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A STUDY ON NEWCASTLE DISEASE VACCINATION : The Immunological Response to Inactivated and Attenuated Virus Vaccines

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INTRODUCTION

Vaccination against Newcastle disease is commonly employed in the prevention of this disease. In this country immunization against Newcastle disease using formalin-inactivated virus with the addition of aluminum hydroxide gel was first reported by Lim and Oh (1) in 1954, and since then this vaccine has been used extensively.

Beach (2), however, reported that the inactivated virus vaccine failed to protect outbreaks of Newcastle disease. In 1952, Fabricant(3) also concluded that a commercial product of inactivated Newcastle disease virus vaccine commonly used in the United States was insufficient to be of practical value for the poultry industry.

Doll(4) experimented with a formalin-inactivated vaccine adsorbed to a commercial aluminum hydroxide gel and found that the vaccinated chickens possessed no significant circulating antibodies as revealed by the Hemagglutination Inhibition Test and Neutralization Test. However, these birds were protected mostly against the challenging dose of 10 MLD 3-4 weeks after vaccination.

The attenuated Newcastle disease vaccine described by Hitchner and Johnson (5) was characterized by low virulence for chickens of any age, and possessed the capacity to provoke substantial immunity when administered intranasally. This vaccine has been given to very young and susceptible chickens without any harmful effects. Hitchner also found that the duration of immunity following the vaccination was longer than that produced by killed vaccine.

Attenuated virus vaccines are now available for use by any of the following methods, namely by spraying in the air, wing web or intramuscular inoculation, intranasal instillation, and by addition to the drinking water. Winterfield(6) reviewed the drinking water method of immunization employing many strains and came to the conclusion that the Lasota strain produced superior immunity to any other strain tested. Moreover, this method of administration seemed to be simple and time saving procedure in comparison with other methods.

The author has been interested in the drinking water method of administration because of its applicability without any particular skill. This paper reports the immunological value

of the Lasota strain as compared with the Seoul strain of Newcastle disease virus.

MATERIALS and METHODS

Two strains of Newcastle disease virus were used in this trial. Seoul strain of Newcastle disease virus isolated during the epidemic outbreaks in winter of 1959 was obtained from the An-Yang Vetrerinary Laboratory. Lasota strain of Newcastle disease virus was given in

Table I. Titration of Seoul Strain

Dilut.	No. of Embryos	Steril.	Examin. by Hours 24	36	48	60	72	HA Activity
10^{-1}	6	S	−	2D	4D	−	−	+ + + + + +
10^{-2}	6	S	−	2D	3D	1D	−	+ + + + + +
10^{-3}	6	S	−	−	3D	3D	−	+ + + + + +
10^{-4}	6	S	−	−	4D	2D	−	+ + + + + +
10^{-5}	6	S	1D	−	1D	4D	−	+ + + + + X
10^{-6}	6	S	−	−	3D	3D	−	+ + + + + +
10^{-7}	6	S	1D	−	2D	3D	−	+ + + + + X
10^{-8}	6	S	−	−	2D	3D	−	+ + + + + +
10^{-9}	6	S	−	−	1D	3D	1D	+ + + + + +
10^{-10}	6	S	−	−	1D	1D	−	+ + • • • •
10^{-11}	6	S	−	−	−	−	−	• • • • • •

(S; sterile, D; dead, −; alive, +; hemagglutination positve,
X; discarded, •; hemaghglutination negative)

Table II. Titration of Lasota Strain

Dilut.	No. of Embryos	Steril.	Examin. by Hours 24	36	48	60	72	HA Activity
10^{-1}	6	S	−	−	−	−	−	+ + + + + +
10^{-2}	6	S	−	−	−	−	−	+ + + + + +
10^{-3}	6	S	−	−	−	−	−	+ + + + + +
10^{-4}	6	S	1D	−	−	−	−	+ + + + + X
10^{-5}	6	S	−	−	−	−	−	+ + + + + +
10^{-7}	6	S	−	−	−	−	−	+ + + + + +
10^{-8}	6	S	1D	−	−	−	−	+ + + + + X
10^{-9}	6	S	−	−	−	−	−	+ + + + + +
10^{-10}	6	S	−	−	−	−	−	+ + + + + •
10^{-11}	6	S	−	−	−	−	−	+ • • • • •

(S; sterile, D; dead, −; alive +; hemagglutination positive,
X; discarded •; hemagglutination negative)

a dry form by the University of Massachusetts. The Seoul strain has titer of 10^8 MLD/ml and $10^{9.6}$ LD_{50}, whereas the Lasota strain has the titer of 10^9 MLD, and $10^{10.5}$ ID_{50} in chick embryos, as determined by embryo mortality and hemagglutination activity. The results are included in Table I and II.

