POTENCY TEST AND CROSS REACTION AMONG IBD VACCINES

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ABSTRACT

From several IBD vaccines which were tested for their potency using Plaque Reduction Test against Lukert strain of IBD virus as a challenge virus standard gave the result as follows : The protection for live IBD vaccines at 3 weeks post vaccination were 0 % (vaccine no 1 and 3), 40% (vaccine no 4) and 80% (vaccine no 2 and 5), whereas for killed IBD vaccines at 4 weeks post vaccination were obtained 0% (vaccine no 10); 20% (vaccine no 7 and 8); 80% (vaccine no 6, 9,11 and 12). Using 5 weeks post vaccination serum of live IBD vaccines against Lukert, mild and intermediate also field strains by Serum Neutralization Test (SNT) showed that they have cross immunity among them.

ABSTRAK

Uji potensi beberapa vaksin IBD dengan Plaque Reduction Test (PRT) menggunakan virus IBD strain Lukert sebagai standar virus tantang, memperlihatkan hasil sebagai berikut : Serum 3 minggu paska vaksinasi terhadap beberapa vaksin IBD aktif memberikan prosentasi proteksi 0% (vaksin no 1 dan 3); 40% (vaksin no 4) dan 80% (vaksin no 2 dan 5), sedangkan dengan menggunakan serum 4 minggu paska vaksinasi terhadap vaksin IBD inaktif diperoleh hasil proteksi sebesar 0% (vaksin no10); 20% (vaksin no 7 dan 8); 80% (vaksin no 6.9, 11 dan 12). Uji Serum Netralisasi (SNT) memakai serum 5 minggu paska vaksinasi terhadap vaksin IBD aktif melawan virus vaksin IBD strain Lukert (standar), strain ringan (mild), strain sedang (intermediate) dan strain lokal memperlihatkan adanya kekebalan silang antar mereka.

INTRODUCTION

IBD in Indonesia was first serologically recognized by Akiba in 1974 (Akiba *et al,* 1976) and there was no clinical case until the first outbreak in 1980 reported from Bogor Area (Partadiredja *et al.,* 1981). The virulent form of IBD was first identified in the outbreak in central Jawa in 1991 (Unruh, 1996) and became rapidly widespread throughout the archipelago within 6 months of its introduction in Sulawesi, Maluku and Irian (Darmadi and Muhammad, 1993) and to NTT and NTB in 1982 (Ketut *et al.*, 1992). Several attempts can be carried out for controlling is through vaccination programs that take into account the parent stock and maternal antibodies in the chick, and the following period when the bird becomes fully susceptible to virulent virus and actual risk of exposure to the field virus (Van den Berg et al., 1991).

In the field, there are many kinds of IBD vaccine which can broadly categorized into groups of mild, moderate or intermediate, attenuated "Hot" or intermediate plus and also killed vaccines, although the vaccine prepared should only be originated from strains of virus which are attenuated virulence or from naturally occurring strains of low virulence (Payla, V. 1991).

There are two serotypes of IBDV : only birds infected with serotype I viruses manifest the disease, while serotype 2 viruses mainly occur in turkeys (Reddy, S.K. *et al.*, 1992).

Regarding to our routine work in quality control of Gumboro vaccines, vaccinated birds immune serum must be challenged by Lukert strain as the challenge virus standard (FOHI, 1995), but since there are many intermediate and intermediate plus which had been distributed in the field, we also challenge the vaccinated serum against homolog virus.

The purpose of this trial was to compare the potency of live IBD vaccines (vaccinated immune serum) against Lukert and also against mild, intermediate and field strain of vaccines.

MATERIALS AND METHODS

Chickens :

Four-day-old SPF DOC and three week-old SPF chickens were used for live and killed IBD vaccines respectively. They were raised in the isolator cage in the room facilitated with HEPA-filtered intake and exhaust air. They were fed with concentrated feed produced in our laboratory.

Vaccines :

The live and killed IBD vaccines of several strains were used in this experiment.

Challenge Virus :

IBD Lukert strain was used as our routine challenge virus standard, beside several strains of respective vaccines were also used throughout this experiment.

Antiserum :

Immune sera were produced from the vaccinated birds at 3-5 weeks post vaccination.

Plaque Reduction Test :

This test was carried out as our routine work to determine the immune response of vaccinated chickens after IBD vaccination. At the end of the potency test, the sera from vaccinated and control birds were inactivated at 56°C for 30 min. The serum were diluted 200 times for live and 128 times for killed IBD vaccinated serum. The diluted serum were mixed with 100 PFU of IBD virus (Lukert strain) in equal volume. These mixture of serum and virus were incubated onto a monolayer of CEF prepared in 60 mm glass Petri dishes and incubated for 1 hour at 37°C in a 5% CO_2 incubator. Then the cultures were covered with a first overlay of agar (containing : autoclaved 2% bacto agar in DW_2 and mixed with the same volume of Eagle medium 78% Tryptose Phosphate Broth 10%, Antibiotic 2%, L-glutamine 2%, Na_2HCO_3 4% and Fetal Calf Serum 4%) and were incubated for 3 days at 37°C in a 5% CO_2 incubator, and then stained with a second overlay agar medium containing 1% Neutral Red. The incubation period for one more day and the plaques were then counted.

