

معهد الأمصال واللقاحات البيطرية - العباسية .  
مدير المعهد : أ . د . سيد سلامه .

### تجارب معملية وحقلية

للتحصين ضد مرض النيوكاسل بـلقاح الـلاسوتا في كل  
من كتاكيت ذات مناعة وخالية منها

ناديه حسن ، كارولي الماس ، عادل الصباغ

- (١) عند تحصين الكتاكيت حديثة الفقس والبالغة من  
المناعة الموروثة ( SPF ) بـلقاح الـلاسوتا عن طريق  
ماء الشرب ( ١٠٠ر٠٠٠ جرعة  $EID_{50}$  ) . كانت  
المناعة المكتسبة ذات فاعلية عالية وصلت الى ٩٥ر٩٠٪ .
- (٢) تحصين الكتاكيت ذات المناعة الموروثة أعمار ١٠ ، ١١ ،  
١٢ يوم بنفس جرعة اللقاح السابق لم تعطى نتائج  
مشجعة بعد التحصين الأول ولكن عند إجراء  
التحصين الثاني بعد ١٤ ، ٢٤ يوم زادت كفاءة  
التحصين .
- (٣) عند تحصين كتاكيت ذات مناعة موروثة عمر ١٤ يوم  
بجرعة ٢٥٠٠ر٠٠٠  $EID_{50}$  لكل كتكوت كانت  
المناعة المكتسبة عالية ووصلت الى ٩٠٪ .
- (٤) رد الفعل المناعي مقدرا بالقوى العيارية لـاليفاف  
التلازن الدموي ( HI titer ) لم تتوافق نتائجه  
مع النسب المثوية للمناعة ضد العدوى المرضية .

Animal Health Research Institute Abasia, Cairo.

**LABORATORY AND FIELD IMMUNIZATION OF CONGENITALLY IMMUNE  
AND SUSCEPTIBLE CHICKS WITH LASOTA NEWCASTLE DISEASE VACCINE**  
(With 2 Tables)

By  
**N.M. HASSAN, K. ALMASSY\* and A. EL-SABBAGH**  
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**SUMMARY**

When Newcastle disease vaccine (La Sota strain) is used in drinking water for vaccination of specific pathogen free (SPF) baby and adult chickens, good protection was obtained even with a small dose of 100,000 EID<sub>50</sub> (at least 95.9%).

Vaccination of maternally immune chicks of 10, 11 and 12 days old with this vaccine, did not give satisfactory results following the primary vaccination dose (protection of 50-55%). When a secondary vaccination dose was given to two groups of birds at two different intervals following the first dose, i.e. after 14 and 24 days, the percentage of immune birds increased to 65 and 80% respectively.

The acquired immunity was quite satisfactory and reached 90%, when maternally immune chicks of 14 days old were primary vaccinated with a dose of 2,500,000 EID<sub>50</sub>.

HI titers in vaccinated birds could not be used as a criterion to judge on the protection percentage in challenged birds.

**INTRODUCTION**

The study of the efficiency of vaccination against Newcastle Disease (ND) in maternally immune and susceptible chicks has been always the aim of many research workers (DOLL *et al.* 1950; LEVINE and FABRICANT 1950, BEAUDETTE and BEVINS, 1953, WINTERFIELD and SEADAL 1957, BANKEWSKI and CORSTVET, 1962 and KEEBLE and WADE 1963).

The purpose of the present investigation is to determine the efficiency of LaSota drinking water vaccine in one day old and adult chickens which were either completely susceptible or congenitally immune to Newcastle disease.

**MATERIAL and METHODS**

Material:

I - Virus strains.

- A) LaSota Newcastle disease vaccine strain originally supplied by Weybridge Reference Lab., U.K., was used as seed virus for the production of vaccine batches. The infectivity titer of the vaccine used in this investigation when titrated in embryonated eggs was found to be  $10^{8.7}$  /0.1. Each lyophilized ampoule of the vaccine contained

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\* Phylaxia Institute, Budapest, Hungary.

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5 ml. of the original suspension.

