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# Human Antibody Response After Rabies Pre- And Post- Exposure Prophylaxis: A Philippine Experience

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**Abstract**

Rabies, though fatal, remains to be a vaccine-preventable disease. A rabies antibody threshold of 0.5 IU/mL is considered protective. Compliance with vaccination, however, continues to pose a challenge to rabies prevention and control. A demographic profile and serum antibody levels were collected and analyzed using a cross-sectional analytic study design. A total of 183 individuals in Davao City, Philippines, who received rabies preexposure and post-exposure prophylaxis were recruited. The mean antibody levels were higher than the protective threshold regardless of the number of doses. A significantly higher antibody level was noted with two and three doses than the single dose (p=0.007). This study revealed that one vaccine dose may confer enough protection against rabies infection. However, Two or three doses can provide higher antibody levels lasting up to more than 6 years post-vaccination. Completing the rabies vaccination series increases the likelihood of developing sufficient protection against rabies disease.

**Keywords:** Rabies, rabies vaccines, pre-exposure prophylaxis, post-exposure prophylaxis, antibody levels, Philippines

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## 1. Introduction

Rabies is a preventable disease with adequate vaccination. However, if left untreated, it has a 100% fatality rate.1 In 2018, the global annual rabies fatality rate was estimated to be 59,000.2 With 250 rabies deaths per year, the Philippines remains one of the top ten countries with the highest cases.3 From 2014 to 2018, the Philippines reported 1,094 rabies cases, with Region XI (including Davao City) ranking sixth in the country for the highest number of cases.4

Efforts in controlling the spread of rabies include pet vaccination, preexposure prophylaxis (PreP), and post-exposure prevention (PEP).4 Vaccination of pets is regarded as the most cost-effective method of preventing rabies in humans. However, this necessitates continued immunization of at least 70% of the animal population

each year.3 With a large number of stray dogs and disregard for responsible dog ownership regulations, this remains to be a difficult task. 5-6 PrEP is administered to high-risk persons on days 0, 7, and 21 or 28. Boosters are recommended every five years. PEP, on the other hand, is administered to those with an animal bite history. A positive protective response is the formation of>0.5 IU/mL of serum antibody levels at least two to four weeks following the final vaccine dose.6,7

Davao City, a 1.8 million-person city in the Philippines, has established Animal Bite Treatment Centers (ABTCs) as part of its rabies prevention and control program, which includes the distribution of PEP and PreP vaccines.5,8 Despite these efforts, Davao City is still far from being rabies-free, with 210 reported rabies cases between 2006 and 2017.9 Challenges in the vaccination program include vaccine availability, vaccine type, vaccination dose schedule, and full vaccination compliance. Each may have a significant implication for rabies disease prevention and control.10 This study determined the antibody levels among vaccinated individuals according to the number of doses received and across time (in years) among vaccinated individuals.

## 2. Methods

The study utilized a cross-sectional analytic design where demographic data and serum antibody levels were collected from the study population at one time only. An ethics approval was secured from the Davao Medical School Foundation, Inc. Hospital Ethics Committee, Grant No. P-12-02-38. A transmittal letter was sent to the Davao City Health Office Animal Bite Center (DCHO-ABTC) for permission to access the patient records from 2012 onwards. The study population for PEP and PreP was recruited purposively from the records provided. The inclusion criteria were the following: (1) PEP: Patients 12 to 59 years old who received complete or incomplete anti-rabies vaccination after a dog bite, (2) PreP: Patients 18 to 59 years old who received anti-rabies vaccine for PreP classified as high-risk for rabies such as health workers handling rabies cases, animal handlers or pet owners and veterinarians with no history of animal bite. Potential participants were contacted by phone, and those who responded were explained the study's purpose and other relevant information. Those who agreed to join were visited in their homes or offices according to their area and time of preference. Participants were then asked to sign a written informed consent after a thorough explanation of the study. This was followed by a collection of demographic data by the research team and blood extraction by a licensed and skilled medical technologist.

