

# ANTI-RABIES ANTIBODIES IN JAPANESE VOLUNTEERS IMMUNIZED WITH IMPORTED VACCINE: A PRELIMINARY STUDY

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**Abstract.** We vaccinated seven Japanese volunteers and collected sera to examine their immunological responses to rabies vaccine. A purified chick embryo cell vaccine, Rabipur (PCECV), provided by Chiron, was used. Subjects were vaccinated by intramuscular injection. The vaccination schedule was Days 0, 7 and 21 we collected blood samples on Day 0, before the vaccination, and after Day 56. The seroprotective titer was measured by a Rapid Fluorescent Focus Inhibition Test (RFFIT). Six subjects had increases in anti-rabies titers to seroprotective levels. We defined seroprotection as  $\geq 0.5$  IU/ml. One subject has a lower response post-titer vaccination of 0.6 IU/ml. A post-vaccination titer was not available in one subject. We did not observe any significant adverse effects. We confirmed that Rabipur is immunogenic in Japanese subjects. This research may contribute to a guide for Japanese patients exposed to rabies outside Japan.

## INTRODUCTION

Except for a few imported cases, there have been no rabies patients for more than 30 years in Japan. No animal cases have been reported since 1956 (Takayama, 2000). Since the government started an official incident report in the early 20th century, there have been several outbreaks of rabies in both humans and animals (Tamashiro *et al*, 2007). The government intensively promoted rabies eradication programs after World War II. The Rabies Prevention Act was passed in 1950 (Act No. 247, 1950). Japan is now a rabies-free country (<http://www.who.int/rabies/human/en/>). Some have pointed out that since Japan is surrounded by water infected animals cannot easily enter Japan (Mannen, 2002). Another explanation is that compulsory rabies inoculation has worked well to control canine rabies (Takayama, 2000; Tamashiro *et al*, 2007).

Since few people in Japan are concerned now about rabies, in 2000 Takayama wrote a book about human rabies entitled “Human rabies, a fatal disease forgotten” (Takayama, 2000). Rabies kills more than 50,000 people each year and is an often neglected disease (Ruprecht *et al*, 2002; WHO, 2006). In 2006, we had two rabies victims in Japan (<http://idsc.nih.gov/idwr/kanja/idwr/idwr2006/idwr2006.46.pdf>; <http://idsc.nih.gov/idwr/kanja/idwr/idwr2006/idwr2006.47.pdf>). They were Japanese who had been bitten by an infected dog while abroad and then developed rabies. It was thus alarming to have rabies patients in “rabies-free” Japan (Tamashiro *et al*, 2007).

Rabies is invariably fatal once the symptoms have appeared (Bleck and Ruprecht, 2002). The only way to escape from death by rabies is vaccination. Both pre- and post-exposure vaccination is effective to prevent rabies.

Pre-exposure vaccination is done for those who intend to stay abroad for extended periods of time in high risk areas. Post-exposure vaccination is done for those exposed to rabies or animals suspected of having rabies. In 1999, more than 65 people received post-exposure vaccination. Most of them were bitten by an

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animal suspected of having rabies while abroad (Takayama, 1997).

More and more Japanese are travelling abroad; 17.4 million Japanese went abroad in 2005 (Immigration Bureau, Immigration Control, Ministry of Justice, 2006). The number of Japanese who may be exposed to rabies or suspicious animals and therefore need to receive a rabies vaccine may increase. We noted a shortage of rabies vaccine after the case in 2006. Narita Airport quarantine depot had to suspend vaccination for rabies in December, 2006. Japan has only one type of rabies vaccine, the Purified Chick Embryo Cell Vaccine (PCECV), manufactured by Chemo-Sero-Therapeutic Institute, Kaketsuken.

It is not as common to vaccinate against rabies in Japan as in Southeast Asian countries, such as Thailand and the Philippines. Not many people are interested in receiving rabies vaccinations. Some Japanese may be given a vaccine not approved by the government. We, therefore, need to understand the immunogenicity and risk of adverse effects of those vaccines. We vaccinated seven Japanese and collected sera to examine their immunological responses to an imported vaccine.

## MATERIALS AND METHODS

Rabipur (Chiron), a Purified Chick Embryo Cell Vaccine (PCECV), was used for this study. In Japan we have another PCECV manufactured by Chemo-Sero-Therapeutic Institute, Kaketsuken. It is approved by the Ministry of Health, Labor and Welfare in Japan. We decided not to use the Human Diploid Cell Vaccine (HDCCV), another major rabies vaccine, to avoid encountering unmanageable adverse effects.

