قسم : الميكروبيولوجيا وصحة الحيوان .
كلية : الطب البيطرى بأدفينا - جامعة الاسكندرية .
رئيس القسم : أ . د . / طه حسن مصطفى .

# دراسة المقاومة الغير تخصصية في الكتاكيت ضد ميكروب كوليرا الطيور

حلمي تركي ، محمود السيد ، محمد عقيلة

تم دراسة دور المقاومة الغير تخصصية ضد ميكروب كوليرا الطيور . ولقد أمكن اثبات وجود علاقة بين المستوى المعيارى للأجسام المناعية في الدم وقوة التخلص من الميكروب بالخلايا المتخصصة ( Phagocytic cells ) وكان لوجود الايستوتين الخاص أثر في وجود الخلايا المتخصصة ( Phagocytic cells ) وزيادة نشاطها . وكانت الخلايا الالتهابية تعتمد اعتمادا كليا على خلايا الدم البيضاء ( Polmorphnuclear ) أكثر من خلايا ( Monocytes ) حيث أن التحليل الاحصائي أثبت ذلك .

*Dept. of Microbiology & Animal Hygiene,*
*Fac. of Vet. Med., Alexandria University,*
*Head of Dept. Prof. Dr. T.H. Moustafa.*

# *IN VITRO ASSAY OF NON-SPECIEIC RESISTANCE TO FOWL CHOLERAIN CHICKEN*

*(With 2 Tables)*

*By*
***H.A. TORKY; M. EYZZAT and M. AKEULO***

*(Received at 19/5/1984)*

## *SUMMARY*

The role of nonspecific resistance to Pasteurella multocida was studied. A correlation between levels of systemic antibodies (titres) and chemotactic and phagocytic activity of phagocytic cells was proved. The presence of specific opsonin not only result in high significant shift of phagocytic cells toward the microorganism (chemotaxis) but also it increase the phagocytic activity of these cells. The phagocytic activity depends mainly on the polymorphonuclear cells (PMN) than monocytes as P/ 0.05 in case of PMN and P 0.05 in case of monocytes.

## *INTRODUCTION*

P. multocida serotypes 5:A, 8:A, and 9:A, were found to be pathogenic for chickens by NAMIAKA and BRUNER (1963), later NAMIOKA (1970) refered to type 2:D and -:D to be associated with chronic fowl cholera. In Egypt EL-GHAWAS (1975) investigated 200 strains of P. multocida from acute fowl cholera and were serotyped 5:A, 8:A and 9:A, while AOUAD (1978) and EYZZAT, (1983) found that 5:A, 8:A, 9:A and 2:D serotypes of P. multocida are the causative agents of chronic fowl cholera. Moreover EYZZAT (1983) reported that chickens vaccinated with bacterins of this serotypes survived challenge by (95.7%) and P. multocida was not recovered from dead birds. While the control group died from fowl cholera after 3 days post-challenge. The extraordinary high virulence exhibited by many chicken and turkey strains seems to correlate with the ability of this organisms to resist phagocytosis, once it enteres the tissues (WOOLCOCK & COLLINS, 1976 and COLLINS, 1977).

Since the mechanism of acquired resistance to P. multocida infection remains poorly understood, specially the role of non specific resistance, studies were performed to measure the phagocytic activity and levels of immunity and protection to challenge. This knowledge could help in devising a more effective immunization program against fowl cholera in chickens.

## *MATERIAL and METHODS*

**Bacterial antigens:**

4 virulant strains of P. multocida serotypes 5:A, 8:A, 9:A and 2:D were used for the preparation of vaccine, challenge and measuring the phagocytic activity of leukocytes from vaccinated as well as from non vaccinated chickens. These strains were isolated, serotyped, tested for their virulence in the Department of Microbiology and Animal Hygiene Faculty of Veterinary medicine university of Alexandria.

**Birds:**

12 Dokki 4 of 10 weeks old broilers were obtained from a local source. They were devided into two groups (6 birds in each group) and housed in separate rooms for three weeks before vaccination. The tested group was subcutaneously vaccinated with 0.5 ml of oil-adjuvant polyvalent bacterins at 13 weeks of age and a second dose at 15 weeks as described by (EYZZAT, 1983).

**Agglutination test:**

An assay was carried out for measuring the humoral immunity using the ordinary tube aggultination test as recommended by NAMIOKA and BRUNER (1963).

**Assay for phagocytosis:**

The test was carried out essentialy as described by the Department of Microbiology, Veterinary Academy, MOSCOW, (1980).

