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Evaluation of cell-mediated immune response in chickens vaccinated with Newcastle disease virus

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Abstract

The leukocyte-migration inhibition test for the evaluation of cell-mediated immunity was developed for the use in chickens vaccinated with newcastle disease vaccine and other infected with local virulant strain of newcastle disease virus. Results indicated that the leukocyte migration test was reproducible and relatively easy assay to be performed .Serological antibody titers were determined to study the correlation between haemagglutination inhibition titers and leukocyte-migration level using the HI macro-assay .By results of this study cellular immunity level very important for evaluation the level of immunity beside the humoral immunity.

***Keywords*:** Cell mediated immunity; Newcastle disease; Vaccine.

تقييم الاستجابة المناعية الخلوية للدجاج الملقح ضد فايروس مرض النيوكاسل

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الخلاصة

تم تقييم الاستجابة المناعية الخلوية في الأفراخ الملقحة بلقاح مرض النيوكاسل وكذلك المخمجة بعتره محلية ضارية لفايروس مرض النيوكاسل وبالاعتماد على اختبار هجرة خلايا الدم البيضاء . أشارت النتائج ان اختبار هجرة خلايا الدم البيضاء يعد قيما وسهل الإجراء وقد اعتمدت عينات المصل في دراسة العلاقة بين معايير الأضداد المثبطة للتلازن الدموي ومستوى هجرة خلايا الدم البيضاء وباستخدام اختبار تثبيط التلازن الدموي . أشارت نتائج هذه الدراسة الى أهمية دراسة المناعة الخلوية في تقييم المناعة بجانب المناعة الخلطية.

Introduction

The Newcastle disease(ND)is an economically one of the important viral diseases(1) of poultry and its a major concern world wide (2) the sensitized lymphocytes from an immune animal when stimulated by specific antigen (3) release factors that are capable of inhibiting the migration of macrophages (macrophage inhibition factor) and blood leukocytes (leukocyte inhibition factor) (4) the level of immunity against NDV determines the severity of the disease (5).The cellular and humeral response have been suggested to play important roles in the hosts defense against NDV infection (6,7) cell mediated immunity (CMI) has been reported as the first immunological response, being detected as early as 2-3 days after ND vaccination (7).in this study the capillary- tube leukocyte-migration inhibition test (LMI) has been used to evaluate cell mediated immunity (CMI) against ND (8), the present study presents simple under agarose LMI technique to evaluate cell- mediated immune response in chickens .

Materials and methods

Chickens: one hundered twenty one day old broiler chicks (faobro-1), were housed and kept adlbidu feed on concentrated feed. These chickens were not given any types of antibiotic or vaccine and were divided in to 4 groups, each one of 30 chicks.

First Group: thirty chicks were vaccinated at three weeks old with 0.1 ml ND vaccine orally (VG GA strain $10^{7.1}$EID50/0.1ml).(9)

Second Group: thirty chicks were vaccinated in three and five weeks old with 0.1 ml ND vaccine orally(VG GA strain $10^{7.1}$EID50/0.1ml). (10)

Third group: thirty chicks inoculated with virulent ND virus in two weeks old oronasally ($2x10^{6.5}$EID50/0.1ml)(11).

Fourth group: thirty chicks remain as control group without vaccinatioin

Haemagglutination inhibtion test (HI)

Blood sample were collected from the wing vein of each bird left for clotting ,serum were separated ,the humoral antibody response was evaluated using Beta procedure(12).The highest serum dilution causing complete inhibtion of haemagglutination was considered as the end point .The geometric mean HI titer for each group was expressed as the Log2 of the serum dilution(13).

Migration inhibition test (MIT)

Peripheral blood leukocytes of all groups were studied by capillary migration inhibition test according to (14). The migration inhibition index was calculated using the following formula:

Migration index(MI)=

Distance of migration with antigen x 100

Distance of migration without antigen

A 30% inhibtion of migration, i.e. MI of ≤ 70% was considered as significant result, the migration index represent the mean of four reading.(15).

