

COMPARISON BETWEEN HI TITERS OF ND IMMUNE SERUM FROM EYE-DROP AND INTRA-NASAL ROUTES IN SPF CHICKEN

PERBANDINGAN TITER HI DARI KEKEBALAN ND ANTARA VAKSIN TETES MATA DAN INTRANASAL

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ABSTRACT

Up till now, there are many complaints from the poultry farmers surrounding our laboratory that they still face Newcastle Disease (ND) problem although they have used a good ND vaccine which be recommended by Technical Service. There are some reasons to answer their questions, such as : vaccine's quality, handling and vaccine misused eyedrop vaccines was applicated according to our standard which was compared to intra-nasal as manufacture recommended for live ND vaccine. Eye-drop route conferred significant higher HI titre than intra-nasal route ($P>0.01$) for two commercial ND Lasota strain and La Sota combined vaccines, but not for Hitchner B1 strain vaccine.

Key words : HI titre, ND

ABSTRAK

Banyak keluhan disampaikan petugas lapang (*Technical Service*) tentang kejadian penyakit Tetelo yang sampai kini masih banyak menyerang para peternak ayam didaerah sekitar laboratorium kami. Beberapa alasan yang menunjang kegagalan program vaksinasi antara lain adalah : mutu vaksin yang kurang baik dan penanganan maupun aplikasi vaksin yang kurang tepat. Vaksinasi dilakukan secara tetes mata seperti standar pengujian kami, dibandingkan dengan tetes hidung yang dilakukan oleh peternak sesuai anjuran pabrik. Hasil titer HI diperoleh lebih tinggi secara tetes mata ($P>0,01$) dibanding tetes hidung setelah aplikasi vaksin komersial bagi ND La Sota dan ND La Sota kombinasi, tetapi tidak untuk ND Hitchner B1.

Kata kunci : Titer HI, ND

INTRODUCTION

Newcastle Disease (*Tetelo*) is still a problem among poultry farmer in Indonesia since 1926 (Kraneveld, 1926). There are 2 types of ND vaccines distributed among the Indonesian farmers : lentogenic types (*Hitchner B1*, *La Sota*, *F*, *Ulster* and *V4*) also mesogenic types (*Komarov*, *Mukteswar* and *Roakin*). Vaccine quality control is often still in doubt about the reoccurring of the disease although they have used good quality vaccines.

Usually, in each description of the biological products, the producer should attach the procedure of how to use their product. For live ND vaccine, the common routes of the vaccine application are : eye-drop; intra-nasal; drinking water or spraying. The farmers can select the vaccines, schedule and vaccination method, however even with those they have, they sometimes still find the disease in their area.

The immune response can be achieved not only from the systemic but also from the mucosal immunity, it makes us think to perform this trial to compare the vaccination routes between eye-drop and intra-nasal as a representative of mucosal immunity (Dept. of Histology, 1994).

The purpose of this experiment is to compare the mucosal immunity between the two vaccination routes (eye-drop and intra-nasal) induced in chicken by three kinds of live ND vaccines.

MATERIALS AND METHODS

Chickens :

All chickens were kept in individual cages. Seventy SPF chickens of 4 weeks old were used in this experiment. They were raised in the cages placed in special room with Air Condition and were fed with concentrate made by our laboratory.

Vaccines :

The combined live vaccine (IB H120 + La Sota), live ND vaccine Hitchner B1 strain and live ND vaccine La Sota strain were used in this experiment.

Challenge Virus :

ND "*Sato*" strain, propagated originally from National Veterinary Assay Laboratory in Japan, was used as a challenge virus.

Antisera :

Immune sera were produced in 4 weeks old SPF chickens by vaccination through eye-drop and intra-nasal routes with one dose of the above-mentioned vaccines. The immune sera were obtained from a two week post vaccinated birds (before

challenged) and re-bled after two weeks post-challenged.

Antigen :

The "*Ishii*" strain of NDV was used as the antigen for Haemagglutination Inhibition (HI) test.

