Vet.Med.J.,Giza. Vol.52, No.4. (2004):627-636

USES OF NON-SPECIFIC IMMUNOSTIMULANT SIMULTANEOUSLY WITH VACCINATION WITH VIRAL AND BACTERIAL POULTRY VACCINES

S.S., SALAMA*; AMAL E. ABD EL-HADY*; N.A., SHERIF* and SAMAR M. MOUNEIR**

* Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo.

** Pharmacology Department, Faculty of Veterinary Medicine, Cairo University.

Received: 22.9.2004.

Accepted: 5.12.2004.

SUMMARY

Two types of non-specific immunostimulant, fowl pox vaccine and dextran sulphate, were used to study the cellular and humoral immune response in chickens. The chickens were vaccinated with live *P. multocida* vaccine and live infectious bronchitis (IB) vaccine. The results proved that such materials enhanced the immune response either on the cellular or humoral levels. Fowl pox vaccine was more effective than dextran sulphate and both treated chicken groups gave significant difference in the immune response than chicken groups receive only either live *P. multocida* or infectious bronchitis(IB) vaccines.

INTRODUCTION

During the last three decades, a great attention was paid towards poultry industry in Egypt to diminish the gap between the increased demand of human population and the shortage in animal protein. Consequently, poultry epidemics become more numerous and caused sever economic losses.

Prophylactic vaccination against infectious diseases remains the clearest cut success of immunopotentiation in the practical application of immunological principles for the improvement of human and animal health.

Specific vaccination has also been used to restrict great numbers of chronic infectious diseases whose response to anti-microbial chemotherapy remains suboptimal (Gatenby, 1998).

In order to develop effective vaccination, it is necessary to induce serum or local antibody mediated and cellular immunity mediated compounds which enhance the immune response to vaccine have been sought by immunologists, for many

years (Samina et al., 1995).

Modern pharmacology has a wide range of immunotropic drugs if they activate the immune system function, they are called immunostimulants and if suppress the immune system function, they are called immunodepressors (Pridybailo et al., 1991). Immunostimulation defined as a process that directly enhance one or more specific immune function or modifies one or more components of the complex immunoregulatory network to achieve its effects through indirect mechanism (Gatenby, 1998).

The use of immunostimulant drugs has been required to enhance the immune response in the development of vaccine and to overcome possible immunological interference resulting from combined use of antigens. Adjuvants may also be important to maximize IgG production in vaccinated birds as a source of antibodies (Han and Park, 2000). Thus, the present work was conducted to prove the effect of fowl pox vaccine and dextran sulphate as immunostimulants for inhancing the immune response during the vaccination of live infectious bronchitis (IB) and fowl cholera vaccines by evaluation of of both humoral immune response using ELISA and cellular immune response using lymphocyte transformation test (MTT).

MATERIAL AND METHODS

1. Immunostimulants:

a. Fowl pox vaccine:

Live attenuated fowl pox vaccine with specific solvent was used, it was obtained from Intervet, Holland and prepared immediately before administration via wing web puncture.

b. Dextran sulphate:

(Sigma, Lot No. 127H.765). It was diluted to concentration of 100ug/ml and used in 2 doses IM with 2 weeks interval (100ug/kg b.wt.).

2. Vaccines used:

a. Live Infectious Bronchitis (IB) vaccine:

Live attenuated infectious bronchitis vaccine ($10^{5.2}$ EID_{50} /dose) was obtained from Intervet Company, Holland.

b. Live fowl cholera vaccine:

Clemson University (CU) *P. multocida* vaccine; the concentration of the vaccine15 x 10^{10} /500 doses; (Schering Plough Animal Health, USA) with its specific diluent vaccination via wing web puncture and boostering after 2 weeks.

3. Experimental Design:

A total of 210, one day old, chickens were reared up to 4 weeks and divided into 7 groups, each containing 30 chickens as follow:

Group	Treatment	Vaccination
1	Dextran Sulphate	Live IB vaccine
2	Dextran Sulphate	Live *P. multocida* vaccine
3	Fowl Pox	Live IB vaccine
4	Fowl Pox	Live *P. multocida* vaccine
5	No Treatment	Live IB vaccine
6	No Treatment	Live *P.multocida* vaccine
7	No Treatment	No vaccine.

4. Blood sampling:

Five blood samples were collected from all groups in sterile and dry tubes then serum separated for humoral immunity evaluation at interval of 3, 7, 14, 21, 28 and 35 days post vaccination.

