Evaluation of the Protection Efficacy of Newcastle Disease Vaccination Programs

[1]Hsiang-jung TSA1, and [2]Dih-Fa LIN

1.Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan 106, ROC
2.Taiwan Animal Health Research lnstitute, Tansui, Taipei, Taiwan 251, ROC (Received: October 27, 1998. Accepted: December 2, 1998.)

ABSTRACT Various Newcastle disease (ND) vaccination programs were tested for their protection efficacy. In trial 1, SPF chicks were vaccinated with an attenuated or inactivated ND vaccine at 4-day-old, then boosted again with an attenuated or inactivated ND vaccine at 7 days of age or 14 days of age. All vaccinated groups showed a good protection rate (80-100%) when they were challenged with a virulent ND virus at 5-week-old. In trials 2 and 3, several groups of broilers with high maternal antibodies (geometric mean hemagglutination-inhibition antibody titer was 1:84.5-1:135 at 4-day-old) were simultaneously immunized with an attenuated and an inactivated ND vaccine at 4 days of age, and then revaccinated with an attenuated ND vaccine at 17-day-old. Other groups of broilers were first vaccinated with a live vaccine at 4-day-old, revaccinated with an inactivated vaccine at 14-day-old, and then boosted again with a live vaccine at 17-day-old. Good protection rate (80-100%) was achieved in both groups when they were challenged at 5-week-old. When they were challenged at 3-week-old and 4-week-old, the latter broiler groups had a significantly higher protection rate (100%) than the formal broiler groups (50-80%) ($p<0.05$).

Keywords: Newcastle disease (ND), Vaccination programs, Protection efficacy

INTRODUCTION

Newcastle disease (ND) has been recognized as one of the most devastating poultry diseases worldwidel [1, 5, 7]. The disease has become an endemic disease in Taiwan since it was first reported in 1951[15]. Major epidemisc occurred in Taiwan in 1969-1971,1984 [11] and in 1995. In these recent outbreaks, the relaxation of vaccination programs has been blamed. However, some also suspected that ND vaccination programs once conferring good protection may not be adequate in recent outbreaks. It has been reported that high maternal antibodies interfere with immune response of chicks to intranasal, intraocular, or oral routes of ND vaccination [4, 6, 9, 12]. In order to improve vaccination efficacy, vaccination programs composed of simultaneously inoculating chicks with live and inactivated ND vaccine gained

popularity in the field. However, few tests have been done to evaluate this kind of vaccination program. In this paper, several ND vaccination programs employing both live and inactivated ND vaccines were evaluated.

MATERIALS AND METHODS

Vaccines Vaccines used in this study included (1) Vaccine V (L), an imported attenuated ND vaccine ; (2) Vaccine V (K), an imported oilemulsion inactivated ND vaccine ; (3) Vaccine VB (L), a bivalent live vaccine containing both ND and IB (infectious bronchitis) viruses ; (4) Vaccine NI (K), a bivalent inactivate vaccine containing both ND (Komorov strain) and IB viruses (local isolate), two forms (oil-emulsion and aluminum hydroxide gel) of vaccines were used ; (5) Vaccine MB, an imported live IBD (infectious bursal disease) vaccine ; (6) Vaccine NB (L), an imported bivalent attenuated vaccine containing ND (Clone 30) and IB (MA5) viruses ; (7) Vaccine D-78, an imported attenuated IBD vaccine ; (8) Vaccine I, an imported oil-emulsion ND vaccine ; and (9) Vaccine B, an imported oil-emulsion ND vaccine. Vaccines are denoted by letter to profect commercial privacy.

Viruses Two velogenic viserotropic ND (VVND) viruses were used in challenge studies. One (Sato strain) is now used as a standard challenge strain for ND vaccine official evaluation in Taiwan. The other (Chiayi strain) is a local isolate which was isolated in the 1995 epidemic from a field outbreak of VVND. The intracerebral pathogenicity index (ICPI) of Chiayi strain is 1.62 (unpublished data). The Sato strain was used at 9th passage in SPF chicken embryos, while Chiayi strain was used at 3rd passage. The challenge dose was 1,000 LD_{50}per chicken via intramuscular route.

Serological tests The ND hemagglutination-inhibition (HI) test was performed as described by Beard〔2〕. The ND HI antigen was purchased from Taiwan Animal Health Research Institute (TAHRI), Tansui, Taiwan.