The acceptable titer of antibodies is fulfilled when *at least 80%* of the vaccinated serum show a *50% or greater* reduction in plaque number from those of *the positive virus controls.*

Serum Neutralization Test :

Two-fold dilution of vaccinated serum was mixed with an equal volume of 100 $TCID_{50}$ of IBD virus (Lukert strain) or the vaccine virus strains for cross reaction challenge. The mixed serum virus solution was allowed to neutralize for one hour at 37°C. Then the mixed sera virus solution were inoculated into the 96 micro wells of the CEF monolayer micro plate already prepared. The micro-plates were incubated at 37°C for one hour in a 5% CO_2 incubator. Maintenance medium (contained : Eagle medium 85%, Tryptose Phosphate Broth 10%, Antibiotic 1%, L-glutamine 1%, Na_2HCO_3 2% and Fetal Calf Serum 1%) were then added to the wells and the plates were incubated at 37°C in a 5% CO_2 incubator for a period of 6 days. The micro plates were then read microscopically to determine the serum dilutions in which CPE (Cyto Pathic Effect) is inhibited.

Potency of live IBD vaccines with SNT will be passed if: **800 times** (9.65 log 2) of serum titers in 80% of vaccinated chickens need to be achieved, while for killed IBD vaccine **512 times** or more of vaccinated serum titer is considered satisfactory (Lestari, I. 1998).

RESULTS

Indeed to determine the level of immunity induced by IBD vaccines, a minimum standard for potency test is Plaque Reduction Test (PRT). Our minimum standard requires that 3 weeks post vaccination for live IBD vaccine or 4 weeks post vaccination for killed IBD vaccine, 80% of the immunized birds must give protective titer against Lukert strain.

The result of potency test in Plaque Reduction Test (PRT) against Lukert strain for immune sera in 3 weeks post vaccination of several live IBD vaccines is presented in table 1. In this test, we used only 5 representative immune serum of each live IBD vaccines to be challenged against Lukert strain. The vaccinated serum were 200 times diluted before challenging by Lukert. The average positive virus control plaque count was 20 PFU/0.2 ml, thus sera showing 10 only will be considered as immune/protective.

From this result, according to our minimum requirement, Live IBD vaccines in number codes 2 and 5 only provided a good protection, since 80% of their serum confer 50% reduction in plaque number.

The result of potency test by Plaque Reduction Test (PRT) against Lukert strain for immune sera 4 weeks post vaccination of various killed IBD vaccines can be seen in the table 2. Also in this experiment, only 5 representative immune serum of each killed IBD vaccines were used to be challenged against Lukert strain. The vaccinated serum has been diluted 128 times before performing of this test. Since the average positive virus control plaque count was 20 PFU/0.2 ml and the negative control plaque was 0 PFU/0.2 ml, sera giving PFU of 10 PFU/0.2 ml only will be considered as immune/protective. From this result, according to our minimum requirement, the killed IBD vaccines in number codes 6, 9, 11 and 12 flavored a good protection, while codes of 7, 8 and 10 were unsuccessful to demonstrate a 80% minimum protection.

Indonesia seems to have many kinds of animal diseases including poultry diseases, moreover there are so many vaccine strains already circulated in the field. Different procedures for potency test used by many producers. In the case of retest, we may adopt the recommended procedures.

Another sensitive test for measuring antibody titer for IBD vaccine in Serum Neutralization Test (SNT) which is not so complicated nor time consuming compare to Plaque Reduction Test (PRT) even the specificity of SNT is less than PRT (Lestari, I. 1998).

Since we have many strains of IBD vaccines and we have difficulties to pass them according to our minimum requirement, we conducted SNT to compare the efficacy of different live IBD vaccines.

In this experiment, results of serology test from vaccinated serum 3-5 weeks after vaccination of mild, intermediate and field virus strains are presented in table 3. It was carried out in January 16, 1996 where vaccine code 002 contained field/local strain, vaccine code 303 was intermediate strain and vaccine code 329 was mild strain. The serum titer was expressed in log 2, where according to our minimum requirement 80% of vaccinated birds serum should give protective titer at least 9.65 log 2 (800 times) or more

At table 3, from 80% vaccinated birds serum, we found that using their virus strain in SNT method, field strain vaccine (code : 002) and mild strain vaccine (code 329) developed protective titer

(800 times or 9.65 log2) at 5 weeks post vaccination, while intermediate strain vaccine (code 303) achieved proper protection titer at 3 weeks post vaccination.