Table III. H.A Titer of Embryos(Allantonic Fluid) Inoculated by Virus Dilutions

Dilut \ Titer	5	10	20	40	80	160	320	640	1280	2560	Cont.
Seoul											
10^{-1}	+	+	+	+	+	+	+	+	+	+	−
10^{-2}	+	+	+	+	+	+	+	+	+	−	−
10^{-3}	+	+	+	+	+	+	+	+	+	+	−
10^{-4}	+	+	+	+	+	+	+	+	+	−	−
10^{-5}	+	+	+	+	+	+	+	+	+	−	−
10^{-6}	+	+	+	+	+	+	+	+	+	−	−
10^{-7}	+	+	+	+	+	+	+	+	+	+	−
10^{-8}	+	+	+	+	+	+	+	+	+	−	−
10^{-9}	+	+	+	+	+	+	+	+	+	−	−
10^{-10}	+	+	+	+	+	+	+	+	+	−	−
Pool	+	+	+	+	+	+	+	+	+	−	−
Inact. Sal. Mix. (Saline 4+Tissue 1)	+	+	+	+	+	+	±	−	−	−	−
Lasota											
10^{-1}	+	+	+	+	+	+	+	+	+	+	−
10^{-2}	+	+	+	+	+	+	+	+	+	±	−
10^{-3}	+	+	+	+	+	+	+	+	+	+	−
10^{-4}	+	+	+	+	+	+	+	+	+	±	−
10^{-5}	+	+	+	+	+	+	+	+	+	−	−
10^{-6}	+	+	+	+	+	+	+	+	+	−	−
10^{-7}	+	+	+	+	+	+	+	+	+	±	−
10^{-8}	+	+	+	+	+	+	+	+	+	−	−
10^{-9}	+	+	+	+	+	+	+	+	+	±	−
10^{-10}	+	+	+	+	+	+	+	+	+	−	−
Pool	+	+	+	+	+	+	+	+	+	−	−

(+: hemaglutination positive, −: hemagglutination negative)

Inactivated virus vaccine was prepared from the Seoul strain. Nine-day-old developing chick embryos were inoculated with 0.1 ml. of 10^{-3} through A.A. route(allantoic cavity) in order to obtain a maximum growth of virus. The embryo which died within 24 hours

were regarded as a result of bacterial contamination and discarded. The remaining embryos that died approximately 60 hours after inoculation were used for vaccine production. Allantoic fluid was harvested and tested of its HA activity. Then the harvested tissue materials (allantoic fluid, embryos and chorio-allantoic membranes) were ground in a Waring blender.

As shown in Table III, the HA titers of each embryo inoculated by various dilutions ranged from 1,280 to 2,560. The pool of macerated tissue was inactivated by 1 per cent of fromaldehyde solution with frequent agitation at room temperature for a period of 48 hours. To one part of pooled tissue materials were added four parts of saline, and the mixture was inoculated into fluid thioglycollate medium (Difco) for the detection of bacterial contamination. In orderto insure complete inactivation of virus in the meterial, 0.2 ml. of tissue-saline mixture was inoculated into developing chick embryos(Table IV).

Table IV. Inactivation and Sterility Test

Inoculum	Inoculated into (Media)	Changes in Media(Day)				
		1	2	3	4	5
0.2 ml.	Embryo	−	−	−	−	−
〃	Thioglycollate	−	−	−	−	−

(− ; no change

To one part of tissue-saline mixture was added 1/5 part of commercially prepared aluminum hydroxide gel. This constituted the inactivated virus vaccine.

For the preparation of the attenuated virus vaccine, 9-day-old developing chick embryos were inoculated with 0.1 ml. of 10^{-3} Lasota strain. The embryos died within 24 hours were regarded as a result of bacterial contamination and discarded. To 100 parts of allantoic fluid were added 4 parts of skimmilk. Each 2.5 ml. was distributed into 10 ml. vials. The material was lyophilized and checked for vacuum and used as attenuated virus vaccine. The final product was stored in refrigerator at 5°C. In test, the dried vaccine material was reconstituted to its original volume. As shown in Table V, the positive HA activity of embryos inoculated with dried attenuated virus vaccine by various dilution ranged from 10^{-1} to 10^{-9}.