Experimental designs

Trial 1 A total of 137 one-day-old SPF chicks was purchased from Branch Institute of Drug Inspection, TAHRI. The birds were randomly allocated into 13 groups and each group was housed separately. The vaccination programs were applied as shown in Table 1. All birds were challenged with Sato strain of ND virus at 5-week-old, and the clinical signs and mortality were observed and recorded daily for one week. Blood samples were taken from all birds when they were 14 days and 28 days of age, respectively. The HI antibody titers against NDV were determined.

Trial 2 A total of 122 one-day-old broilers was purchased from a broiler breeding company. These birds were allocated into 6 groups, and each group was housed separately. The vaccination program of each group of chicks is shown in Table 2. An attenuated ND vaccine and an inactivated vaccine were given simultaneously to the chicks in groups 1 and 2 at the age of 4 days. In group 3 and 4, live vaccine was given at the age of 4 days, and inactivated vaccine given at the age of 14 days. In the trial, the inactivated vaccine was given to 4-day-old broilers at a dose of 0.1-0.2 mL, and was given to 14-day-old broilers at a dose of 0.5mL. All ND vaccinated groups (groups 1-4) were boosted again with an attenuated bivalent vaccine at 17-day-old. All birds were challenged with Sato strain or Chaiyi strain of ND virus at 5-week-old, and the clinical signs and mortality were observed and recorded daily for one week. Blood samples were taken from birds when they were 4, 24, and33 days of age. The HI antibody titers against NDV were determined.

Trial 3 A total of 135 one-day-old broilers was purchased from the same broiler breeding company as mentioned in trial 2. These birds were divided into 5 groups, and each group was housed separately. The vaccination program of each group is shown in Table 3. Each group was divided into 3 subgroups, and each subgroup was challenged with Sato strain of ND virus at 3-, 4- or 5-week-old. Blood samples were taken from birds when they were 4, 24 and 31 days of age. The HI antibody titers against NDV were determined.

Table 1. Efficacy of the Newcastle disease vaccination programs in SPF chickens

	Age of Vaccination						ND HI titers (GMT)		Protection Rate (%)*	
	4		7		14					
	vaccine route		vaccine route		vaccine route		14 days	28 days		
Groups	(type)	[dose]	(type)	[dose]	(type)	[dose]				
1	V (L)	IO [1]			V (L)	IM [2]	194.0	128.0	13/15	86.7 (a)
2	V (L)	IO [1]			V (K)	IM [1]	294.1	891.4	8/10	80.0 (a)
3	V (L)	IN [1]			V (L)	IM [2]	304.4	84.4	19/19	100 (a)
4	V (L)	IN [1]			V (K)	IM [1]	388.0	1024	9/9	100 (a)
5	V (K)	SC [3/5]	V (L)	IO [1]			147.0	55.7	9/10	90.0 (a)
6	V (K)	SC [3/5]			V (L)	IM [2]	76.1	256.0	11/12	90.9 (a)
7	I (K)	SC [3/5]	V (L)	IO [1]			388.0	430.5	9/10	90.0 (a)
8	B (K)	SC [3/5]	V (L)	IO [1]			84.5	512.0	10/10	100 (a)
9	NB (L)	IN [1]	V (K)	SC [1]			NT	NT	5/6	83.3 (a)
10	NB (L)	IN [1]			V (L)	IM [1]	256.0	891.4	9/10	90.0 (a)
11	NB (L)	IN [1]			V (K)	IM [1]	891.4	1552.1	6/6	100 (a)
12					V (L)	IM [1]	<2	38.1	10/10	100 (a)
13							NT	2.4	0/10	0 (b)

*Each bird was challenged at 5-week-old with Sato strain of NDV with a dose of 1,000 LD_{50} via intramuscular route. Protection rates were indicated by numbers of birds survived/numbers of birds challenged. Values within a column followed by different ltters are significantly different ($p < 0.05$).
IO = intraocular ; IN = intranasal ; SC = subcutaneous ; IM = intramuscular ; NT = not tested.

