THE HUMORAL IMMUNITY RESPONSE OF DOG VACCINATED WITH ORAL SAG2 AND PARENTERAL RABISIN AND RABIVET SUPRA92

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ABSTRACT
This research aims to determine whether SAG2 oral vaccine induced antibody response as high as that of parenteral vaccine (Rabisin and Rabivet Supra 92). The experimental design applied was randomized pre and post control group design with 4 treatments (oral SAG2 vaccine, parenteral rabisin, parenteral Rabivet Supra 92 and control without vaccination) and with 9 replication. As many as 36 kampung dogs used and the anti rabies antibody was determined by enzyme-linked immunosorbent assay (ELISA) at day 0, 21, 56, 84, 119, and 147 post vaccination. The antibody titer induced by oral SAG2 vaccine raised more slowly but persisted longer than parenteral vaccine. On day 21 after vaccination, the average antibody titers induced by oral SAG2 vaccines (0.501 IU/ml) was still significantly lower than those induced by parenteral Rabisin (3.504 IU/ml) and Rabivet Supra92 (2.545 IU/ml). However, by day 119 after vaccination the average antibody titer induced by SAG2 oral vaccine (0.889 IU/ml) was significantly higher (p <0.05) as compared to those of parenteral Rabisin (0.625 IU/ml) and Rabivet Supra 92 (0.223 IU/ml). The antibody response induced by SAG2 oral vaccine appears to persist longer than parenteral vaccines and it can therefore be used as an alternative vaccine to combat rabies in animals, especially for dogs kept in free range.

Keywords: Rabies, antibody titer, SAG2, Rabisin, Rabivet Supra 92

INTRODUCTION
Rabies is a fatal zoonotic disease attacking all warm blooded animals including humans. At this time the rabies continues to spread and difficult to control, not only in developing country but also in developed countries. Until now, the methods used to control rabies are vaccination, mostly by parenteral routes. Parenteral vaccination route faces many problems when applied to dogs. Such problems cause difficulties in achieving vaccination coverage of greater than 70%. Recently oral vaccination has been recommended by the World Health Organization (WHO) and has been successfully used to eradicate rabies from many countries, especially for control of rabies in wild animals and dogs. Oral vaccines that have been guaranteed for its safety are the Rabies Virus Glycoprotein (VRG) and Street Alabama Dufferin Gif 2 (SAG2). The use of oral vaccines in Indonesia is expected to improve vaccination coverage by more than 70% and its use in the field appears to be very practical as it does not require many vaccinators in the field, especially in wild animals and dogs that are kept removable and hard to catch. Up to now, research on the use of oral vaccines to control rabies in free range dog in Indonesia has not been conducted yet. It is therefore necessary to conduct a study on the humoral antibody of dogs vaccinated with oral SAG2 vaccines as comparison to those induced by parenteral rabisin and Rabivet Supra 92.

METHODS
Vaccines
In this study, the vaccine used were oral dose of SAG2 rabies vaccine (Virbac, France), parenteral Rabisin (Merial, France), and parenteral Rabivet Supra 92 (Pusvetma, Surabaya, Indonesia). They were all commercial vaccines. Th SAG2 vaccine was a modified-live rabies vaccine included in a bait for oral administration to kampung dogs. The rabies virus strain SAG2 was selected from SAD Bern-a subclone of a virus isolated from salivary glands of a rabid dog in 1935- in two-step process of amino acid mutation (Lafay., et al. 1994). The vaccine liquid suspension is presented within a PVC/aluminium sachet, which is coated with an appetent matrix made of mineral fat, fish ingredients and tetracycline as a biomarker. The bait measures 4.9 cm x 4.4 cm x 1.5 cm and weights 28 g. Rabisin was inactivated vaccine consisted of rabies virus glykoprotein ≥ 1 IU), 0.1 mg Thiomersal, 1.7 mg Aluminium (as hydroxide) with the Lot number

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L360155. Rabivet Supra 92 rabies was inactivated tissue culture vaccine with butch number: Deptan RI D.06121430 VKC.1.

