Immune State of Dogs Injected with Rabies Vaccines in the West Java, Indonesia

Norio HIRAYAMA, Enuh RAHARJO USA1, Mastur AENY ROCCHMAN NOOR1, Kaoru SAKAKI2, and Munee OGATA3

National Veterinary Assay Laboratory, Kokubunji, Tokyo 185, Japan, 1Veterinary Drug Assay Laboratory, Gunung Sindur, Bogor 16340, Indonesia, 2Nihon Vaccine Co., Ltd., Sakura, Chiba 285, and 3Japan International Cooperation Agency, Shinjuku-ku, Tokyo 160, Japan

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Rabies is widely prevalent throughout Indonesia, especially in Sumatra, Java and Sulawesi. More than 800 cases in animals have been recognized and 60 humans die from rabies each year [3]. Dogs in enzootic areas have been vaccinated against rabies by the government free of charge to eradicate the disease. In spite of these efforts, the outbreaks of rabies continue up to now. Although there are a number of reasons for such epidemics, the main reason is that rabies vaccines are not produced sufficient doses in Indonesia. There is another possibility that the quality of the rabies vaccines used may be low. The quality of the vaccines used to date is uncertain, but relevant information can be obtained by a serological survey of vaccinated dogs. Therefore, we collected sera from vaccinated dogs and measured neutralizing antibodies against the rabies virus to determine whether or not rabies vaccines used in Indonesia are effective.

The serum samples tested were collected in December 1986 from 93 dogs in the west Java (Jakarta, Bandung and Garut). Dogs in the Jakarta and Garut areas had received inactivated goat brain emulsion vaccines 12 months and 8–10 months ago, respectively and these in Bandung area had received inactivated suckling mouse brain emulsion vaccines 1–7 months ago. The goat brain emulsion vaccines were produced by the Pasteur strain in Center for Veterinary Biologics (Surabaya) and the suckling mouse brain emulsion vaccines were produced by the Pitman-Moore strain in Bio Farma (Bandung). Date of vaccination and type of vaccine could be confirmed by a card published by the Indonesian government at the time of vaccination. The age of the dogs was learned directly from the owner. As for the kind of dogs, the majority were local mixed breeds with a few pointers and German shepherds.

For the neutralization test, chick embryo fibroblasts (CEFs) were prepared from 7-day-old chick embryo by the method described previously [4], and 0.1 m/l of cell suspension (1.5 × 10² cells) was distributed to each well of microplates (Nunc).

The CVS strain of rabies virus adapted to CEFs [8] was used for the neutralization test. It had been passaged 87 times in CEFs. Serial two fold dilutions of the serum inactivated at 56°C for 30 min were mixed with equal volumes of virus suspension containing 200 TCID₅₀/0.025 m/l of virus. The virus-serum mixture was incubated at 37°C for 60 min and inculcated with 0.025 ml amounts of mixture into 4 wells per serum dilution. After incubation at 37°C for 60 min, the cell monolayers in wells were washed once with Hanks balanced salt solution, and then maintained with medium 199 supplemented with 0.11% bovine albumin, 0.4% NaHCO₃ and antibiotics. The culture was incubated at 37°C in 5% CO₂ for 4 days and determination was conducted by the indirect immunoperoxidase technique [7] with some modification. In brief, monoclonal antibody against glycoprotein of rabies virus and the peroxidase conjugated antimouse IgG goat serum (Cappel) were used as the first and second serum, respectively. Each incubation was carried out at 37°C for 60 min. Enzymatic activity was detected by using o-phenylenediamine as a substrate. The color development was terminated with 5N H₂SO₄, and then judged by the naked eye. The neutralizing antibody titer was calculated by the method of Reed and Muench [9] from the highest dilution of serum that inhibited virus growth. A neutralizing antibody titer of 1:4 or higher was considered positive [1, 6, 10].

A titer 1:4 or higher was detected in 46 (49%) of the 93 dogs tested (Table 1). The geometric mean (GM) titer was 8.5. The positive rate and GM titer were highest in the Bandung area. This
Table 1. Detection of neutralizing antibody against rabies virus in vaccinated dogs in the west Java, Indonesia

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of sera</th>
<th>No. of sera with antibody at indicated titer</th>
<th>Positive (≥4)</th>
<th>Geometric mean titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;4</td>
<td>4&lt;16</td>
<td>16&lt;64</td>
</tr>
<tr>
<td>Jakarta</td>
<td>12</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Garut</td>
<td>41</td>
<td>22</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Bandung</td>
<td>40</td>
<td>15</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>47</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

a) Significant difference from the Bandung area at p<0.01.
b) Significant difference from the Bandung area at p<0.05.