Table 2. Vaccination efficacy of the Newcastle disease vaccination programs in broilers with high maternal antibody titers

	Age of Vaccination*			ND HI titer (GMT)		Protection rate (%)**	
Group	4	14	17	24-day-old	33-day-old	Sato strain	Chiayi strain
1	VB (L)	NI (K; oil)	VB (L)	8.0	34.3	10/10 (100)	8/10 (80)
	NI (K,0.2mL)		VB (L)	4.0	27.9	(a)	(a)
2	VB (L)		VB (L)	7.5	337.8	9/9 (100)	9/10 (90)
	NI (K, 0.1mL)					(a)	(a)
3	VB (L)					9/9 (100)	9/9 (100)
						(a)	(a)
4	VB (L)	NI (K; gel)	VB (L)	4.0	27.4	10/10 (100)	9/10 (90)
						(a)	(a)
5				2.2	2.0	0/9 (0)	1/10 (10)
						(b)	(b)
6				0.9	3.5	1/14 (7.2)	0/12 (0)
						(b)	(b)

*Groups 1-5 received a live IBD vaccine (MB) at 12-day-old. All live vaccines were inoculated via ocular route; all inactivated vaccines were inoculated via subcutaneous route. The doses of the inactivated vaccine given was 0.5mL per chicken, if not specifically denoted.

**Values within a column followed by different letters are significantly different ($P < 0.05$).

Table 3. The efficacy of the Newcastle disease vaccination programs in broiler with high maternal antibody titers

	Age of Vaccination*				ND HI titer (GMT)		Protection Rate (%)**		
Group	4	12	14	17	24-day-old	31-day-old	21-day-old	28-day-old	35-day-old
1	VB (L) NI (K)	MB		VB (L)	9.3	68.6	7/10 (70) (b)	8/10 (80) (ab)	5/6 (83) (a)
2	VB (L) NI (K)	D-78		VB (L)	12.1	13.0	6/10 (60) (b)	5/10 (50) (b)	7/8 (88) (a)
3	VB (L) NI (K)			VB (L)	4.0	8.6	7/10 (70) (b)	7/10 (70) (b)	6/7 (86) (a)
4	VB (L)	MB	NI (K)	VB (L)	12.1	222.9	10/10 (100) (a)	10/10 (100) (a)	4/4 (100) (a)
5					1.2	3.0	0/10 (0) (c)	0/10 (0) (c)	0/10 (0) (b)

*All live vaccines were inoculated via ocular route; all inactivated vaccines were inoculated via subcutaneous route. The dosage of the inactivated vaccine received by each 4-day-old bird was 0.1mL, and 0.5mL was given to each 14-day-old bird.

**Values within a column followed by different letters are significantly different ($p<0.05$).

Trial 1 As shown in Table 1, all vaccinated groups (groups 1-12) showed the protection rates of (80-100%) against VVND challenge. No significant difference in protection ability was found among vaccinated groups ($p > 0.05$). Otherwise, the mortality of the unvaccinated control group (group 13) was 100%. Although the difference in the protection efficacy was not statistically significant, it seems that intranasal inoculation (groups 3&4) at 4 days of age gave better protection than intraocular inoculation(groups 1&2).

Trial 2 The geometric mean titer (GMT) of maternal HI antibody titer against ND in 4-day-old broilers was 84.5. A good protection rate (80-100%) was observed in all ND vaccinated groups (groups 1-4), while mortality of the unvaccinated controls was 90-100% (groups 5 and 6). The results of challenge using different challenge virus (Sato or Chiayi strain) did not differ significantly ($p > 0.05$).

Trial 3 The GMT maternal HI antibody titer against ND in 4-day-old broilers was 135.0. Good protection rates (83-100%) were observed in all ND vaccinated groups (groups 1-4), when they were challenged at 35-day-old. When the challenge was performed at the age of 3 weeks and 4 weeks, Group 4 had a significantly higher protection rate than groups 1-3 ($p > 0.05$). The mortality of the unvaccinated control group (group 5) was 100%, no matter the broilers were challenged at 3-, 4- or 5-week-old.

DISCUSSION

In this study, most of the vaccination programs evaluated gave good protection (80-100%) when the vaccinated chickens were challenged at 2-3 weeks after last vaccination. The challenge strain (Sato strain) and challenge dose (1,000 LD_{50}) used in this study were according to the National Standard For Animal Drug Assay (NSFADA; revised by the Council of Agriculture, Executive Yuen on March 26, 1994). The NSFADA indicated that ND vaccine should have a protection rate higher than 75%. Lin *et al*. [10] have evaluated various commercial ND live and inactivated vaccines by various vaccination routes and programs in a series of trials in Taiwan. His results indicated that different ND live vaccine conferred similar protectivity, but protection conferred by different ND inactivated vaccines varied greatly. In trial 1, protection conferred by different ND inactivated vaccine did not show significant differences ($p > 0.05$). However, since only 3 brands of inactivated ND vaccines were tested in this study, evaluation tests on more vaccines may be needed.