Other heparinized blood samples were collected from all groups at the same interval for the evaluation of cellular immunity.

5. Evaluation of humoral immunity:

Preparation of *P. multocida* ELISA antigen was conducted according to Higgins and Whithear (1985). While, the IB antigen was prepared according to Lonal et al. (1983) and the results were calculated according to Briggs and Skeels (1984). The buffers were prepared according to Henrick (1994) and the assay was performed according to Richard (1995).

6. Evaluation of cellular immunity:

The procedure was performed according to Lucy (1978).

7. Statistical analysis

The obtained data were statistically analyzed using the method of Snedecor (1971) for calculating the mean values, standard error of the mean and significance between values of different groups (Student -T test)

RESULTS AND DISCUSSION

The immunostimulants are used to potentiate the immune response of poultry against vaccination and to avoid the adverse effect of environmental immunosuppressive factors. In the present study, two different non-specific immunostimulant were used; fowl pox vaccine and dextran sulphate.

The data given in tables (1 and 3) showed the effect of fowl pox vaccine as immunostimulant on the immune response of chickens against live *P. multocida* vaccine measured by MTT assay and ELISA.

Concerning the blastogenic lymphocyte response of chicken vaccinated with live *P. multocida* vaccine and stimulated by fowl pox are shown in table (1), the mean absorbance value was 1.042 three days post vaccination increased to 1.502 after 14 days post vaccination and reached its maximum level (1.624) after 28 days post vaccination. These values showed a clear difference than the group of chicken vaccinated with *P. multocida* and not vaccinated with fowl pox vaccine which showed 0.962 three days post vaccination raised to 1.269 at 14 days post vaccination and reached 1.546 at 28 days post vaccination with a special reference to significant increase from control untreated and unvaccinated group at $p < 0.05$.

These results coincide with that of Meyer and Mayer (1981) who reported that PINDAVI pox led to T-cell activation which resulted in increase stimulation index. Richter (1983) added that PIND Orf (Ovine para pox) reduced the fall in total leucocyte and lymphocyte counts and the phagocytosis rate as compared with control birds. Also, Mayer-Bibrack (1980) stated that the use of PIND-Avi induced non-specific immunity and increased the resistance to infection.

Regarding the humoral immune response of live *P. multocida* and fowl pox vaccines, the data in table (3) showed that the mean ELISA antibody titers was 1225 increased to 2185 after 14 days post vaccination and reached 2983 after 28 days post vaccination in case of chicken group vaccinated with *P. multocida* alone compared with 1279 after 3 days post vaccination, increased to 2337 at 14 days post vaccination and reached maximum level (3102) after 28 days post vaccination in chicken group vaccinated with *P. multocida* and fowl pox vaccines, showing a significant increase in antibody response that received *P. multocida* alone. Charles et al. (1991) reported that antibody titers to *P. multocida* significantly higher in groups vaccinated with live CU P. multocida vaccine which may referred to the fact that the fowl pox was considered as an immunostimulant agent. Meanwhile, Gergis et al. (1994) concluded that the neutralizing index in birds vaccinated with the combined fowl cholera and fowl pox vaccine did not differ significantly from the group vaccinated with *P. multocida* vaccine alone.

The same results were obtained with IB-Pox vaccination as shown in table (2). Chicken group received both IB and fowl pox vaccine showed a blastogenic lymphocyte activity as early as 3rd day post vaccination, 1.024 increased to 1.294 after 14 days post vaccination, compared with 1.001, 1.216 and 1.558 at the same intervals for chicken group received IB vaccine alone. The same picture obtained on measuring antibody titers using ELISA for the same groups as shown in table (4), where the chicken group vaccinated with both IB and pox vaccines gave 1279 ELISA

antibody titer 3 days post vaccination compared with 1159 with chicken group received IB vaccine only raised to 2273 and 2116 after 14 days post vaccination and reached 3091 and 2792 at 28th day post vaccination, respectively. Samina et al. (1995) recorded that live fowl pox vaccine increased the protection rate by 20-30% compared with vaccine alone after challenge with the reference to virulent strains.

