DETECTION OF MAREK'S DISEASE (MD) ANTIBODIES USING CVI AND HVT INFECTED CELLS

Soedijar, Ida Lestari and Siti Marinda
Veterinary Drug Assay Laboratory, Gajahmungkur, Bogor 16340. T. (021)7560489. E-mail: bpropok@indosat.net.id, idased@indos.net.id

ABSTRACT

Fifty sera representative from several chicken farms in Java, Lampung and Bali were detected against Marek's Disease (MD) by Indirect Fluorescence Antibody Technique (IFAT). More than 80% of the chickens which were suspected to receive MD vaccination showed positive antibodies.

Key words: Marek's disease, CVI and HVT infected cells

ABSTRAK

Telak diperiksa 50 contoh serum ayam terhadap penyakit Marek (MD) dengan metoda Indirect Fluorescence Antibody Technique (IFAT) yang berasal dari peternakan di daerah propinsi Jawa, Lampung dan Bali. Didapatkan lebih dari 80% serum ayam yang diduga divaksin MD memberikan titr antibodi yang cukup testosterone MD.

Kata kunci: Penyakit Marek, sel terinfeksi CVI dan HVT
INTRODUCTION

Marek's disease (MD) is a neoplastic and neuropathic disease of poultry caused by a highly contagious, cell associated herpes virus. The disease to occur in chickens over 20 weeks old and mortality rate about 70% (Eskerpin et al, 1983).

In Indonesia, MD occurred for the first time in 1951 as fowl paralysis (Djioenodin and Koekanja, 1956). Since 1990 till now, incidence of acute MD especially in broiler is very rare, but 67% of sample tissue from broiler farm based on histopathological changes, showed lesion which similar to MD (Tabbo, C.R, 2001).

At present, in Indonesia MD case reports are merely based on pathological anatomy and histopathological changes. In this report, detection of MD antibodies was performed by using Indirect Fluorescence Antibody Technique (IFAT) against serotype 1 (Central Veterinary Institute/CVI 988) and serotype 5 (Herpes Virus Turkey/HVT).

MATERIALS AND METHODS

Chicken's sera :
Sera were obtained from 5 farms of 5 provinces (West Java, East Java, Bali, Lampung and Yogyakarta). SPF chicken sera were used as negative control.

Infected Cells :
Monolayer of primary CEF cultures is Ø 45 X 15 mm dishes containing coverslip were infected with 8 X 10⁴ PFU/dish of MD vaccine (from Merial Laboratory – Singapore). After showing more than 75% cyto pathetic effect (CPE) within 2-3 days incubation, the infected cells were harvested.

Table 1. Percentage positive sera against serotype 1 (CVI) and 3 (HVT) of MD in chickens by IFAT

<table>
<thead>
<tr>
<th>No</th>
<th>Chicken strain</th>
<th>Age (week)</th>
<th>Provinces</th>
<th>No of tested sera</th>
<th>Against CVI + CVI %</th>
<th>Against HVT + HVT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Layer</td>
<td>21</td>
<td>West Java</td>
<td>10</td>
<td>10 / 0 / 100</td>
<td>10 / 0 / 0 / 100</td>
</tr>
<tr>
<td>2</td>
<td>Layer</td>
<td>8</td>
<td>East Java</td>
<td>10</td>
<td>10 / 0 / 100</td>
<td>10 / 0 / 100</td>
</tr>
<tr>
<td>3</td>
<td>Layer</td>
<td>4</td>
<td>Bali</td>
<td>10</td>
<td>9 / 1 / 90</td>
<td>8 / 2 / 80</td>
</tr>
<tr>
<td>4</td>
<td>Layer</td>
<td>26</td>
<td>Lampung</td>
<td>10</td>
<td>10 / 0 / 100</td>
<td>10 / 0 / 100</td>
</tr>
<tr>
<td>5</td>
<td>Layer</td>
<td>8</td>
<td>Yogyakarta</td>
<td>10</td>
<td>9 / 1 / 90</td>
<td>10 / 0 / 100</td>
</tr>
<tr>
<td>6</td>
<td>SPF</td>
<td>8</td>
<td>Bogor</td>
<td>10</td>
<td>0 / 10 / 0</td>
<td>0 / 10 / 0</td>
</tr>
</tbody>
</table>

The coverslip contain infected cells were taken out and washed once in Phosphate-buffered-saline (PBS) for 15 minutes and fixed for further 15 minutes in cold acetor. These cells are kept at −20°C until ready to use.