It was also found that broilers vaccinated with live vaccine at 4-day-old then revaccinated with an inactivated vaccine at 14-day-old had a superior protection efficacy to broilers simultaneously vaccinated with a live and an inactivated vaccine at 4-day-old. For example, broilers that were vaccinated simultaneously with a live vaccine and an inactivated vaccine at 4-day-old reached a protection rate of only

80-90% when they were challenged with Chiayi strain VVND virus. Although not significantly different ($p > 0.05$), the other non-simultaneously vaccinated broiler groups reached a higher protection rate (100%) (trial 2). Also, broilers simultaneously vaccinated with a live vaccine and an inactivated vaccine at 4-day-old had a significantly lower protection rate when the challenge study was performed at 3 or 4-week old ($p > 0.05$) (trial 3). However, Hsien [8] found that one-day-old or 14-day-old broilers with medium-high maternal antibodies (GMT HI titer 1:50 at one-day-old) simultaneously vaccinated with a live and an oilemulsion inactivated vaccine both gave a 100% protection rate when they were challenged at 4-week -old. The different results obtained by the present study may be due to the differences in the levels of maternal antibodies or the brand and dosage of the vaccine used. The dosages of the inactivated vaccine used by Hsien [8] were 0.3mL at one-day-old and 0.5mL at 2-week-old. Wang et al. [14] showed that the maternal antibodies in most of the one-day-old broilers in Taiwan range from 1:16 to 1:64. If the high maternal antibodies are able to interfere with the vaccine efficacy of the inactivated vaccine, the broilers with very high maternal antibodies may need to be vaccinated with the higher dose of the inactivated vaccine or immunized at a later time in order to achieve better vaccine efficacy. In trial 1, groups of SPF chicks were given live vaccine two times first at 4-day-old via intranasal or intraocular route, and then at 14-day-old via intramuscular route. A good protection rate (80-100%) was achieved when they were challenged at 5-week-old. Beard et al. [3] had a similar finding that SPF chicks vaccinated with live ND vaccine at 1-day-old once or revaccinated at 17-day-old had an 87.5-100% protection rate when they were challenged at 30-day-old. Hsien *et al.* [8] has shown broiler chicks inoculated with live ND vaccine via ocular route 2-3 times could achieve 60-90% protection. If the live vaccine was given to broiler intramuscularly under 2 weeks of age 1-2 times, a protection rate of only 20-73% could be achieved. Compared to our study of SPF chickens, their results may indicate that maternal antibodies could interfere with the vaccine efficacy of the intramuscular route inoculation of the live vaccine.

Lin *et al*. [10] evaluated 4 VVND viruses isolated in Taiwan in 1985 and found that their virulence was similar to that of the Sato strain by the standard characterization (intracerebral pathogenic index, ICPI and intravenous pathogenic index, IVPI).

In our study, the 1995 isolate (Chiayi strain) also had a similar virulence to that of broilers. Thus, this may indicate that no significant virulence change has occurred to the ND virus in the field. In this study, chickens previously primed with live ND vaccine (L) and then revaccinated with an inactivated ND vaccine (K) were found to have a higher antibody response than those with the live vaccine alone. This is in agreement with the findings of Partadiredja *et al*, [13]. Lin *et al.* [10] also demonstrated that merely using live or inactivated ND vaccine did not confer a good protection in broilers, whereas the 4 (L), 14 (L), or 24 (K) vaccination programs gave 100% protection when the challenge study was done at 45-day-old. In this study, the best protection was achieved by the 4 (L), 14 (K), and 17 (L) vaccination programs when the

challenge study was performed at 21-, 28-, and 35-day old. The evaluation of the protection efficacy beyond 35-day-old may be needed, especially in those birds such as native chicken which are marketed at a much higher age.