Concerning the using of Dextran sulphate as non-specific immunostimulant, the results in table (1) showed that the lymphocyte transformation from live *P. multocida* vaccine-dextran sulphate treated chicken group was 1.042 at 3rd day post vaccination increased to 1.502 at 14th day and reached 1.624 after 28 days post vaccination showing marked increase in absorbance unit than those of chicken group received only live *P. multocida* vaccine at the same intervals. The same picture appeared concerning the antibody titer measured by ELISA, as shown in table (3). The results are agreed with Kishima et al. (1985) who concluded that the cell mediated immune response to mycoplasma in pigs was effectively enhanced by dextran sulphate. Also, Takai et al. (1990) added that the leukocytic count increased by 6 hours after injection with dextran sulphate, then fell to control level.In addition Kishima et al. (1987) recorded that the use of dextran sulphate was effective in enhancing antibody production in mice immunized with a mixture of *M. pulmonis* and dextran sulphate than in mice immunized with *M. pulmonis* alone.

Regarding IB vaccination, the same picture appeared as the chicken group received both dextran sulphate and IB vaccine as shown in table (2) showing higher rate starting as early as the 3rd day post vaccination 1.003 compared with 1.001 for group received IB vaccine only, raised to 1.289 and 1.216 at 14th day post vaccination then to 1.614 and 1.558 at 28th day post vaccination respectively. These results referenced to significant increase from control untreated and unvaccinated group at $p < 0.01$.

Concerning the humoral immune response, the same results obtained on using dextran sulphate in the group of chicken received both IB vaccine and dextran sulphate than those received IB vaccine alone . As early as the 3rd day post vaccination, as shown in table (4). The chicken group received both dextran sulphate and IB vaccine showed higher antibody titer (1194) than those received only IB vaccine (1159) increased up to 3013 compared to 2792 at 28th day post vaccination respectively. Mastromarino (1997) reported that dextran sulphate was known for its antiviral activity against different viruses. Also, Han and Park (2000) concluded that dextran sulphate adjuvant showed higher antibody titer after immunization. Wetzel and Ketman (1981) found that dextran sulphate act as B-cell mitogen.

From the above mentioned data, it is clear that the use of fowl pox vaccine as non-specific immunostimulant more or less better than dextran sulphate and both is better to be used with vaccination programs than the use of vaccines only.

Table (1): Lymphocyte transformation (Optical Density) in chicken groups vaccinated with live *P. multocida* vaccine measured by MTT

Group	Days Post Vaccination					
	3	7	14	21	28	35
2**	1.042 ±0.02	1.251 ±0.02	1.502 ±0.03	1.598 ±0.01	1.624 ±0.03	1.599 ±0.13
4**	1.054 ±0.04	1.297 ±0.05	1.581 ±0.01	1.648 ±0.02	1.682 ±0.02	1.642 ±0.03
6*	0.962 ±0.04	1.047 ±0.04	1.269 ±0.02	1.492 ±0.006	1.546 ±0.003	1.521 ±0.008
7	0.922 ±0.003	1.001 ±0.001	1.012 ±0.004	1.007 ±0.001	1.002 ±0.001	1.007 ±0.001

* Significant increase from control group at P. < 0.05
** Significant increase from control group at P. < 0.01

Table (2): Lymphocyte transformation (Optical Density) in chicken groups vaccinated with live infectious bronchitis (IB) vaccine measured by MTT

Group	Days Post Vaccination					
	3	7	14	21	28	35
1**	1.003 ±0.03	1.097 ±0.04	1.289 ±0.03	1.417 ±0.02	1.614 ±0.03	1.589 ±0.02
3**	1.024 ±0.05	1.119 ±0.04	1.294 ±0.02	1.588 ±0.11	1.699 ±0.03	1.591 ±0.03
5*	1.001 ±0.03	1.036 ±0.03	1.216 ±0.05	1.385 ±0.003	1.558 ±0.004	1.542 ±0.014
7	0.922 ±0.002	1.001 ±0.003	1.012 ±0.002	1.007 ±0.002	1.002 ±0.001	1.007 ±0.004

* Significant increase from control group at P. < 0.05
** Significant increase from control group at P. < 0.01

Table (3): Antibody titer in chicken groups vaccinated with live *P. multocida* vaccine measured by ELISA

Group	Days Post Vaccination					
	3	7	14	21	28	35
2	1289	1432	2281	2481	3077	3010
4	1362	1524	2337	2563	3102	3029
6	1225	1384	2185	2371	2983	2957
7	60	71	70	70	65	72