Experimental animals and vaccination procedures

As many as 36 Kampung dogs aged 3 month to 1 year old were used in the study. They were randomly allocated into four groups: group A (n = 9 kampong dogs) was vaccinated by Oral SAG2, group B (n = 9 kampong dogs) was vaccinated by Rabisin, group C (n = 9 kampong dogs) was vaccinated by Rabivet Supra 92, and group D (n = 9 kampong dogs) was not vaccinated and kept as controls. Serum samples were collected from the dogs on days 0 (prior to vaccination) and on days 21, 56, 84, 119, and 147 post vaccination.

Enzyme-linked immunosorbent assay (ELISA)

Antibody titer was determined by the enzyme linked immunosorbent assay (ELISA) (PLATELIA™ RABIES II KIT, ad usum veterinarium) according to manufacturer procedure described. In brief; 100 µl sera samples (diluted 1:100 in dilution buffer) were added into each well of ELISA plates coated with glycoprotein-rabies virus and incubated for 1 hour at 37°C. After that, the wells were washed 3 times with ELISA wash buffer, then 100 µl anti-canine IgG-horse radish peroxidase was added into each well and incubated at 37°C for 1 hour. Then, the wells were washed again (2 times) as above and 100 µl ABTS substrate was added and incubated at room temperature for 30 minutes. Stop solution was then added and absorbant was recorded using ELISA reader at 450 nm. Antibody titer were calculated by comparing absorbant of each sample with standard positive and negative samples and expressed in international units (IU).

Experimental design and analysis data

Experimental design applied in this study was completely randomized pre and post control group design consisted of 4 treatments (A: oral SAG2 vaccine, B: parenteral rabisin, C: parenteral rabivet Supra 92, and D: without vaccination). Each treatment consisted of 9 replications. The data obtained were analysed by Analysis of Variances (Anova) and proceeded with Least Significant difference (LSD) if there were significant differences among treatment groups.

RESULTS

The antibody titer induced by oral SAG2 vaccine raised more slowly but persisted longer than parenteral vaccine. On day 21 after vaccination, the average antibody titers induced by oral SAG2 vaccines (0.501 IU/ml) was still significantly lower than those induced by parenteral Rabisin (3.504 IU/ml) and Rabivet Supra92 (2.545 IU/ml). However, by day 119 after vaccination the average antibody titer induced by SAG2 oral vaccine (0.889 IU/ml) was significantly higher (p<0.05) compared to those of parenteral Rabisin (0.625 IU/ml) and Rabivet Supra 92 (0.223 IU/ml). Mean Rabies Humoral Antibody Titres on day 0 (before vaccination) and on days 21, 56, 84, 119 and 147 after vaccination (before challenge) were presented in Table 1 and Figure 1.

Table 1

<table>
<thead>
<tr>
<th>Days</th>
<th>Mean rabies antibody after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>21</td>
<td>0,501* c</td>
</tr>
<tr>
<td>56</td>
<td>1,406 a</td>
</tr>
<tr>
<td>84</td>
<td>1,389 a</td>
</tr>
<tr>
<td>119</td>
<td>0,889 a</td>
</tr>
<tr>
<td>147</td>
<td>0,675 a</td>
</tr>
<tr>
<td>Mean</td>
<td>-0,03</td>
</tr>
</tbody>
</table>

A: oral SAG2, B: Rabisin, C: Rabivet Supra 92, D: Control, same alphabetic indicates insignificant (p > 0.05).

Figure 1

Mean antibody titer was vaccinated by oral SAG2, parenteral Rabisin and Rabivet Supra 92 on at days 0, 21, 56, 84, 119 and 147 after vaccination.

