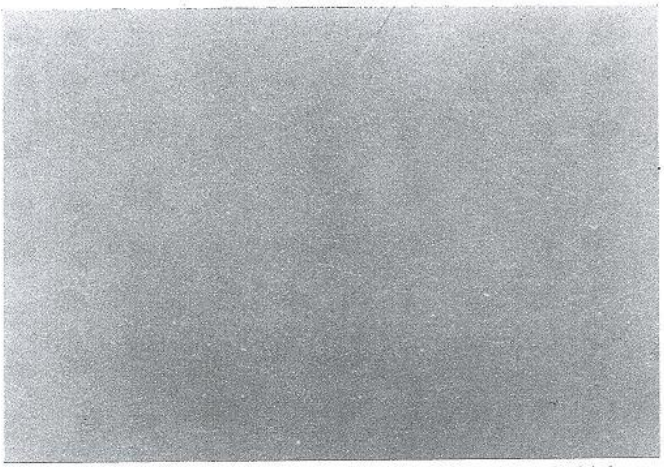


Fig. (6): Dot blot ELISA of fowl cholera vaccinated chicken