Table (4): Antibody titer in chicken groups vaccinated with live infectious bronchitis (IB) vaccine measured by ELISA

Group	Days Post Vaccination					
	3	7	14	21	28	35
1	1194	1372	2172	2394	3013	2999
3	1279	1487	2273	2481	3091	3004
5	1159	1263	2116	2301	2792	2731
7	60	71	70	70	65	72

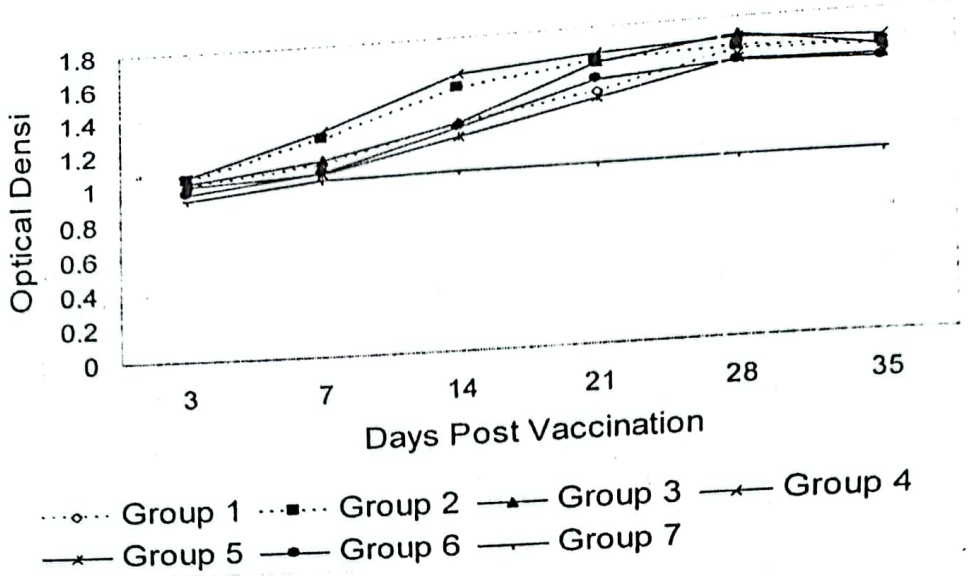

Fig.(1): Lymphocyte transformation in chicken groups vaccinated with *P.multocida* and IB vaccine and received fowl pox and dextran sulphate

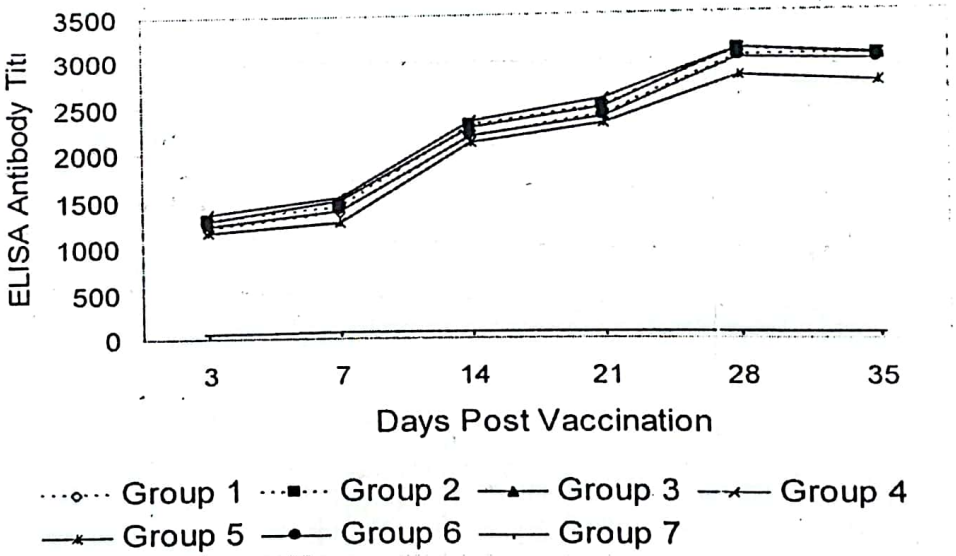

Fig.(2): Antibody titres in chicken groups vaccinated with *P.multocida* and IB vaccine and received fowl pox and dextran sulphate

REFERENCES

Briggs, D.J. and Skeels, J.K. (1984): An ELISA for detecting antibodies to *P. multocida* in chickens. Avian Dis., 28 (1): 208-215.