**Chemotaxis studies:**

A modified technique of chemotaxis assay under agarose as described by NELSON et al. (1975), was used. Where leukocyte suspension containg PMN and mononeuclear cells were utilized. Chemotaxis was measured after 2 hours of incubation i.e. measuring the distance of migrating cells towards antigens, using blood leukocytes from immunized birds. Quantitation of the results was carried out by calculating the mean surface area of migration to antigen and comparison to the control tubes which contained only blood leukocytes from non immunized birds not containing any agglutinating antibodies.

**Antigen for chemotaxis Assay:**

P. multocida antigens were prepared as described by NAMIOKA and BRUNER (1963). They represented the packed cells obtained from 16 hours old culture, suspended in 10ml of NHcl saline (86ml conc. Hcl + 9gm Nacl + 914 distilled water) and incubated in a screw capped bottles at 37°C for 16-18 hours. The suspension was centrifuged at 3000 r.p.m. for 15 min. and the sediment was twice washed with 0.3% formalinized buffer saline. The final pH of the suspension was adjusted to 7 using 10% sodium carbonate solution.

## *RESULTS*

P. multocida serotypes 5:A, 8:A, 9:A and 2:D were not equally phagocytosed by blood leukocytes before and after vaccination, i.e. in the absence of specific opsonins, phagocytosis activity were very low and statistically was not significant (P 0.05). After vaccination the activity became high and was significant statistically where P/ 0.05 (Table I). However it appears that the phagocytic activity depends mainly on the PMN than monocytes as P/ 0.05 in case of PMN and P> 0.05 in case of monocytes (Table II).

A correlation between levels of systemic humoral antibodies and phagocytic activity was found (Table II).

The results of chemotaxis assay showed that the migration was increased segnifically post-vaccination where P/ 0.05 (Table I).

## *DISCUSSION*

The nature of the defence mechanism by various immunoprophylactic (vaccines) agents used against fowl cholera in chickens remains enigmate. DUA and MAHESWARAN (1978) found a correlation between levels fo systemic humoral antibodies (titres) and cell-mediated immunity responses and resistance to challenge with a virulent strain of P. multocida. However independent studies by HEDDLESTON and WATKO (1965), BHASIN and BIBERSTEIN (1968) and ALEXANDER and SOLTYS (1973) found no correlation between serum titres and resistance. The data generated from statistical analysis of correlation between levels of antibody titres and phagocytic activity indicated that systemic immunity played a significant role (r = 0.52-0.57). These results also confirm and explain earlier finding of ALEXANDER and SOLTYS (1973), BIERER and DERIEUX (1975), and HEDDLESTON et al. (1975) that turkeys which were passively immunized with antisera raised from immunized birds were protected against challenge. The extraordinary high virulence exhibited by many poultry strains are correlated with the ability of this organism to resist phagocytosis (COLLINS, 1977). These seems that vaccination cannot protect chickens against P. multocida, unless the phagocytic activity of the phagocytic cells is increased. The presence of specific opsonin not only results in high significant shift of phagocytic cells towards the microorganisms (chemotaxis) but also there is an increase in the phagocytic activity. Unfortunately, it should also be pointed out here that the significant statistical difference ($P/ 0.05$) between the phagocytic activity of PMN to phagocytose the different serotypes of P. multocida, can destroy the cross protection.

A commercial bacterins, only contain a few of available strains, so it cannot protect against P. multocida infection, under all field conditions and protection is often fully effective when the autologous vaccine strain is used.

## *REFERENCES*

Alexander, M.; and Soltys, M. (1973): Relationship of serum agglutinins to protective immunity produced in turkeys immunized against fowl cholera. J. Comp. Path., 83, 191-198.

Aowad, J. (1978): Studies on the somatic antigen of P. multocida in carrier and chronic fowl cholera in poultry. M.V.Sc. Microbiology thesis presented to Cairo University.

Bhasin, J. and El-Biberstein (1968): Fowl cholera in turkeys-the efficancy of adjuvant bacterins. Avian Diseases, 12, 159-168.

Bierer, B. and Derieux, W. (1975): The ability of blood plasma from drinking water vaccinated turkeys to protect against a lethal challenge of P. multocida. Poultry Sci., 24, 2091-2093.

Collins, F. (1977): Mechanism of aquired resistance to P. multocida infection. Cornell Vet., 67, 103-138

Department of Microbiology, Vet. Academy (1980): Metodichecki Okazanie Potestirovanic iestistivenoy resistentnocti teliat. Fsecaioznaia Akademia Selckokhazaiestiveniki Nauk immeni. D.N. Lenina Otdelenie Veterinary. Moskovckia Veterinarnia Akademia. Immeni K.N. Skrabina. Moskva.