Table V. HA Activity of Embryos Inoculated with Dried Attenuated Virus Vaccine by Various Dilutions.

Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
HA Activity	+++	+++	+++	+++	+++	+++	+++	+++	++−	−−−

(+ ; hemagglutination positive,
; hemagglutination negative)

The majority of chickens used in this study were White Leghorn approximately 5 months of age. These were divided in two groups, each group consisting of one hundred birds. None

of these birds had been previously vaccinated against Newcastle disease. Five birds from each group were set aside as control. Prior to the vaccination, blood samples were obtained and tested for the presence of antibody by beta HI test(7).

Each bird in the first group was injected intramuscularly with 1 ml. of the inactivated virus vaccine. Each of the socond group received about 10 ml. of drinking water before food intake in which the attenuated virus vaccine was diluted at the ratio of 1: 400. All the birds were bled two weeks after the vaccination and subsequently at one month interval for three month, and then sera were tested for antibody.

Three-and-half months after the vaccination ten birds from each vaccinated group and ten control birds were exposed to the challenge of the field strain of Newcastle disease virus. The challenge virus used was a recently isolated strain from the field outbreak of Newcastle disease in Seoul by the An-Yang Veterinary Laboratory. Each challenge bird received 0.5 ml. of 10^{-3} dilution of the challenge virus into the muscle of thigh(8), and all of the challenge birds were observed for a period of one week.

RESULTS

The HI titers obtained from the two groups of chickens before the vaccination and 15, 45 75, and 105 days after the vaccination were as follows: Before the vaccination the titers ranged from 5 to 20(Table VI). All of the HI titers 15, 45, and 75 days after the vaccnation were 640 except the one in which the titer decreased to 320(Table VII, VIII, and IX).

Table VI. The HI Titers Before the Vaccination

Birds	Titer								Control
	5	10	20	40	80	160	320	640	
I-1	−	−	+	+	+	+	+	+	−
I-2	−	+	+	+	+	+	+	+	−
I-3	−	−	−	+	+	+	+	+	−
I-4	−	+	+	+	+	+	+	+	−
I-5	−	+	+	+	+	+	+	+	−
A-1	−	−	+	+	+	+	+	+	−
A-2	−	+	+	+	+	+	+	+	−
A-3	−	−	−	+	+	+	+	+	−
A-4	−	+	+	+	+	+	+	+	−
A-5	−	−	−	+	+	+	+	+	−

(I : Inactivated virus vaccine group,
A : attenuated virus vaccine group,
+ : hemaglutination positive,
− : hemmagglutination inhibited)

Table VII. The HI Titers 15 Days After the Vaccination

Birds	Titer								Control
	5	10	20	40	80	160	320	640	
I-1	–	–	–	–	–	–	–	–	–
I-2	–	–	–	–	–	–	–	–	–
I-3	–	–	–	–	–	–	–	–	–
I-4	–	–	–	–	–	–	–	–	–
I-5	–	–	–	–	–	–	–	–	–
A-1	–	–	–	–	–	–	–	–	–
A-2	–	–	–	–	–	–	–	–	–
A-2	–	–	–	–	–	–	–	–	–
A-3	–	–	–	–	–	–	–	–	–
A-4	–	–	–	–	–	–	–	–	–
A-5	–	–	–	–	–	–	–	–	–

Table VIII. The HI titers 45 Days After the Vaccination

Birds	Titer								Control
	5	10	20	40	80	160	320	640	
I-1	–	–	–	–	–	–	–	–	–
I-2	–	–	–	–	–	–	–	–	–
I-3	–	–	–	–	–	–	–	–	–
I-4	–	–	–	–	–	–	–	–	–
I-5	–	–	–	–	–	–	–	–	–
A-1	–	–	–	–	–	–	–	–	–
A-2	–	–	–	–	–	–	–	–	–
A-3	–	–	–	–	–	–	–	–	–
A-4	–	–	–	–	–	–	–	–	–
A-5	–	–	–	–	–	–	–	–	–

The HI levels in the vaccinated group were appreciable degree as compared with the non-vaccinated group of chickens. The antibody titers obtained from the groups of chickens that were vaccinated with the inactivated and attenuated virus vaccines are shown comparatively in Graph I.