Erythrocytes :

Blood for HI test was collected from cardiac puncture of mature SPF chicken. The cells were collected in Alsever's solution, sedimented at 1000 rpm for 5 minutes and washed 3 times in Phosphate Buffered Saline pH 7.2. Erythrocyte suspensions were prepared volumetrically in packed cells.

Experimental Design :

Seven experimental groups of chickens were made from 70 of 4 weeks old SPF chickens. There were 10 unvaccinated as controls and 60 vaccinated chickens in each of the six groups. The initial vaccination of the chickens were given 0.1 ml/dose intra-nasally (IN) and intra-ocular/eye-drop (ED). Two types of three live ND vaccines were used in this experiment. After two weeks post vaccination, all the vaccinated and control birds were challenged with the *Sato* (10^4 CLD₅₀) challenge strain of ND Virus. All control chickens (group 7) died between the second to the fifth day of post challenge. This procedure is the same with our minimum requirement as a potency test. (F.O.H.I., 1995).

The birds were bled at 14 days post vaccination and 14 days post challenged.

Serologic procedure :

The immune serum were inactivated at 56°C for 30 minutes. HA and HI titres were estimated using manual 0.025 ml microtitre-tips and was undertaken in U shaped microtitre trays using 4 Haemagglutination Units of ND *Ishii* antigen and 0.5% of Chicken Red Blood Cells where the reaction was read after letting it stand for 60 minutes at room temperature (Allan, 1974).

RESULTS AND DISCUSSION

All the vaccines tested in this experiment have been passed according to our minimum requirement for safety, potency and virus content tests we applied eye-drop (as the usual method) and also intra-nasal to compare them in mucosal immunity for potency test. While the condition of raising the chickens, challenge virus and serological procedure were also done in the same way in our routine work of laboratory.

We bled the birds before and after the challenge to know if there was increasing immunity or not. All the unvaccinated (control) group became paralyzed or died while none of the 60 vaccinated

chickens were died or became paralyzed which indicated that the vaccines gave 100% protection.

GMT calculation (Soedigdo, 1977) was carried out to find out the different eye-drop from intra-nasal application for ND vaccination.

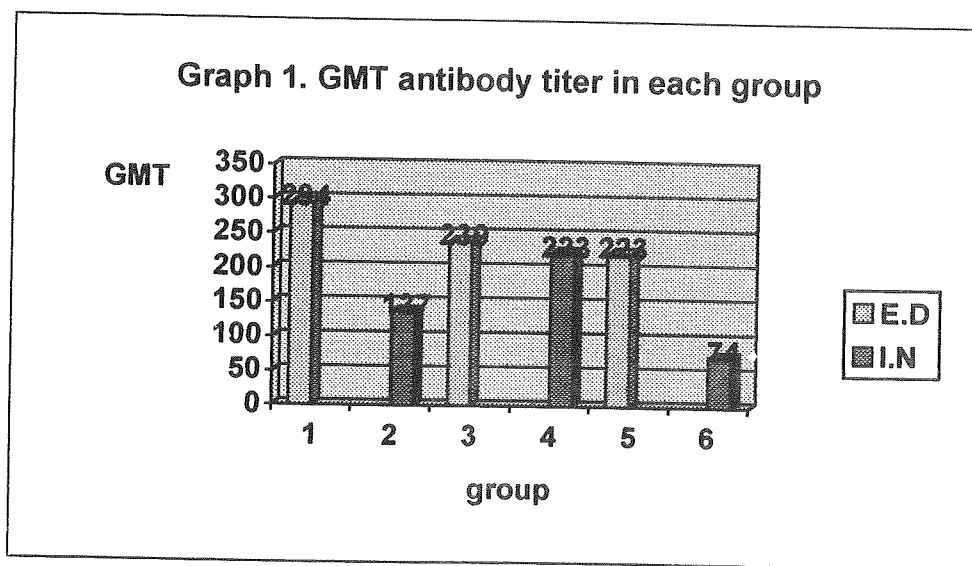
peak between eye-drop and intra-nasally (Graph 1), La Sota strain and La Sota combined vaccines gave significant difference peak where eye-drop route gave higher titre than intra-nasal. In eye-drop application, the highest antibody titre (GMT) was achieved from group