Based on the presented result, where protective titer resulted from 80% vaccinated birds serum were almost at 5 weeks post vaccination, we performed subsequent experiment using 5 weeks post vaccination

Table 1. Potency test of live IBD vaccines (3weeks post vaccination) using Lukert strain by Plaque Reduction Test (PRT).

Virus / Vaccine Serum no	Vaccine no 1 (123) S706 strain	Vaccine no 2 (144) 1-65-pv strain	Vaccine no 3 (157) cheville strain	Vaccine no 4 (171) 2512 strain	Vaccine no 5 (174) 2512 strain
1**	33*	1*	22*	0*	8*
2	23	10	26	1	5
3	19	23	16	26	28
4	12	4	21	19	4
5	16	7	23	13	4
Percentage ***	0%	80%	0%	40%	80%

* : number of plaque.

** : serum collected from birds vaccinated by it's vaccine respectively.

*** : percentage of sera showing plaque number of $\subseteq$ 10 PFU (50% reduction).

Table 2. Potency Test of killed IBD vaccines (4 weeks pos t vaccination) using Lukert strain with Plaque Reduction Test.

Virus Serum Code	Vac no 6 (011) IBD-ND 2512 str	Vac no 7 (037) 2512 str	Vac no 8 (096) 2512 str	Vac no 9 (121) VNJO str	Vac no 10 (147) 1-65 pv str	Vac no 11 (207) boxendale str	Vac no 12 (211) 2512 str
No 1**	3*	23*	15*	14*	17*	3*	0*
No 2	6	23	0	6	18	6	0
No 3	8	29	16	0	13	16	0
No 4	16	29	17	1	28	6	3
No 5	1	9	16	6	17	9	11
Percentage***	80%	20%	20%	80%	0%	80%	80%

* : number of plaque
** : immune serum collected from birds vaccinated by it's vaccine respectively.
*** : no of sera showing plaque number of $\subseteq$ 10 PFU (50% reduction).

serum by SNT method against Lukert virus strain and their vaccine virus strains as can be seen in table 4. This test had been done in January 30, 1996. Under this experiment, five representative vaccinated sera of 5 weeks post vaccination of each live IBD vaccines, were chosen randomly.

In table 4, we can observe that 5 weeks post immune serum from each vaccines can match with Lukert and they conferred to 9.65 log 2 as minimum protective titer, except what was unexpected data for immune serum code 329 (mild strain) gave 6.6 log 2 titer only.

Immune serum code 002 (from field IBD vaccine strain) gave response to its virus strain and also match with other virus vaccines strain (intermediate, mild and Lukert).

Immune serum code 303 (inherited from intermediate IBD strain) offered the highest titer with its virus strain and also match with other virus vaccine strain (field, mild and Lukert).

The result presented herein was unexpected for immune serum code 329 (mild IBD strain), though it had responded to its vaccine virus, but it has failed to give a sufficient serum conversion to Lukert, field and intermediate even at 5 weeks post vaccination.

DISCUSSION

From these results, it was found that some of immune serum induced by live/killed IBD vaccines still did not give enough protective titer against Lukert by PRT method within optimum time which is requested in our minimum requirement, such as vaccine number 1,3 and 4 (live) and 7,8 and 10 (killed) as shown in table 1 and 2.

Depending on the IBD strain virulence, the efficacy test is actually different in using SPF chickens age. To say that the strain is included in intermediate or intermediate plus, it is still to be debated, although there

Table 3. Serum Neutralization Test (SNT) of three kinds of sera using their respective virus strains.

Vaccine code - 002			Vaccine code - 303			Vaccine code – 329		
3 week	4 week	5 week	3 week	4 week	5 week	3 week	4 week	5 week
S1= 3	S1= 6	S1= 9	S1= 10	S1= 10	S1= 10	S1= 6	S1= 9	S1= 6
S2= 3	S2= 7	S2= 10	S2= 10	S2= 10	S2= 10	S2= 11	S2= 4	S2= 11
S3 =3	S3 =9	S3 =11	S3 =7	S3 =10	S3 =10	S3 =9	S3 =6	S3 =11
S4 =3	S4 =7	S4 =10	S4 =9	S4 =8	S4 =11	S4 =9	S4 =4	S4 =11
S5 =3	S5 =8	S5 =11	S5 =9	S5 =11	S5 =10	S5 =3	S5 =8	S5 =6
S6 = 3	S6 = 3	S6 = 10	S6 = 10	S6 = 10	S6 = 9	S6 = 3	S6 = 11	S6 = 11
			S7 = 10	S7 = 11	S7 = 10	S7 =3	S7 = 8	S7 = 10
				S8 = 10	S8 = 11		S8 = 3	
					S9 = 11			
N : 6	N : 6	N : 6	N : 7	N : 8	N : 9	N : 7	N : 8	N : 7
*3	*6.7	*10.17	*9.29	*9.88	*10.2	*6.29	*6.63	*9.4
**3	**8.3	**12.7	**11.6	**12.3	**12.7	**7.9	**8.3	**11.8

* : average titer (GMT) of 100% serum (expressed in log2).
** : average titer (GMT) of 80% serum (expressed in log 2).