- B) The velogenic ND virus used in the challenge test was the "Hearts strain" which was supplied by the same Lab.

## II - Birds.

- A) 208 day old SPF chicks used in these experiments were supplied by an SPF farm Budapest, Hungary.
- B) 57,999 congenitally immune baby and adult chickens from different farms were chosen and some of them were selected to be used in the present investigation. The distribution of these chickens is given in table (1).

### Methods:

The Beta procedure of HI test was applied to demonstrate the immune response after vaccination and challenge. Such technique was accomplished by using a standard 4 HA units of ND virus suspension, against double fold dilutions of each serum together with 1% washed chicken RBCs in PBS solution.

## EXPERIMENTAL STUDIES

### 1- Vaccination:

The vaccine was put into the drinking water. The birds were deprived of water overnight before given the water containing vaccine. After reconstituting the lyophilized vaccine, the dose per bird was calculated to be as follows:

A. 10-14 days old chicks	10 ml. water/bird.
B- 60 days old chicks	20 ml. water/bird.
C- Adult hens	40 ml. water/bird.

Since each lyophilized vial contains 5 ml. of the original suspension having a titer of  $10^{8.7}$  EID<sub>50</sub>/0.1, thus various dilutions in drinking water were prepared to give final concentrations of 100.000, 400.000 and 2.500.000 EID<sub>50</sub> per dose per bird. The number and age of birds as well as the programme of vaccination used in laboratory and field experiments are presented in table (1).

### 2- Protection test:

It was carried out 14 days post vaccination. The challenge dose was 0.5 ml. of the velogenic strain containing  $10^6$  ELD<sub>50</sub>, given by I/M or S/C routes. This dose was found sufficient to kill chicks within 4-6 days after showing nervous manifestations. Challenged birds were isolated and kept under observation for a period of 15 days post-challenge with recording of any symptoms and/or deaths.

## RESULTS

Results of this investigation are summarized in tables (I and II). The data demonstrate that chicks receiving 2.500.000 EID at 12 and 60 days old as well as those receiving 100.000 EID<sub>50</sub> at 13 days old proved to have acquired sufficient immunity according to the following criteria:

- A- In the group of 12 days old having received 2.500.000 EID<sub>50</sub> 31.5% gave reliable HI titers (20 or more), whereas 95.9% were refractory to infection with the velogenic strain.



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- B- In birds of 13 days old having received 100.000 EID<sub>50</sub>, 75% showed reliable HI titers, whereas 98% were solidly immune to challenge with the virulent virus.
- C- In the group of 60 days old having received 2.500.000 EID<sub>50</sub>, 94.7% gave reliable HI titers and at the same time 98.7% were solidly immune.
- D- Finally in the group of 18 months old (receiving 400.000 EID<sub>50</sub>), 95% gave reliable HI titers and they were 100% immune.

Meanwhile, the congenitally immune chickens having received 500.000 or 2.500.000 EID<sub>50</sub> showed the following results according to their age:

- A- In group 5 (table 1) of 10 days old having received 500.000 EID<sub>50</sub>, none of them gave reliable HI titers (0%) and only 50% proved to be immune after primary vaccination. Following the second vaccination carried out 14 days later, the immune response slightly increased reaching up to 65%.
- B- In the sixth group of 11 days old receiving 500.000 EID<sub>50</sub>, only 10% showed reliable HI titers and 50% were refractory to infection following the primary vaccination. After the booster dose which was given after 24 days, reliable HI titers were manifested in 35% whereas 80% were solidly immune to challenge infection.
- C- In the seventh group of 12 days old (having received 2.500.000 EID<sub>50</sub>), none of the birds demonstrated reliable HI titers and only 55% were immune after the first dose. When the booster dose was given after 21 days, 40% of the birds showed reliable HI titers, while those resisting challenge infection were 85% of the group.
- D- In birds of 8-14 days old receiving 2.500.000 EID<sub>50</sub>, 54.7% showed reliable HI titers whereas 92% were refractory to challenge infection.