The subjects were four males age 30 to 60 years old and three females in their 20's. The vaccine was given in three doses, either at "0, 7 and 21 days" or at "0, 7 and 28 days". The injection was given intramuscularly in the deltoid muscle.

Table 1  
Immunogenicity of Rabipur

Subject	Pre- vaccination IgG titers (IU/ml)	post- vaccination IgG titers (IU/ml)
F1	0.05	0.60
M1	0.05	2.14
F2	N/A	9.90
M2	0.60	N/A
F3	0.05	2.79
M3	N/A	2.68
M4	N/A	2.14

Anti-rabies antibody titers by the Rapid Fluorescent Focus Inhibition Test (RFFIT) method: US CDC-tissue culture chamber slide. Virus challenge was CVS-11; 07/10/06 stock. Cell line was mouse neuroblastoma. Reference serum US standard rabies immune globulin.

A Rapid Fluorescent Focus Inhibition Test (RFFIT) was used to measure antibody titers (Khawplod *et al*, 2005). The tests were carried out at the Rabies Research Laboratory, Veterinary Research Department, Research Institute for Tropical Medicine, Department of Health, Republic of the Philippines.

## RESULTS

We obtained pre- and post-vaccination sera in 3 subjects. Four subjects dropped out of the study. The WHO defines a seroprotective titer as more than 0.5 IU/ml. Five post-vaccination sera showed increases in rabies IgG titers, greater than the WHO seroprotective criteria. We observed one low responder whose post-vaccination titer was 0.6 IU/ml (Table 1). We observed no significant adverse effects due to the vaccinations.

## DISCUSSION

We measured anti-rabies antibody titers

to evaluate the pre-exposure effectiveness of vaccination against rabies in Japanese volunteers as a preliminary study. We used Rabipur, which is not approved in Japan but widely used in many countries. We intend to recruit more volunteers to evaluate another rabies vaccine, such as the IMOVAX, a human diploid cells vaccine by Aventis Pasteur, to compare immunogenicity and adverse effects.

We had one low responder from whom we collected further information. The questionnaire addressed whether the subject had other vaccinations recently, such as HAV, HBV, tetanus or measles, or other medications, such as anti-malaria agents, steroid drugs or antibiotics, an immunosuppressive condition, or other diseases, such as chronic diseases, metabolic disorders, cardiovascular diseases or diabetes mellitus, in addition to smoking and drinking or abnormal nutritional conditions. The low responder had received HAV and tetanus inoculation and had urticaria. This may have suppressed immunoactivation. Two other subjects also had HAV and a tetanus vaccine in addition to the rabies vaccination at the same time.

Barth *et al* (1990) evaluated the potency of Rabipur *in vivo* by an NIH standard test to show that Rabipur is able to induce anti-rabies antibodies. Vodopija *et al* (1999) tested the immunogenicity and safety of Rabipur in Croatia. They followed the WHO guidelines (WHO Expert Consultation on Rabies, 2005). The 23 subjects vaccinated with the pre-exposure regimen obtained sufficient antibody titers, more than 0.5 IU/ml in 90 days and did not develop any severe adverse effects. Kamoltham *et al* (2007) reported they vaccinated 703 children (5-8 years old) and observed sufficient antibody titers after the first vaccination. They did not observe any serious adverse effects from the vaccination.

Although we only had seven subjects and three post-vaccination sera, we verified that Rabipur is immunogenic in Japanese. No adverse reactions were observed. This study supports that Rabipur is effective and safe

in Japanese. We will conduct further testing to monitor how anti-rabies antibodies are maintained in the serum.

The WHO recommends three types of rabies vaccines. One is the human diploid cell vaccine (HDCV), another is a purified vero cell a vaccine for humans (PVCV) and the third is a purified chick embryo cell vaccine, one of which is Rabipur, which was used in this study.

There are no reports of immunogenicity of either HDCV or PVCV in Japanese. Takayama (1997) reports that Japanese bitten while travelling abroad were vaccinated in other countries. He interviewed 93 patients who visited his hospital for post-exposure vaccination. His report shows that the patients were vaccinated with variety of products, including PCECV, HDCV and PVCV. A few of the patients were vaccinated with brain tissue vaccine, which is not recommended because of the risk for severe adverse effects.