REFERENCES

1. Alexander DJ. Newcastle disease and other avian paramyxovirus infections. In BW Calnek, HJ Barnes, CW Beard, LR McDougald, YM Saif. (eds.) Diseases of Poultry 10th ed. lowa State Univ Press, Ames, Iowa, 541-569, 1997.
2. Beard CW. Serological procedures. In HG Purchase, LH Arp, CH Domermuth, JE Pearson, (eds), Isolation and Identification of Avian Pathogens, 3rd ed. Am Assoc Avian Pathologists, College Station, Texas, 192-200, 1989
3. Beard CW, Villegas P, Glisson JR. Comparative efficacy of the B-1 and VG/GA vaccine strains against velogenic viscerotropic Newcastle disease virus in chickens. Avian Dis 37:222-225, 1993.
4. Box PG. The influence of maternal antibody on vaccination against Newcastle disease. Vet Rec 77:246, 1965.
5. Brandly CA, Moses KE, Jones EE, Jungherr EL. Epizootiology of Newcastle disease of poultry. Am J Vet Res 7:243, 1946.
6. Eidson CS, Kleven SH. A comparison of various routes of Newcastle disease vaccination at one day of age. Poultry Sci 55: 1778-1781, 1976.
7. Hanson RP. World wide spread of viscerotropic Newcastle disease. Proc. 76th Ann Meeting, US Animal Health Assoc., Miami Beach, Fla. pp. 276-279, 1972.
8. Hsien JH. Personal communication. Dept. Vet. Med., National Chung-Hsing University, Taichung, Taiwan, 1996.
9. Kelvin SH, Eidson CS. Efficacy of intratracheal administration of Newcastle disease vaccine in day-old chick. Poultry Sci 55:1252-1267, 1976.
10. Lin MY, Chung YF, Hung SK, Cheng MC, Sung HT. Comparison of the immunity conferred by different vaccination programs and routes of commercial Newcastle disease vaccines against challenge with recent isolates of velogenic viscerotropic Newcastle disease virus from Taiwan. J Chin Soc Vet Sci 16:33-45, 1990.
11. LuYS, Tsai HJ. Epizootiology of Newcastle disease in Taiwan in 1984. J Chin Soc Vet Sci 12: 197-207, 1986.
12. Partadiredja M, Eidson CS, and Kelvin SH. A comparison of immune responses of broiler chickens to different methods of vaccination against Newcastle disease. Avian Dis 23:622-633, 1979.
13. Partadiredja M, Eidson CS, Kleven SH. Immunization of broiler breeder chickens against Newcastle

disease with an oil-emulsion vaccine. Avian Dis 23:597-607, 1979.
14. Wang CH, Chang JM, Tseng CC, and Hsu HY. Serologic profiling of chickens in Taiwan from 1993 to 1994. J Chin Soc vet Sci 21 (2) :75-81, 1995.
15. Yeh MT, Kao CY. Occurrence of Newcastle disease in Taiwan. Taiwan Prov Res lnst Anim Hith Exp Rep. 50-51, 1951.

新城雞病免疫計畫保護效力之評估

蔡向榮[1]　林地發[2]

1.國立台灣大學獸醫學系
2.行政院農業委員會家畜衛生試驗所

摘要

在本研究中對數種新城雞病（Newcastle disease）之免疫計畫以強毒株攻毒的方式評估其保護效力。在試驗1中，12組SPF雞隻在4日齡時以ND活毒(L)或不活化疫苗(K)免疫後，在7日齡或14日齡再以活毒(L)或不活化疫苗(K)補強一次，各組在5週齡時以10^3 LD_{50}佐藤株ND病毒株攻毒時都有良好的保護力(80-100%)。在試驗2及3中，具有高移行抗體之肉雛雞(在4日齡時之幾何平均ND血球凝集抑制抗體力價為1:84.5-1:135)，部份組別在4日齡同時以活毒及不活化疫苗(L+K)接種，然後在17日齡時接種活毒疫苗(L)，其他組別則是在4日齡以活毒疫苗(L)接種後，再在14日齡以不活化疫苗(K)接種，在17日齡時再以活毒疫苗(L)接種一次。活毒疫苗皆以點眼的方式接種，不活化疫苗皆以皮下的方式接種。二種疫苗接種計畫在5週齡攻毒時都有很好的保護效果(80-100%)，但如果在3週齡或4週齡攻毒時，後者〔4(L),14(K),17(L)〕之保護率(100%)顯著較前者〔4(L+K),17(L)〕之保護率為高(50-80%)($p<0.05$)。〔*蔡向榮、林地發。新城雞病免疫計畫保護效力之評估。中華獸醫誌25(1):1-7 1999。*聯絡人TEL:02-23692844,FAX:02-23661475, e-mail:tsaihj@ccms.ntu.edu.tw〕

關鍵詞：新城雞病、免疫計劃、保護效力