Table 2. Distribution of neutralizing antibody against rabies virus at different periods after vaccination

<table>
<thead>
<tr>
<th>Month after vaccination</th>
<th>No. of sera</th>
<th>Positive No.</th>
<th>Positive %</th>
<th>Geometric mean titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>23</td>
<td>17</td>
<td>74</td>
<td>28.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4–6</td>
<td>14</td>
<td>5</td>
<td>36</td>
<td>5.1</td>
</tr>
<tr>
<td>7–9</td>
<td>23</td>
<td>10</td>
<td>43</td>
<td>6.4</td>
</tr>
<tr>
<td>10–12</td>
<td>33</td>
<td>14</td>
<td>49</td>
<td>5.4</td>
</tr>
</tbody>
</table>

a) Significant difference from three other groups at p<0.01.

Table 3. Distribution of neutralizing antibody against rabies virus at different age groups of dogs

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>No. of sera</th>
<th>Positive No.</th>
<th>Positive %</th>
<th>Geometric mean titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>32</td>
<td>16</td>
<td>50</td>
<td>7.5</td>
</tr>
<tr>
<td>2, 3</td>
<td>29</td>
<td>15</td>
<td>52</td>
<td>8.7</td>
</tr>
<tr>
<td>4, 5</td>
<td>16</td>
<td>8</td>
<td>50</td>
<td>12.8</td>
</tr>
<tr>
<td>≥6</td>
<td>16</td>
<td>7</td>
<td>44</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Table 2 result may depend upon months after vaccination rather than the kind of vaccine used.

When these results were grouped according to time after vaccination (Table 2), the positive rate and GM titer in dogs 1–3 months after vaccination were 74% and 28.9, respectively. In the other groups vaccinated more than 4 months earlier, however, the positive rate and GM titer were lower. In particular, the positive rate was 17% and GM titer was less than 4 in dogs vaccinated 12 months previously in the Jakarta area (Table 1).

The distribution of neutralizing antibodies by age of dogs was also examined (Table 3). All age groups showed the same positive rate and GM titer, indicating that these dogs received vaccine only once.

Judging from the positive rate of neutralizing antibodies in dogs 1–3 months after vaccination, rabies vaccines used in Indonesia are somewhat effective. However, decrease of the positive rate to about 40% more than 4 months after vaccination indicates that the immunity is not maintained long enough.

Baer and Wandeler [2] described that a herd immunity of over 70% was enough to stop the spread of rabies among dogs. Minamoto [6] also reported that 75.1% of pet dogs had neutralizing antibodies in Japan. At the time of the survey, dogs received an inactivated goat brain emulsion vaccine (Nishigahara strain) twice a year. Japan has maintained a rabies-free status from 1957 by mass vaccination, catching stray dogs and severe quarantine regulations. This suggests that a positive rate of over 75% is necessary to eradicate rabies and maintain a rabies-free condition.

The present data suggest that vaccination once a year is not sufficient to maintain the protective level of antibody against rabies virus in Indonesia. A basic immunization of two injections less than one year old dogs has been effective to give a strong immunity. However, the such vaccination program is troublesome for mass vaccination. Therefore, it is considered that dogs should be revaccinated regularly the present vaccines at 6 months interval or an improved vaccine (such as one which is adjuvant-added or tissue culture vaccine used in Japan) which would give dogs long term immunity once a year.

It is important to select the strain for neutralization test in the survey of antibody such as the
present study. We used the CVS strain in this survey from following reasons. This strain has been used in the world as a challenge virus for the potency test of rabies vaccine. Moreover, Lafon et al. reported that all mice developed a high seroneutralizing activity against the CVS strain whichever vaccine strain was used for immunization, although the antigenic similarity between the Pitman-Moore and CVS strains were demonstrated and the Pasteur strain was classified in another group by analysis using neutralizing monoclonal antibodies [5]. It is adequate to use the CVS strain which reacts widely with the other strains, when the survey was done in area used several kinds of vaccines.

This survey is a first trial in Indonesia. The survey should be demonstrated periodically, since to know the immune state against rabies in dogs will be very useful for the decision of control strategies.

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REFERENCES