The results indicate that both inactivated and attennuatted virus vaccines produced the same degree of HI titers so far as tested 15, and 45 days after the vaccination. The peak of titer reached with both two vaccines at two weeks after the vaccination and maintained the same level for at least two months except the one in which the titer decreased to 320, and then diminished there after. As shown in Graph I, the antibody titers produced by the

Table IX. The HI Titers 75 Days After the Vaccination

Birds	Titer								Control
	5	10	20	40	80	160	320	640	
I-1	–	–	–	–	–	–	–	–	–
I-2	–	–	–	–	–	–	–	–	–
I-3	–	–	–	–	–	–	–	–	–
I-4	–	–	–	–	–	–	–	–	–
I-5	–	–	–	–	–	–	–	–	–
A-1	–	–	–	–	–	–	–	+	–
A-2	–	–	–	–	–	–	–	–	–
A-3	–	–	–	–	–	–	–	–	–
A-4	–	–	–	–	–	–	–	–	–
A-5	–	–	–	–	–	–	–	–	–

Table X. The HI Titers 105 Days After the Vaccination

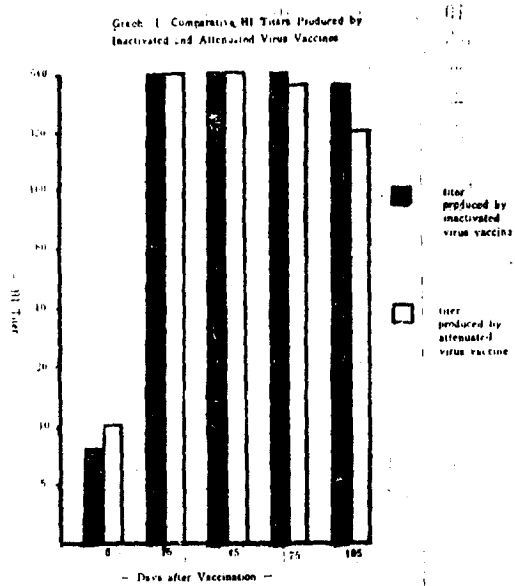
Birds	Titer								Control
	5	10	20	40	80	160	320	640	
I-1	–	–	–	–	–	–	–	–	–
I-2	–	–	–	–	–	–	–	+	–
I-3	–	–	–	–	–	–	–	–	–
I-4	–	–	–	–	–	–	–	–	–
I-5	–	–	–	–	–	–	–	–	–
A-1	–	–	–	–	–	–	+	+	–
A-2	–	–	–	–	–	–	–	+	–
A-3	–	–	–	–	–	–	–	–	–
A-4	–	–	–	–	–	–	–	+	–
A-5	–	–	–	–	–	–	–	+	–

Table XI. Challenge Examination

Group Ident.	No. of Birds	Challenge Dose & Dil.	1	2	3	4	5	6	7 (Examin. Days)
Inact Virus V.	10	10^{-3}, 0.5ml.	–	–	–	–	–	–	–
Atten. Virus V.	〃	〃 〃	–	–	–	–	–	–	–
Control	〃	〃 〃	–	–	4D	5D	1D	–	–

(D: dead, –: alive)

Lasota strain declined slowly from 75 days after the vaccination, while the inactivated virus vaccine antibody titer remained high.



As shown in Table XI, all of the control birds were killed by the challenge virus within 5 days, while the birds vaccinated with both inactivated and attenuated virus vaccines all survived.

DISCUSSION

The experiment conducted in this study demonstrates the degree and duration of immunity for Newcastle disease following the application of inactivated and attenuated virus vaccines.

A group of chickens received a single injections of the in activated virus vaccine generally developed a significant level of immunity against Newcastle disease as determined by the serological method and challenge exposure.

The attenuated virus vaccine prepared from the Lasota strain can be favorably compared with the inactivated virus vaccine as long as 105 days after the vaccination, but appeared to have a slight disadventage only in serological procedure. Both groups of chickens resisted the challenge dose of Newcastle disease virus given 105 days after the vaccination. It seems, however, that the duration of immunity produced by the attenuated virus vaccine is shorter than that produced by inactivated virus vaccine as manifested by the decline of HI titer 75 days after the vaccination.

Although application of Newcastle disease vaccine in drinking water is simple because it is not necessary to handle individual birds. It should be pointed out that the exact amount of vaccine consumed by individual birds has never been determined because of the handling difficulties. A further study is suggested to determine as to whether the ingestion of

insufficient quantities of vaccine in drinking water would cause lower degree of immune response.