### DISCUSSION

It is known that baby chicks susceptible to ND infection are more liable to acquire immunity by vaccination (ALLAN *et al.* 1973). Yet, day-old maternally immune (MI) chicks can successfully withstood Newcastle disease infection. (DOLL *et al.* 1950 b; MARKHAM *et al.* 1951; EISSA *et al.* 1961; ZAHER *et al.* 1962; ALMASSY *et al.* 1972 and ALLAN *et al.* 1973). The induced immunity becomes more efficient as maternal antibodies becomes hardly detected.

Investigation proved that recently hatched chicks possess an immature and still undeveloped immune system which gradually become more efficient reaching its maturity and efficient capacity by the sixth week. This fact was confirmed by KEEPLE and WADE (1963), who found that young chicks with congenitally derived antibodies against Newcastle disease virus were refractory to early vaccination. This refractiveness is generally attributed to incomplete immunological competence of the chicks as well as to an abrogating effect of maternal antibodies on the vaccinal antigen. These informations may explain the variation in results with respect to the immunity acquired by (MI) baby chicks following a single dose of vaccine (Table I and II).

In our experiments a total of 208 SPF and 75.999 (MI) chicks were vaccinated by LaSota vaccine in the drinking water. The induced immunity that withstood challenge with the virulent ND virus after primary vaccination was at least 95.9% percent among SPF chicks of 12 days old whereas it was only 55% in MI chicks of the same age (Table II groups 1 and 7 respectively).

According to our results we can't deduce a sort of correlationship between the different values of HI titers and percentages of resistance following challenge after primo-vaccination.



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For instance when the percentage of birds giving HI titers of 1/20 or more was only 31.5% the percentage to challenge was found to be over 95.9% in SPF 12 days old chicks, yet in the maternally immune chicks of the same age, no reliable HI titers were found, and only 55% of them were refractory to virulent infection. Nevertheless Table II demonstrates that in 3 groups the percentage of reliable HI titers were very low being between zero and 10, although the resistance to challenge following this primary vaccination was never below 50%.

This phenomenon postulates the existence of local immunity induced by vaccination with live virus vaccine. PONTECORVO and LOMBARDI (1966) reported that intranasal instillation with ND live virus vaccine induced the appearance of neutralizing antibodies in the respiratory tract even in birds showing a relatively low serum antibody titer. Such results were confirmed by ISAO YOSHIDA et al. (1971).

After giving the booster dose of vaccine in passively immune birds, the immune response has slightly increased and was ranging between 65-85% as detected by the challenge test. The main reasons for this weak immune response in farm flocks may be due, either to the maternal immunity in young chicks or to the possibility of the presence of other latent virus infections.

From the above mentioned results, the authors may recommend to carry the immunization process at the age of two weeks when the inherited antibodies decline, or when the immune status of the flock becomes unperceptible at the time of vaccination.

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Table (II)

Correlation between HI status and challenge immunity in SPF and maternally immune chicks

Number of groups	Age of birds	First immunization		Second immunization	
		% of birds giving reliable HI titer after first immunization	% of birds resisting challenge after first immunization	% of birds giving reliable HI titers after second immunization	% of birds resisting challenge after second immunization
SPF					
1	12 d.	23/73 = 31.5%	70/73 = 95.9%	-	-
2	13 d.	45/60 = 75 %	59.60 = 98.3%	-	-
3	60 d.	71/75 = 94.7%	74/75 = 98.7%	-	-
4	18 m.	67/80 = 95 %	80/80 = 100 %	-	-
MI					
5	10 d.	0/20 = 0 %	10/20 = 50 %	-	13/20 = 65 %
6	11 d.	2/20 = 10 %	10/20 = 50 %	7/20 = 35 %	16/20 = 80 %
7	12 d.	0/20 = 0 %	11/20 = 55 %	8/20 = 40 %	17/20 = 85 %
8	14 d.	41/75 = 54.7%	69/75 = 92 %	-	-

- x = By reliable mean an end titer of 20 or more  
 - = Birds not given the second vaccination dose  
 SPF = Specific pathogen free  
 MI = Maternally immune chicks