Charles, W.; Beare, C.; William, M.; Schmitzlein, N.; Deoki, N. and Tripathy, N. (1991): Protection of chickens against highly pathogenic avian influenza virus (H5N2) by recombinant fowl pox viruses. Avian Dis., 35: 356-359.

Gatenby, N. (1998): Encyclopedia of Immunology. Acad. Press, N.Y., USA.

Gergis, S.M.; Souad M. Soliman; Samira, A.A.; Suzan F. Gorgi and Michael, A. (1994): Combined vaccination against fowl cholera and fowl pox diseases. J. Egypt. Ass. Immun., 1: 104-106.

Han, H.R. and Park, H.M. (2000): Effect of adjuvant on the immune response of staphylococcal alpha toxin and capsular polysaccharide (CPS) in rabbit. J. Vet. Med. Sci., 62 (3): 237-241.

Henrick, (1994): Methods in Practical Laboratory Bacteriology. Laboratory of Enteric Pathogens. Central Public Health Laboratory, London, England, CRC Press, London.

Higgins, P.A. and Whithear, K.G. (1985): Detection and differentiation of M. gallisepticum antibodies in chicken serum using ELISA. Avian Dis., 30 (1): 160-168.

Kishima, M.; Ross, R.F. and Kuniyasa, C. (1985): Cell mediated and humoral immune response to M. hypopneumoniae in pigs enhanced by dextran sulphate. Amer. J. Vet. Res., 46 (2): 456-462.

Kishima, M.; Kuniyusu, S.; Eguchi, M. and Nakgwa, M. (1987): Humoral immune response to non-viable M. pulmonis in mice enhanced by dextran sulphate. Vet. Microbiol., 13 (4): 335-342.

Lonal, D.P.; Marquardt, W.W. and Synder, D.B. (1983): Comparison of whole blood dried on filter paper and serum for measurement of the temporal antibody response to avian infectious bronchitis virus by ELISA. Avian Dis., 27: 813-821.

Lucy, F. Lee (1978): Chicken lymphocyte stimulation by mitogen: A microassay with whole blood culture. Avian Dis., 22 (2): 296-307.

Mastromarino, M. (1997): Dextran sulphate as a potent antiviral drug against several viruses infection. Poult. Sci., 3: 15-19.

Mayer-Bibrack, B. (1980): Paraimmunization of newborn animals. Prakt. Tierarzt, 61 (9): 714-718.

Meyer, H. and Mayer, A (1981): Clearance and phagocytosis of Pseudomonas aeruginosa in inoculated perfused livers of germ. Free rats after pretreatment with the non-specific immunostimulant. Pind-Avi Zbl Vet. Med., B 28 (1): 46-58.

Pridybailo, N.; Korovim, R.N. and Afanasieva, G.E. (1991): Efficacy of combined administration of vaccine against Marek's disease and immunostimulant thymolin. Vestnik Selskokhozyaistvennoi Nauki Moskoi, 7: 136-138.

Richard, L.M. (1995): Immunology. A Laboratory Manual 2nd Ed., Chapter II: Enzyme Linked Immunosorbent Assay (ELISA), W.M. Brown Publishers.

Richter, R. (1983): Increasing the immune response in poultry by means of the paraimmunity inducer pind orf. Inaugural Dissertation Tierarzliche Fakultat Ludwing Maximilians Universitat Munchen, pp. 78-92.

Samina, I.; Peleg, B.A.; Schwyzer, M. and Ackermann, M. (1995): Live fowl pox vaccine as immunopotentiator for enhanced immunization against fowl cholera (*P. multocida*) in turkeys. Immunobiol. Viral Infect., 47: 410-412.

Snedecor,G.W.; (1971) : Statistical method .14th ed. Ames,Iowa , The Iowa State Univ. Press .USA.

Takai, S.; Yokoyama, Y. and Tsubaki, S. (1990): Enhancement of natural resistance of mice to Rhodococcus equi infection by dextran sulphate 500. Microbiol. Immunol., 34 (8): 697-701.

Wetzel, G.D. and Kettman, J.R. (1981): Activation of r rine B-cells II. Dextran sulphate remove the requirem for cellular interaction during lipopolysaccharide duced mitogenesis. Cell Immunol., 61: 176-189.