SUMMARY

Immune response to two methods of Newcastle disease virus vaccine, one inactivated and the other attenuated, was observed and the data presented.

(1) Administration of inactivated virus vaccine in an amount of 1.0 ml. by intramuscular route gave an appreciable immunity to Newcastle disease for a period of at least three-and-half months.

(2) The chickens given attenuated virus vaccine in the drinking water produced satisfactory immunity as manifested by the fact that immunized birds showed resistance when challenged 105 days after the vaccination and maintained high degree of HI titer for a period of 75 days.

(3) Vaccination with the attenuated virus vaccine in drinking water is very simple and time saving in procedure, although the duration of immunity seems to be slightly shorter than that proced by inactivated virus vaccine.

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抄　錄

Newcastle病의 豫防接種에 관한 硏究;

死毒백신 및 減毒된 生毒백신에 대한 免疫學的 反應

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鄭　吉　澤

著者는 韓國에 特히 많이 流行하고 있는 家鷄의 流行病인 Newcastle disease에 着眼하였다. 豫防方法으로서 從來 使用된 Formalin-inactivated vaccine은 水酸化「알미늄·겔」을 賦加하므로서 筋肉內注射方法으로 使用할수 있게되어 使用方法이 比較的 便利하게 되었으나 그 免疫效果에 對하여서는 아직 異論이 있으므로 Hitcher等이 提唱한 Attenuated Vaccine에 對하여 더 興味를 가지게 되었다. 特히 이와같은 生毒 Vaccine은 닭의 age에 差別없이 毒力이 거의 없을뿐만 아니라 經鼻的으로 大端히 簡易한 方法으로도 投與할수 있고 高度의 免疫을 長期間 賦與할수 있는 點을 들어 其 實用性을 注目하게 되었다. 그러나 vaccine投與方法은 其免疫率과 vaccine投與로 因한 損失을 左右하게 되므로 重要한 硏究課題임을 認定하고 給水法을 採擇하여 一定量의 vaccine을 投與한後 免疫抗體의 出現을 血球凝集阻止抗體와 强毒防禦能力을 觀察하여 具體的方法을 檢討하려 하였다

먼지 生毒vaccine으로 使用한 Lasota strain과 死毒으로 使用한 서울株를 각각 10^{-1}에서 10^{-11}까지 稀釋하여 9日卵의 Allantoic Cavity로 接種하여 60時間이 經過한 後 Allantoic fluia를 採取하여 比較한 結果 서울株는 10^{8}MLD/ml, $10^{9\cdot6}LD_{50}$/ml였고 Lasota strain은 10^{9}MID/ml, $10^{10\cdot5}ID_{50}$/ml였다. Formalin-mactivated Vaccine을 만들기 爲하여 서울株로 感染致死된 鷄胎兒, 羊尿膜, 尿水等의 混合乳劑에 Formaldehy를 1% 加하여 48時間後에 四倍의 生理食鹽水를 加하고 이 混合物 100ml에 4%「알미늄.겔」을 20ml.의 比로 加하여 Inactivation Test (9日卵接種) 및 Sterility Test (Thioglycollate Medium에 接種)를 한 結果 完全히 inactivation 되었고 無菌狀態임을 認定한 後 使用하였다. Attenuated Vaccine을 만들기 爲하여는 Lasota strain을 9日卵의 Allantaic carity로 接種하여 3日間 發育케 한 後 Allantoic fluid를 採取하여 Allantoic fluid 100ml.에 脫脂乳 4ml.의 比로 加하여 2.5ml.式 分注한 後 冷凍乾燥하여 4°C에 保管하였다

五個月八日齡의 白色레구홍 200首를 100首式 二群으로 나누어 各各 死毒 Vaccine (1ml.I.M.) 및 生毒 Vaccine (1 : 400, 10ml 經口)을 投與한 後 105日間 觀察한 結果:

1. 死毒 Vaccine은 HI抗體나 感染防禦作用이 105日間의 실험기간 동안 모두 高度로 維持되었다
2. 生毒 Vaccine은 感染防禦作用은 完全히 維持되었으나 HI抗體는 75日까지 高度로 維持되었으나 75日後에는 若干 低下되었다
3. 給水에 타서 經口的으로 投與하는 生毒豫防接種은 免疫期間이 死毒豫防接種에 比해 약간 짧은듯 싶지만 그接種方法이 대단히 간단하며 時間도 짧게 걸린다