DETECTION OF MD ANTIBODIES OF CHICKEN IN BOGOR AND TANGERANG DISTRICTS USING HERPES VIRUS TURKEY (HVT) INFECTED CELLS

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SUMMARY

Using HVT infected cells, sera from chickens in Bogor and Tangerang districts were titrated for antibodies against MD by IFAT.

In native chickens which had no experience of vaccination of MD, 58% of them in Bogor district showed the presence of antibody and 6.8% of them in Tangerang district showed positive reaction.

In the broiler and layer chickens which were suspected to received vaccination against MD, positive rates were considerably higher compared with native chickens.

In 85.7% of broiler and 100% of layer in Bogor district, and 72.7% of layer in Tangerang district, positive reaction were observed.

INTRODUCTION

The casual agent of MD is herpes virus and characterized by infiltration of lymphoid cells in the peripheral nerves, visceral organs and skin (Rivas and Fabricant, 1988). MD tended to occur in chickens over 20 weeks old and mortality rate about 66.7% (Ekperigin *et al,* 1983).

DR. J. Marek called this disease firstly as neuritis interstitialis/polyneuritis and had been seen firstly in USA 1914. In Indonesia, incident of MD which occured for the first time in 1951 as fowl paralysis (neurolymphomatosis gallinarum). In 1956, special neurolymphomatosis (paralysis in the neck and tail) were found in 4 Indonesian native chickens (Djaenoedin and Koerjana, 1956). During 1972-1976 MD has been diagnosed based on gross lesion and histopathological changes, followed by one case report concerning indonesian (Kedu) chicken (Ginting, 1985). Directorate of Animal Health in 1977 reported that MD almost spread in Indonesia as follow : BOGOR and circumstance (46%); BANDUNG and circumstance (43%); CENTRAL JAVA (50%); YOGYAKARTA (39%); EAST JAVA (98%); BALI (35%); MEDAN and circumstance (29%); PALEMBANG (51%) and SOUTH SULAWESI (45%) (Sofyan, 1984).

At present, MD case reports are merely based on pathological anatomy and histopathological observation in Indonesia. The Agar Gel Precipitation Test (AGPT), although specific and easy to perform, but not sensitive (Chubb and Churchill, 1968). In this report, detection of MD antibodies was performed by using Indirect Fluorescent Antibody Test (IFAT).

MATERIAL AND METHODS

Chicken's sera:

Sera were obtained from farms of some villages of 2 districts (BOGOR and TANGERANG). Chicken's strains were: SPF, native, Broiler and Layer within various age.

Antigen/HVT infected cells :

Monolayers of primary CEF cultures in 45 x 15 mm dishes containing coverslip were infected with 8 x 10^4 PFU/dish of MD vaccine (from Handai Biken laboratory - Japan). After 2 days of incuba-

tion, coverslip were taken off and washed once with phosphate-buffered saline (PBS) for 15 minutes, fixed for 15 minutes in cold aceton, dried at room temperature and kept at -80^{0}C before use.

Indirect Fluorescent Antibody Test (IFAT) :

IFAT was performed with slightly modification according to the method described by Purchase and others (Purchase, 1985). Each chicken sera (x40) were mounted onto the coverslip of monolayer cultures infected with HVT at 37^{0}C for 1 hour. After sensitization, the cells were washed 3 times (5, 10 and 10 minutes) with PBS.

Four units of fluorescein-isothiocyanate rabbit anti-chicken immunoglobulin (ICN laboratory - Israel) was added, and the cells were sensitized again for 45 minutes. The coveslips were washed in PBS for 3, 5 and 7 minutes and mounted on slide glass with glycerin-buffer. Observation was made under the ultraviolet microscope. The tested sera which gave positive result in IFAT were further titrated again until x320 according to the same procedure as mentioned above.

RESULT

Detection of MD antibodies in each places :

Four types of chickens from 2 districts (BOGOR and TANGERANG) were examined. In SPF chickens from Bogor, no antibody titer was detected. However, in the native chickens from Bogor which have no experience of MD vaccine, MD antibodies were observed with range 20-100%. The highest positive percentage was obtained in chickens in Cibinong I (100%) and followed Kebon Kopi, Cibinong I and Pabuaran (68.4%, 60% and 50%) respectively. Two others places were less than 50% in positive rate, (Cimangir was 33.3% and it was 20% for Prumpung). By the way, the native chickens from Tangerang had lowered positive percentage of MD antibodies than Bogor district (7.7% for Balaraja and for Desa Perahu was 0%). Broilers in Bogor showed high titer (87.5% adn 83.3% for 2 areas from Cibinong). In layers from both districts were also showed more than 50% of the chickens were positive against MD antigen (100% for 2 places at Cibinong - Bogor, 57.1% and 100% for Cikupa and Ciputat - Tangerang (tabel 1).