Dua, S. and Maheswaran, S. (1978): Studies on P. multocida - VI Nature of systemic immunity and Analysis of the correlation between levels of immunity induced by various fowl cholera vaccines and protection against challenge. Avain Diseases, 22 (4), 748-764.

Eyzzat, M. (1983): Some studies on pasteurella group of microorganisms in birds. Ph.D. Thesis (Microbiology) University of Alexandria.

Heddleston, K. and Watko, L. (1965): Fowl cholera: comparison of serologic and immunogenic responses of chickens and turkeys. Avian Dis., 9, 367-376.

Heddleston, K.; Robers, P. and Wessman, G. (1975): Fowl cholera: Immunologic and Serologic response in turkeys to live P. multocida vaccina administered in the drinking water. Poultry Sci. 54, 217-221.

Namioka, S. and Bruner, D. (1963): Serological studies on P. multocida. IV. Type distribution of the organisms on the basis of their capsule and o-groups. Cornell Vet., 53, 41-53.

Namioka, S. (1970): Antigenic analysis of P. multocida. National Institute of Animal Health Quarterly 10 supplement, 97-108.

Nelson, R.; Quie, P. and Simmons, R. (1975): Chemotoxin under agarose: a new and simple method for measuring chemotoxis and spontaneous migration of human polymorphonuclear leucocytes and monocytes. J. Immunol., 155, 1650-1656.

Woolcock, J. and Collins, F. (1976): Studies of the immune mechanism in Pasteurella multocida infected mice. Infect. Immuno., 13, 949-958.

Table (I): Effect of immunization with bacteria of P. multocid on phagocytosis of chemotaxis

| Neutrophil | | | | Monocyte | | | | Chemotaxis | | | | t.tabulated | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Before vaccin. x ± S.D. | After vaccinat x ± S.D. | Difference D ± SD | t.cal. | Before Vaccin. x ± S.D. | After vaccin. x ± S.D. | Difference D ± SD | t.cal. | Before vaccination. x ± x ± S.D. | After vaccination. x ± S.D. | Difference D ± SD | t.cal. | 0.05 | 0.01 |
| 60.00 ± 12.59 | 84.61 ± 12.40 | 24.67 ± 14.09 | 4.35 ++ | 63.00 ± 20.06 | 132.17 ± 51.89 | 69.17 ± 48.74 | 3.48 + | 0.25 ± 0.08 | 0.63 ± 0.14 | 0.38 ± 0.15 | 6.33 ++ | 2.02 | 4.03 |
| 49.83 ± 18.71 | 93.17 ± 12.83 | 43.30 ± 22.40 | 4.77 ++ | 56.67 ± 8.91 | 126.00 ± 33.63 | 69.33 ± 27.67 | 6.14 ++ | 0.18 ± 0.08 | 0.52 ± 0.16 | 0.33 ± 0.15 | 5.50 ++ | | |
| 67.50 ± 19.46 | 98.33 ± 10.11 | 30.83 ± 12.83 | 5.88 ++ | 60.67 ± 18.83 | 121.00 ± 38.03 | 60.33 ± 47.06 | 3.14 + | 0.18 ± 0.16 | 0.57 ± 0.14 | 0.38 ± 0.08 | 11.50 ++ | | |
| 72.67 ± 18.52 | 107.17 ± 9.58 | 34.50 ± 19.97 | 4.23 ++ | 76.67 ± 13.49 | 118.00 ± 10.95 | 41.33 ± 17.10 | 5.92 ++ | 0.13 ± 0.05 | 0.57 ± 0.15 | 0.43 ± 0.19 | 5.51 ++ | | |

n = 6

++ = Highly significant at level P ≤ 0.01

+ = Significant at level of P ≤ 0.05

Table (II): Analysis of variance of phagocytic activity and correlation coefficient with titre of antibody

| S.O.V. | Degree of freedom | Mean Square | | | | Correlated Item | Neutrophile | | Monocyte | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neutrophile | | Monocyte | | | Before vaccin. | After vaccin. | Before vaccinat. | After vaccinat. |
| | | Before vaccinat. | After vaccint. | Before vaccinat. | After vaccinat. | | | | | |
| Between treatment | 3 | 290.11 | 533.01* | 452.17 | 230.71 | Titre of antibody | -0.21 | -0.52* | -0.17 | -0.57* |
| Experment Error | 20 | 307.58 | 128.12 | 254.7 | 1347.34 | | | | | |

n = 24

* = Significant at P/ 0.05.