Three milliliters of blood samples were collected and placed in gold-top or red-top blood tubes labeled with the corresponding identification number and collection date. These were then stored in an icebox maintained at a temperature between 4 to 8°C. Samples were then transported to the Davao Medical School Foundation, Inc. College of Medicine, Research Center. Samples were centrifuged at 1000 x g for ten (10) minutes for serum separation. The serum was then stored at -20°C until the ELISA procedure was carried out.11 Rabies antibodies were detected from the serum using the quantitative Platelia Rabies II Kit (Bio-Rad,3, B Boulevard Raymond Poincaré 92430 Marnes-La-Coquette – France), following the manufacturer's specifications. Absorbance was measured at 450 nm using a microplate reader, and rabies antibody titers were determined. Antibody titer equal to or greater than 0.5 iu/L was considered protective or sufficient.12 Data were reported as frequency, percentage, and means and analyzed using a licensed SPSS® Statistics 23.0 software.

## 3. Results

The demographic profile of the participants shows a preponderance of males in both PreP and PEP at 65.4 and 54.6%, respectively. Females comprised only 33.3% in PreP and 44.4% in PEP. The mean age of 39.63 years is higher for PreP compared to 30.77 years for PEP (Table S1).

The total mean antibody levels of the participants across doses revealed a statistically significant increasing trend ($p= 0.007$) (Table 1). However, Tukey Post-hoc analysis showed that the mean of the two- and the three-dose groups were significantly higher than the one-dose group. A similar trend was noted for the PEP group across doses ($p=0.038$), while no significant difference was demonstrated for the PreP group ($p=0.227$).

**Table 1**. Comparison of mean rabies antibody titers of the participants at various dose levels post-vaccination for pre-exposure (PreP) and post-exposure prophylaxis (PEP).

| Number of Doses | Total | PreP | PEP | p-value[2] |
|---|---|---|---|---|
| | n=183 | n=106 | n=77 | |
| One Dose | 0.94±0.78[b] | 1.48±0.56[b] | 0.66±0.75[b] | 0.009** |
| Two Doses | 1.28±0.86[a] | 1.21±0.92[a] | 1.32±0.85[a] | 0.379 |
| Three Doses | 1.51±0.81[a] | 1.62±0.81[ab] | 1.3±0.76[a] | 0.009** |
| P-value | 0.007** | 0.227 | 0.038* | |

*0.05 significant

**<0.01 significant

[1]Comparison of mean antibody titers at various dose levels post-vaccination

[a,b,]Superscripts with same the letters are not significantly different (Tukey post hoc test)

[2]Comparison of mean antibody titers between PreP and PEP groups at various dose levels post-vaccination

Figure 1 presents the mean antibody levels post-vaccination across time (in years). The overall trend showed that the mean antibody levels remained above the critical protective threshold (0.5 IU/mL) up to more than six years post-vaccination. A similar direction was also noted with the PrEp group. The PEP group, on the other hand, showed a decreasing trend over time.