Corelation between positive percentage of Marek's disease and strain of chickens :

Fig.1. shows that sera from SPF chickens did not contain MD antibodi entirely. For native chickens from Bogor showed higher positive than Tangerang (6%), (57%) respectively. These are 57% and 6%, respectively. Eighty percent of broiler from

Table 1 : A Survey of antibody titer against marek's disease in chickens by indirect flourescent antibody test.

Chicken's Strain	Place	Number of District	Number of Sera Used	Number of		Positive %
				Sera Positive	Sera Negative	
SPF	Bogor	Bogor	10	0	10	0 %
Native	Cibinong I	Bogor	10	10	0	100 %
	Cibinong II	Bogor	10	6	4	60 %
	Prumpung	Bogor	10	2	8	20 %
	Kebun Kopi	Bogor	19	13	5	68.4 %
	Cimangir	Bogor	9	3	6	33.3 %
	Pabuaran	Bogor	8	4	4	50 %
	Ds. Balaraja	Tangerang	39	3	36	7.7 %
	Ds. Perahu	Tangerang	4	0	4	0 %
Broiler	Cibinong	Bogor	8	7	1	87.5 %
	Cibinong	Bogor	6	5	1	83.3 %
Layer	Cibinong	Bogor	12	12	0	100 %
	Cibinong	Bogor	7	7	0	100 %
	Ciputat	Tangerang	4	4	0	100 %
	Ds. Cikupa	Tangerang	7	4	3	57.1 %

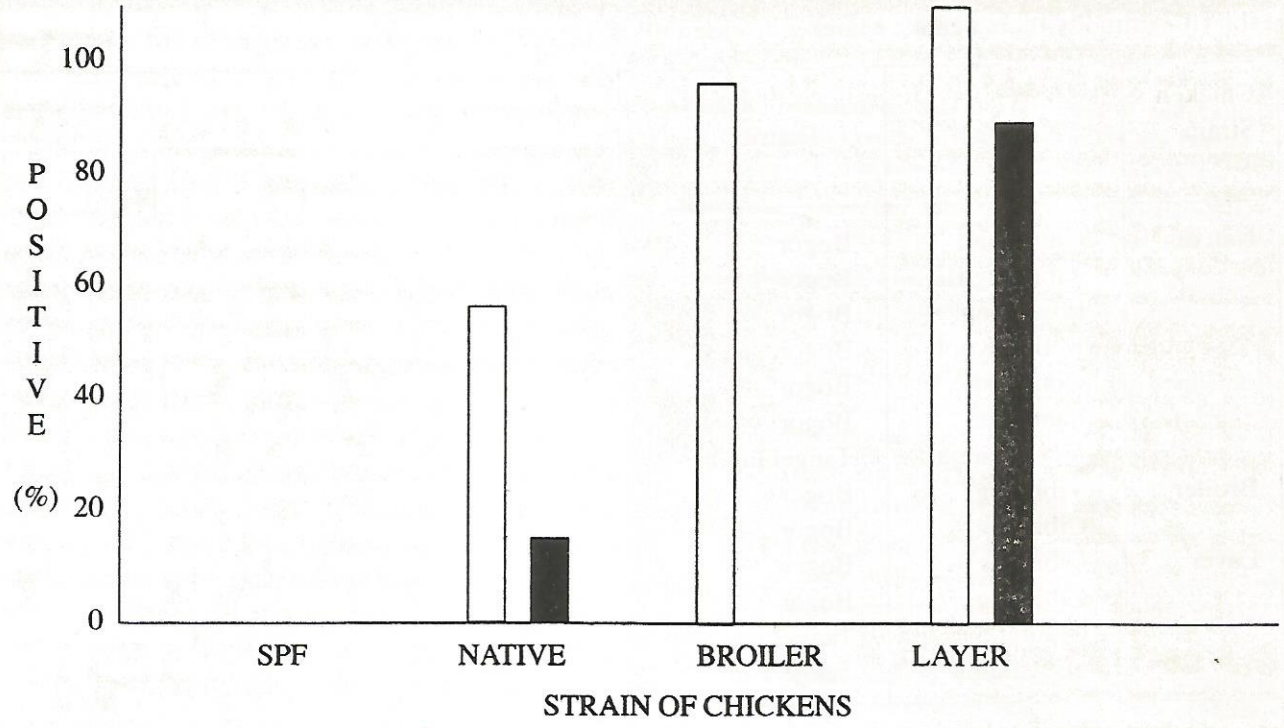

Fig. 1. Correlation between positive percentage of Marek's disease and strains of chickens.