Challange test

The vaccinated groups (one and two) were challanged with virulent strain ND virus ($2x10^{6.5}$EID50/0.1ml) (Virology Lab , Depatment of microbiology , College of veterinary medicine , University of Mosul) at the age of five weeks and the protection was calculated according to the percentage of mortality %, ,the non vaccinated group three was challanged according to the same procedure at the age of three weeks.(16).

Protection

was evaluated by the calculation of % of protected chickens after challanging.

Post mortum observation

The gross examination changs after PM was performed on different organs (Brain , Proventriculus , Spleen , Duedenum , Trachea , Cecal tonsils) and the pathological changes were reported using semi quantity.

Results

The result of cell–mediated immunity by migration inhibition test gave an inhibition of migration of leukocyte with significant index and this inhibition is increased in group two more than other groups, the third group gave an inhibition of migration more than in the vaccinated group after the stimulation with ND antigen when compared with control group. Table (1).

Table 1: Result of migration indices in different groups

Groups	Age				
	1 Day	3 weeks	4 weeks	5 weeks	6 weeks
First	0.9 NS	0.8 NS	0.5 S	0.45 S	0.40 S
Second	0.87 NS	0.83 NS	0.63 S	0.56 S	0.43 S
Third	0.85 NS	0.85 NS	0.40 S	0.40 S	0.38 S
Fourth	0.88 NS	0.87 NS	0.85 NS	0.80 NS	0.80 NS

S= Significant NS=Non Significant

The titer of antibody which was measured by haemagglutination inhibition test showed a significant increase in titer of the vaccinated groups but this increase was more remarkable in third group while the infected group presented highest titer after inoculated with virulent strain. Table (2). The challenge with virulent strain virus gave highest mortality in the non vaccinated group , the animals of this group present after PM severe gross lesions in different organs , while the vaccinated group gave high protection rate with mild changes after post mortum, in vaccinated group when compare with one vaccination, the protection rate was higher in boostered animal group (Table 3).

Table 2: Variation of haemagglutination inhibtion test titer in different groups.

Group NO.	Age				
	1 Day	Three weeks	Four weeks	Five weeks	Six weeks
First	2Log 2	1Log 2	3.8Log 2	3.1Log 2	2.7Log 2
Second	2.2Log 2	1.1Log 2	4Log 2	3.7Log 2	5.5Log 2
Third	1.9Log 2	1.2Log 2	4.7Log 2	6.2Log 2	5.6Log 2
Fourth	2.2Log 2	1Log 2	0Log 2	0Log 2	0Log 2

Table 3: the protection rate and mortality with post mortum lesion In different groups

Group No.	Organs							% Protection rate
	Brain	Proventriculus	Spleen	Duodenum	Trachea	Cecal tonsils	% mortality rate	
First	+	++	++	+	+	++	10/30	66.9%
Second	—	—	+	—	+	+	4/30	86.6%
Third	++	—	++	++	++	+++	17/30	56.6%
Fourth	—	—	—	—	—	—	—	—

(__) = no changes
(+)= mild congestion
(++) = moderate congestion with enlargment
(+++)= severe inflammation with bleeding

Discussion

Various approach have been used for identifying the specific components of the immune system involved in protection.

Results of present study indicate that the cellular immune response of chicken vaccinated with ND vaccine can be evaluated by the migration inhibtion test, this result is an agreement with that of (3,15). The leukocyte migration inhibition test does not require any expensive equipment and advantage of this technique is the plates can be fixed , stained and evaluated at any time and / or save as apermanent record . Although an inverted microscope was used to measure the migration area in the present study. A ruler could have sufficed (3,8) so cell mediated immunity (CMI) is been suggested to be an important factor in the development of protection in chicken vaccinated with ND vaccine (11,12,13).

The humoral immunity was proved to be protected too by HI antibodies against ND (3).

The objective of this study was to ascertain weather (humoral and cellular) immunity are key components in the protection of chicken against ND (12,13) .Therefore vaccination programs should be directed toward eliciting and maintaining high immunity level to NDV in flock of bird also the test of the migration inhibition could be used to evaluate cell mediated immunity against other infectious disease , in addition to ND (8,16).

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