We have not had a rabies case acquired in Japan for more than 30 years. The reasons are as follows. First, since the Japanese government strictly enforces the vaccination of dogs with anti-canine rabies vaccine, dogs cannot serve as reservoirs for the rabies virus. Second, since Japan is surrounded by water, infected animals cannot easily enter.

Mannen (2002) pointed out it is curious that Japan continues to be a rabies-free country. According to statistics, the rate of rabies vaccination for registered dogs has been decreasing. In 2003, 75% of dogs registered were inoculated compared to 81.1% in 1999 ([http://www.forth.go.jp/mhlw/animal/down/2to/2to\\_f.xls](http://www.forth.go.jp/mhlw/animal/down/2to/2to_f.xls)).

In Japan, local government enforces registration of dogs by their owners according to a registration act (Cabinet order No 236, 1953). In 2006, 6,635,807 dogs were registered and 74.0% of them were vaccinated (<http://www.mhlw.go.jp/bunya/kenkou/kekaku-kansenshou10/01.html>). However, there are numerous unregistered dogs in Japan.

According to the Pet Food Manufactures Association of Japan, 12,089,000 dogs live in Japan, therefore 50% of dogs in Japan are not registered (<http://www.jppfma.org/shiryo/shiryo-set.html>). These statistics suggest that only 40% of dogs in Japan are vaccinated (Tamashiro *et al*, 2007).

Although Japan is an island, it is not impossible for animals infected with rabies to be smuggled into the country. Approximately 8,000 dogs are quarantined each year in Japan (<http://www.maff-aqs.go.jp/tokei/nenpo/2006/H18doubutu-3y.xls>). Some of these may pass through quarantine with rabies virus. Although Britain is a rabies-free country, in 1969 a dog imported from West Germany was found to be infected by rabies after it had been released from quarantine. Another dog in the same kennel died of rabies while in quarantine. Another dog from Western Germany was destroyed at the owner's request during quarantine because it showed symptoms of rabies. A dog imported from Pakistan also developed rabies and infected two other dogs in quarantine in 1970 (Hill, 1971). As Mannen (2002) warned, once rabies infected animals enter Japan, the virus may spread quickly and infect humans.

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#### REFERENCES

Barth R, Franke V, Müller H, Weinmann E. Purified chick-embryo-cell (PCEC) rabies vaccine: its potency performance in different test system and in humans.

*Vaccine* 1990;8:41-8.

Bleck T, Ruprecht C. Rhabdoviruses. In: Richman D, Whitley R, Hayden F eds. Clinical virology. New York: ASM Press, 2002:857-73.

Hill F. Keeping Britain from rabies. *Proc Nat Sci Med* 1971;64:231-3.

Kamoltham T, Thinyounyong W, Phongchamnaphai P, *et al*. Pre-exposure rabies vaccination using purified chick embryo cell rabies vaccine intradermally is immunogenic and safe. *J Pediatr* 2007;151:105-22.

Khawplod P, Inoue K, Shoji Y, *et al*. Novel rapid fluorescent focus inhibition test for rabies virus using a recombinant rabies virus visualizing a green fluorescent protein. *J Virol Methods* 2005;125:35-40.

Mannen K. Rhabdovirus -The possibility of rabies reemergence in rabies free Japan. *Uirusu* 2002;52:21-5. [in Japanese with English abstract].

Ruprecht CE, Hanlon CA, Hemachudha T. Rabies re-exceminced. *Lancet Infect Dis* 2002;2:327-43.

Takayama T. Rabies control in Japan. *Jpn J Infect Dis* 2000;53:93-7.

Takayama N. Types of rabies vaccine which were locally injected to the subjects bitten by animals abroad. *J Jpn Assoc Infect Dis* 1997;71:751-5.

Takayama N. Human rabies, a fatal disease forgotten. Tokyo: Jikuu.syuppan, 2000. (in Japanese).

Tamashiro H, Matibag G, Ditangco R, Kanda K, Ohbayashi Y. Revisiting rabies in Japan: Is there case for alarm? *Travel Med Infect Dis* 2007;5:263-75.

Vodopija I, Baklaić Ž, Vodopija R. Rabipur: A reliable vaccine for rabies protection.

*Vaccine* 1999;17:1739-41.

WHO Expert Consultation on Rabies. First report. *WHO Tech Rep Ser* 2005;931:1-88.

WHO. Rabies. *WHO Fact Sheet* 2006; 99. [Cited 2007 Nov 20]. Available from: URL:<http://www.who.int/mediacentre/factsheets/fs099/en/index.html>