DISCUSSION

In this study, rabies virus antibodies examined by ELISA test and titer is measured in international units (IU) per ml. Antigen used in ELISA test is part of the rabies virus glycoprotein, so the result is more specific because it reflects the titer of antibodies against the rabies virus proteins that play an important role in neutralization. As it is known that rabies virus has some kind protein, such as glycoprotein (G), nucleoprotein (N), phosphoprotein (P), and matrix (M). Glycoprotein (G) is an important protein that plays a role in attachment of rabies virus in a suitable target cells. Adhesion to target cells required rabies virus to infect target cells. Antibodies against this protein can prevent infection and neutralize the virus enters the body susceptible host. Therefore, the antibody titers obtained in this study have reflected more or less neutralizing antibodies induced by the above three types of vaccines. According to WHO (1997) that a protective antibody titer was 0.5 IU /
ml. In this study, three types of vaccines applied were able to induce antibodies with titer exceed the number endorse by WHO. However, at the end the titer was declined and some of them below this value.

There are differences in pattern of antibody responses induced by oral and injection vaccine. In oral vaccines, its antibodies appear and achieve maximum peak and declined more slowly than the inject able vaccine. Antiviral antibody titers of dog vaccinated with injection rabies vaccine gain maximum peak in the third week or day-21 after vaccination, while antibody titers in dogs vaccinated with the oral vaccine increase in the eighth weeks or on days 56 after vaccination. This difference appears because of the preparations different of virus used in oral and injectable vaccines. Injectable vaccine contains inactive virus which does not require replication to induce humoral immunity in dogs, while the oral vaccine containing active virus that must be replicated before they were able to induce humoral immunity in dogs vaccinated.3 5

The humoral immune response mediated by B cells is assisted by a helper T cell type 2 (T-helper-2/Th-2). Injectable vaccine virus that has been deactivated will be processed quickly by the antigen presenter cells (antigen presenting cells / APC) and then presented via MHC-2 molecules to the cell Th-2 (CD4+) so that the cells are activated. The cell Th-2 is activated will be trigger B cells to divide and differentiate into plasma cells to produce antibodies and memory cells as a backup if there is a subsequent infection.6 The sooner the vaccine antigen is processed by the immune system, the sooner the response appeared.

Observations from week to week show that the oral vaccine able to induce humoral immunity that last longer than the parenteral vaccine. For example at weeks 12 or on days 84 after vaccination it was able to induce antibody titer higher than oral vaccine with a vaccine injection. In oral vaccine containing active virus will bully humoral immune system after the virus replicates.6 The existence of replication induce the immune last longer and also represents the excess of the oral vaccine compared to vaccine injection.

For parenteral vaccine type, in this case and Rabivet Rabisin Supra 92, averaging humoral antibody titers increased sharply at day 21 after vaccination. This is different from the tiny fraction of that stated in the literature that the striking peak antibody titers of between three months and six months after vaccination.7 Research also showed that the humoral antibody response after vaccination in dogs experiments in the laboratory is always better than in pet dogs.5 Therefore, it should be anticipated that laboratory-scale research may differ from its application in the field.

Antibody titer by vaccine induces Rabisin and Rabivet 92 Supra visible decline started at day 56, day 84 and day-to-119. Especially for flats Rabisin vaccine antibody titers at day-119 is still above 0.5 IU/ml according to OIE or WHO reference, while for the vaccine Rabivet Supra 92, the average antibody titer humoralnya has been below the level of 0.5 IU / ml. These results indicate that vaccination with the vaccine Rabivet 92 Supra requires revaccination (booster) is faster than the vaccine Rabisin. Generally, the first on the pet vaccination induces lower antibody titers and decreased rapidly compared with the second vaccination or more.8 9 According to Lambot et al., (2001) rabies vaccine dosing only once on the wolf not only produced fewer memory cells, but also takes a long time to maintain the memory cells and preparation to protect the body from virus attacks challange.10

CONCLUSION

SAG2 oral vaccine is safe and can be used as an alternative vaccine to combat rabies in animals, especially Kampung dogs. SAG2 oral vaccine can be recommended for use in order to control rabies cases in Indonesia

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