□ : BOGOR District

■ : TANGERANG District

Bogor district were positive to MD antibody. In layers, it was also showed that Bogor had higher positive (100%) compared with Tangerang (71%).

Distribution of MD antibody titer in native, broiler and layer chickens :

Native chickens from both districts tended to have low antibody titre (20 cases and 3 cases for Bogor and Tangerang) respectively. It was also showed that number of positive chickens decrease according to the increase of antibody titer (from 52% decrease to 34% for x80 and became 10% for x160 and no chickens were positive for x320 from Bogor; even 100% of x 40 titre for Tangerang appeared no chickens were positive for more than x40 of sera dilution). In the case of broilers from Bogor, the number of positive chickens was distributed in 41% of x80 and 50% of x 160. Meanwhile, the layer chickens from both districts showed the tendency to give the highest titre (50% for Bogor and 31% for Tangerang) (table 2 and Fig. 2).

DISCUSSION

Detection of MD antibodies in Bogor and Tangerang districts were not fully representative, because both farms selected were not far away from our laboratory. Until now, in Indonesia, the MD case reports were merely based on history, clinical symptom, gross lesion and histopathologically changes (Ginting, 1983 dan Sofyan, 1984), although one survey was based on gel diffusion test (Akiba dkk, 1976) but this test is not sensitive (Chubb and Churchill, 1968). The test was performed by using four types of chickens (SPF, native chicken, broiler and layer) in the various ages (23-45 weeks). SPF and native chickens had no experience of MD vaccination, but broilers and layers were suspected to have MD vaccination since Indonesian policy ordered to vaccinate the day-old chicks (8).

In this experiment, it was demonstrated that SPF chickens had no antibody against MD and it is

Table 1 : Antibody titers against Marek's disease in Postiive sera.

Chicken's Strain	Place	District	Number of Sera Positive			
			X40	X80	X160	x320
Native	Cibinong I	Bogor	4	5	1	0
	Cibinong II	Bogor	2	1	2	0
	Prumpung	Bogor	2	0	0	0
	Kebun Kopi	Bogor	9	4	0	0
	Cimangir	Bogor	1	2	0	0
	Pabuaran	Bogor	2	1	1	0
	Ds. Balaraja	Tangerang	3	0	0	0
Broiler	Cibinong	Bogor	0	2	4	1
	Cibinong	Bogor	0	3	2	0
Layer	Cibinong	Bogor	1	3	4	4
	Cibinong	Bogor	3	1	1	2
	Ciputat	Tangerang	1	1	1	1
	Ds. Cikupa	Tangerang	0	1	0	3