Indirect Fluorescent Antibody Technique (IFAT) :
Indirect Fluorescent Antibody Technique (IFAT) was performed with slightly modification to the method described by Soedijar and others (Soedijar, I.I, et al, 1995). Each chicken sera (x40) were mounted onto the coverslip of monolayer cultures infected with CVI or HVT of MD virus strains. The samples were placed in the moisture box and kept for 45 minutes at 37°C. After sensitization, the cells were washed 15 minutes in PBS.

Four units of fluorescein-isothiocyanate conjugate rabbit anti-chicken immunoglobulin G catalyzed no. F4137 (Sigma laboratory--USA) was added, and the cells were resensitized for 45 minutes at 37°C. The coverslips were washed in PBS for 15 minutes, then with glycercin buffer, coverslips were mounted on slide glass before observation under ultra violet microscope. The positive sera were further rehydrated until x40 according procedure as above mentionned.

RESULTS AND DISCUSSION

Ten chicken sera were randomly tested from five farms of 5 provinces in Indonesia. In our laboratory, originally these sera were tested against Mycoplasma gallisepticum as the first purposed. More than 80% of each province showed all of the farms favour good response against serotype 1 (CVI) and serotype 3 (HVT) of Marek's Disease as is shown from table 1.

The positive sera are more diluted from 40 times to 80 X, 160 X until 320 X with Phosphat Buffer Saline. Lampung province demonstrate to have the highest titre (320 times) of MD antibody against serotype 1 (CVI), whereas Bali province delivered the lowest titre (40 times).

In the case of response MD antibody against serotype 3 (HVT), Yogyakarta province gave the highest titre (140 times) and again Bali province delivered the lowest titre (40 times) as showed in (Graph 1).
Graph 1. Distribution of antibody titre against CVI and HVT serotypes of Marek’s Disease from 5 province chicken farms.

From our serological result, chickens from all of the provinces delivered at least 80% positive antibodies against Marek Disease in both serotype 1 (CVI) and serotype 3 (HVT) (table 1).

Based on our interview to the farmers in the field, even the experience of vaccination program was not clear, but our result indicated that all the chickens have had appropriate antibodies against MD.

According to our minimum requirement, we can say that the MD vaccine is satisfied when it favours at least 80% of vaccinated chickens deliver appropriate antibodies.

As we know, MD vaccination program should be done at day old chick. The vaccinated chickens usually will have enough MD antibodies at least 3 weeks post vaccination and it can be detected during their life span as our experience from the randomly sample field chickens (unpublished).

From table 1, we agreed that all of the farmers have had successfully to gain the chickens which have appropriate MD antibody titre in their bodies. From graph 1, we find that chickens from Bali province showed the lowest antibody titre against both serotypes. It is assumed that is due to the youngest chickens age from Bali province (4 weeks) are sampled, so the level antibody titre still moderate/low in consequence before they go to the peak level.

However from a newspaper (Kompas, june 6, 2002), it is said that 2.8 million broiler died caused by MD and the farmers lost about 19.6 milliard in rupiah. It is very interesting since the report mentioned the MD case attacked broiler which is fairly young in age compared to the usual case (about 20 weeks) (Ekperigin et al. 1985).

It is suspected that might be the failure MD vaccination is occurred?

In Indonesia, we have several MD vaccines distributed among the farmers and be tested in our laboratory, which are Indonesian product and mostly from imported ones. The vaccines usually single MD strain per vial (serotype 1 or 2 or 3 individually), but some of them also combined MD strains such as serotypes 1 + 3 or 2 + 3 per vial.

We have to be aware with this founding and pay strict in the MD vaccination program to anticipate the problem becoming wide and it is wise to judge the MD case not only based on histopathological changes, but also need confirmation through serologically testing and virus isolation either.

For further study, we also need to perform detecting antibody titre against serotype 2 (SB1) of Marek’s Disease to get a full picture of distribution MD antibodies of Indonesia chicken farms.

REFERENCES