Table 1. HI titers before and after challenged in each group.

group	No of birds	Code and vaccine strain	Route	HI titers 2 wks post vaccination	G.M.T	HI titers 2 wks post challenge	G.M.T
1	10	(141) H120 + La Sota	E.D	256;128;512;128; 32;256;512;256; 64;512	194.01	256;256;512;128; 64;256;256;512; 1024;512	294.07
2	10	(141) H120 + La Sota	I.N	16;128;16;64;256; 256;64;2048;128; 128	111.43	64;1024;128;64; 256;256;16;128; 128;256	137.19
3	10	(142) B1 Hitchner	E.D	256;128;64;512; 64;128;1024;256; 64;256	181.02	256;256;512;64; 512;128;128;256 512;256	238.86
4	10	(142) B1 Hitchner	I.N	128;128;32;128; 128;64;2048;32; 256;128	128.00	128;512;32;64; 256;512;128; 1024;256;512	222.86
5	10	(145) La Sota	E.D	64;128;256;256; 512;8;128;256; 512;128	147.03	128;1024;1024; 64;1024;256;128; 64;128;128	222.86
6	10	(145) La Sota	I.N	16;128;64;16;256; 256;64;2048 128;128	111.43	32;256;64;256 32;64;16;512; 64;32	73.52
7	10	Control	-	-	-	All died	

Table 1 gave a picture of HI titres, where we found that, there were increasing antibody titres after challenged except group 6.

1 (combined vaccine) and followed by group 3 (Hitchner B1) and the last was group 5 (La Sota) which gave the lowest titre. In intra-nasally application, the



Among three different commercial lentogenic Newcastle Disease vaccines, we can directly see that using ND strain Hitchner B1, there was no difference

highest antibody titre (GMT) was observed from group 4 (Hitchner B1) and followed by group 2 (combined vaccine) while group 6 (La Sota) showed the lowest

titre. There was a significant difference ($P>0.01$) between the chicken group vaccinated by eye-drop route as compared to the intra-nasal route for La Sota and La Sota combined but not for Hitchner B1 vaccine.

The conclusion are the vaccine tested in our experiment stimulated satisfactory haemagglutination inhibition (HI) antibody response before challenged (2 weeks post vaccination) and increasing antibody occurred at 2 weeks post-challenged. In the field, we can say the vaccinated birds have sufficient immune response against NDV when they give antibody HI titre > 32 times. ND Hitchner B1 gave a more stable result (no significance difference) between the application vaccination routes of eye-drop and intra-nasal. There was a significant difference in HI titres obtained ($P>0.01$) between the eye-drop and intra-nasal routes when we used single live ND La Sota strain vaccine or combined live ND La Sota and IB strains vaccine. Relating to the field condition, they have a motto : expenses must be less than their income. Also possibly the farmers prefer drinking water and aerosol spray to cut expenses and time consuming. To achieve the goal of the bird industry, we have to pay more attention to the vaccination program, and how well the program is administered and monitored.

Referring to Kim et al (1977), he observed that the lentogenic NDV strain could be isolated from conjunctivitis original samples compared to the chickens showing respiratory disease or proventriculitis as the typical ND symptoms. Those results support our experiment where NDV is more replicated in the conjunctiva compared to other target organs of ND virus. Thus eye-drop route might give high HI titres since the lentogenic NDV vaccine is applied in a proper target organ.

Our result indicated that conjunctiva (via eye-drop) and respiratory tract (via intra-nasal) as the representative of mucosal immunity provided sufficient protection against invasion NDV *Sato* challenge. This

study has only compared two live ND strain vaccines. We should perform other ND strains and distribute our results especially to the poultry farmers.

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