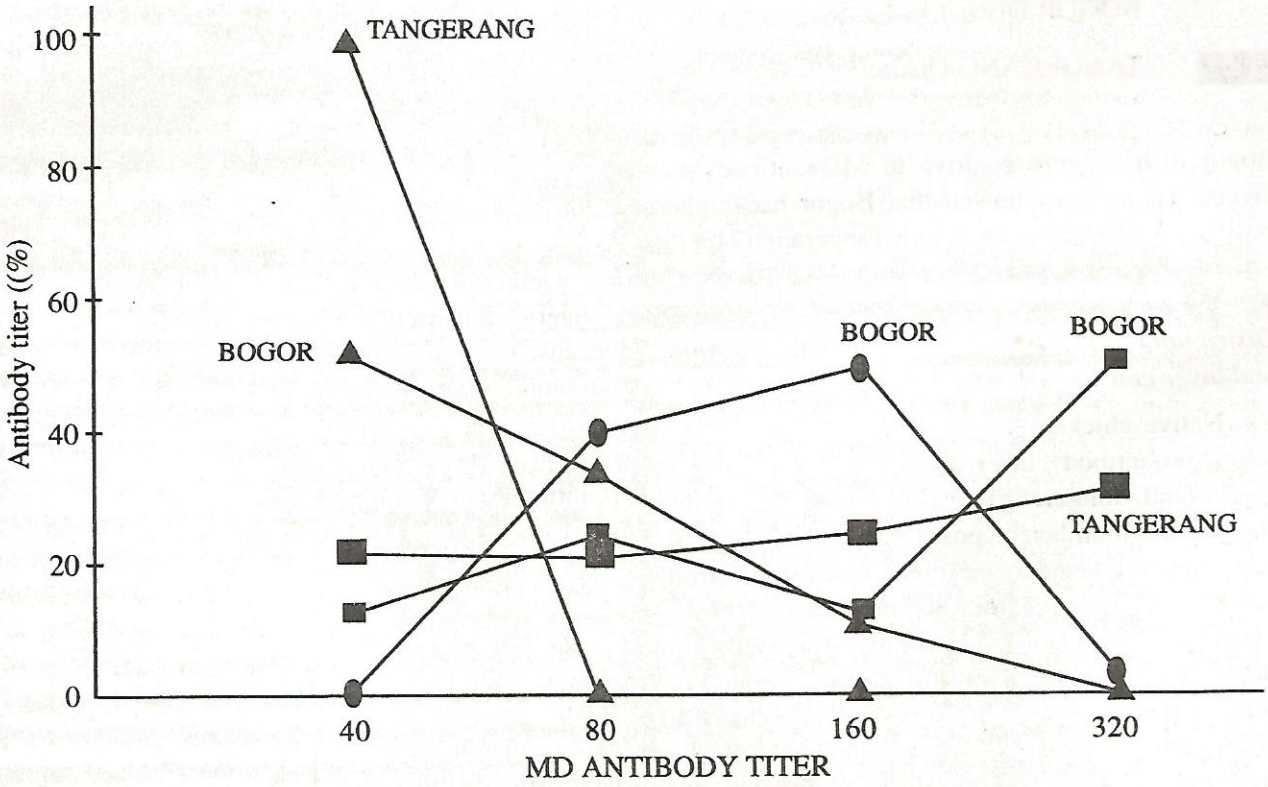

Fig. 2 : Correlation of positive percentage and antibody titers between type of chickens and districts.

▲ : native chickens ● : broiler ■ : layer

agreed since the SPF chickens in our laboratory had no MD vaccination experience. In Bogor, native chickens has positive MD antibodies within range of 20-100% or 55.3% in average (Table 1). The highest positive percentage was observed is x 40 dilution of sera and there was a tendency to decrease according to the higher of titre (Fig. 2). In the case of native chickens in Tangerang, it seemed that they have very few MD antibodies within range of 0-7.7% or 3.9% in average (Table 1). Also 100% of the positive MD antibody in native chickens in Tangerang appeared in x40 dilution of sera, and no chickens showed the higher titer (Fig. 2). From these, it was shown that the native chickens were naturally infected with MD because these chickens had no experiences of MD vaccination. For broilers in Bogor, the MD antibody titre was higher than native chickens (almost in x160 titre) (Fig. 2), and 85% of these were postiive against MD antibodies (Fig. 1). Whereas in the layers, the MD antibody titre distributed among x40 till x320, and had highest titre was x320 for both districts (50% and 31% for Bogor and Tangerang, respectively (Fig. 2).

REFERENCES

Akiba, K., Iwatsuki, K., Sasaki, Y., Furuya, Y. and Ando, Y. 1976. Report on the Investigation of Poultry Disease in Indonesia. Japan International Cooperation Agency.

Chubb, R.C. and Churchill, A.E. 1968. Precipitating Antibodies Associated with Marek's Disease. Vet. Rec. 83:4-7.

Djaenoedin, R. and Koerjana, R. 1956. Beberapa kelajuhan yang agak jarang ditemukan pada neurolymphomatosis. Hemera Zoa. 63: 249-250.

Ekperigin, H.E., Fadly, A.M., Lee, L.F. and Mc Capes, R.H. 1983. Comb lesions and mortality patterns in white leghorn layers affected by Marek's. Avian Dis. 27: 503-512.

Ginting, N. 1983. Marek's Disease in Kedu Chickens (Case Report). Penyakit Hewan. 15: 79-81.

Purchase, M.G. 1985. Immunofluorescence in the study of Marek's disease. I. Detection of antigen in cell culture and an antigenci comparison of eight isolates. J. Virol. 3 : 557-565.

Rivas, A. and Fabricant, J. 1988. Indications of immunodepression in chickens infected with various strains of Marek's disease virus. Avian Dis. 32: 1-8.

Sofyan, S.D. 1984. Penyakit Marek di Indonesia. Poultry Indonesia. 50:11-12.