Table 4 : Cross Reaction amongst live IBD vaccines by SNT (using 5 weeks post vaccination serum).

Vaccine virus / Vaccinated serum	Lukert virus (standard)	002 IBD virus (field)	303 IBD virus (intermediate)	329 IBD virus (mild)
Serum 002	S1 = 11	S1 = 10	S1 = 10	S1 = 11
	S2 = 10	S2 = 10	S2 = 11	S2 = 11
	S3 =11 X = 10.4*	S3 =11 X = 10.2*	S3 =11 X = 10.8*	S3 =11 X = 11*
	S4 = 10	S4 = 10	S4 = 11	S4 = 11
	S5 = 10	S5 = 10	S5 = 11	S5 = 11
Serum 303	S1 = 8	S1 = 10	S1 = 11	S1 = 11
	S2 = 11	S2 = 11	S2 = 11	S2 = 11
	S3 =10 X = 9.8*	S3 =11 X = 9.8*	S3 =11 X = 10.8*	S3 =11 X = 10.6*
	S4 = 10	S4 = 8	S4 = 10	S4 = 9
	S5 = 10	S5 = 9	S5 = 11	S5 = 11
Serum 329	S1 = 3	S1 = 8	S1 = 7	S1 = 11
	S2 = 3	S2 = 6	S2 = 10	S2 = 11
	S3 =9 X = 6.6*	S3 =9 X = 8.2*	S3 =11 X = 9.2*	S3 =11 X = 11*
	S4 = 9	S4 = 9	S4 = 7	S4 = 11
	S5 =9	S5 = 9	S5 = 11	S5 = 11

• = GMT expressed in log 2.

is a statement that the intermediate strain can be said to be mild after passaging the virus at least 60 times in adapted tissue culture.

There are "naughty" manufactures which mentioned that their IBD vaccine strain is the same as our Lukert derivate to be passed through The Veterinary Drug Commission in order The Commission please them to sell or use it in Indonesia after testing the quality of the final product in the National Veterinary Assay Laboratory. We sometimes repeat the test procedure as the same as the manufacture recommended when it is rejected under our minimum requirement (FOHI).

More interestingly, in the using of SNT method with their respective vaccine virus. We found that some IBD vaccines still failed to produce a proper immune response at 3 weeks post vaccination. For example vaccine code 002 (field strain) and vaccine code 329 (mild strain) could not induce sufficient immune response at 3 and 4 weeks post vaccination, but they gave a proper SNT titer at 5 weeks post vaccination (table 3), while vaccine code 303 (intermediate strain) obtained suitable SNT titer at 4 weeks post vaccination from 100% vaccinated birds or at 3 weeks post vaccination if taken from 80% of vaccinated birds.

It is indicates that we have to reconsider our minimum requirement where our minimum standard for live IBD vaccine must give immune response at 3 weeks post vaccination.

However it is important to note that immune serum of each vaccinated birds conferred cross reaction against other strain at 5 weeks after vaccination although we found unexpected result from mild IBD vaccine code 329, which gave insufficient titer against Lukert strain (table4).

We have also performed immune cross reaction among them using Agar Gel Precipitation Test (AGPT) and showed they have cross reaction but we were not successful to produce Lukert antigen standard control for AGPT (unpublished).

Our result is supported by the former researchers' results that there are cross reaction between serotypes of IBDV by Elisa (Ismail, N.M. et al., 1996) and by radio immunoprecipitation (Reddy, S.K. *et al.*, 1992) also informed by the founder Lukert strain (Lukert.P.D, 2001).

According to our minimum requirement, the efficacy (potency) of live IBD vaccine will be released when they give 800 times (9.65 log2) of SNT titer against Lukert virus within 3 weeks observation period as one of several other requirements from the final product. It needs further studies to compare each live/killed IBD vaccine strains from 3-5 weeks post vaccination with Plaque Reduction Test. (PRT).

As we know, vaccination is the best way to protect the chickens against IBD, it is of great importance to know if the current available vaccines are efficient for Indonesia.

At present, we have had not only mild and intermediate IBD vaccines, but it needs further study for each vaccine for their potency beside the most important point is the safety of the vaccinated chickens and surrounding vaccination fields.

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