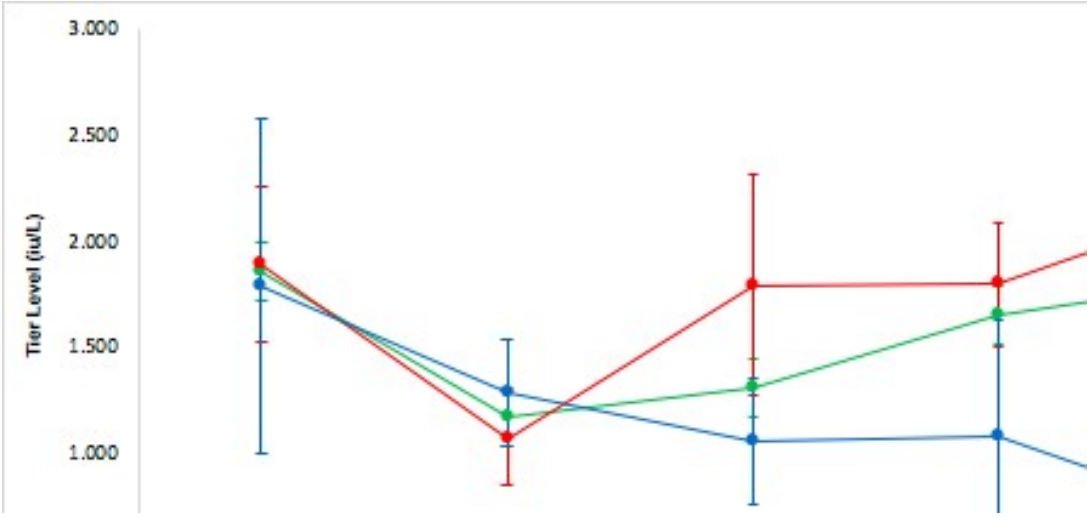


**Figure 1**. Mean rabies antibody titers based on periods post-vaccination

When compared statistically, a significant difference was noted in the total mean antibody levels across time (p=< 0.01) (Table 2). The lowest level was seen at 1-2 years and the highest at one year and more than six years post-vaccination. Significant differences were also noted for the PrEP group (P<0.01). Post Tukey analysis showed comparable antibody levels across time save for a significantly lower level at 1-2 years post-vaccination. The PEP group, however, demonstrated no significant difference across post-vaccination periods (p= 0.135).

**Table 2**. Comparison of mean rabies antibody levels of the participants at various periods post-vaccination for pre-exposure (PreP) and post-exposure prophylaxis (PEP).

| Years Post-vaccination | Total | PreP | PEP | p-value[2] |
|---|---|---|---|---|
| | n=183 | n=106 | n=77 | |
| Within the year | 1.85±0.79[a] | 1.89±0.60[a] | 1.79±1.00 | 0.406 |
| 1 to 2 years | 1.17±0.77[b] | 1.07±0.74[b] | 1.28±0.80 | 0.104 |
| 3 to 4 years | 1.30±0.77[ab] | 1.79±0.79[a] | 1.06±0.60 | 0.008 |
| 5-6 years | 1.65±0.82[ab] | 1.80±0.79[a] | 1.07±0.70 | 0.011 |
| more than 6 years | 1.82±0.81[a] | 2.20±0.36[a] | 0.68±0.70 | <0.01 |
| P-value[1] | <0.01** | <0.01** | 0.135[ns] | |

*0.05 significant

**<0.01 significant

[1]Comparison of mean antibody titers at various periods (years) post-vaccination

[a,b,c]Superscripts with same the letters are not significantly different (Tukey post hoc test)

[2]Comparison of mean antibody titers between PreP and PEP groups at various periods (years) post-vaccination

Most of the vaccinated individuals (141; 75.81%) completed 3 doses, while 45 or 24.19% received only 1-2 doses (incomplete) (Table S2). Odds ratio analysis revealed that individuals with complete vaccination are 5.471 times more likely to have sufficient seroconversion (95% CI 2.559-11.694, p-value < 0.01).

## 4. Discussion

This study provided an overview of human antibody response (IgG) towards rabies vaccination given as PreP or PEP. One dose of the vaccine may be enough to produce protective antibody levels for some individuals. The higher antibody levels of the two and three-dose groups, however, imply higher protection. Rabies-specific IgG antibodies could usually be detected at increasing levels from day 7 to 10 after the first dose (Blanchard-Rohner et al., 2009; Ueki, 1994). In the study conducted by Mills et al. (2021), 38.0 and 31.8% of participants aged <50 and ≥50 years were found to be antibody-positive after one intramuscular (IM) dose of the vaccine as PreP for priming the immune system of travelers under time constraints in different age groups (Mills et al., 2021). The higher IgG titers after the second and third vaccination reflect the most rapid memory B-cell response (Blanchard-Rohner et al., 2009). The PreP group in this study had relatively high titers even at the first dose, unlike the PEP group, whose titers started low and elevated only with increasing doses. High-risk individuals with preexposure prophylaxis, such as veterinary practitioners, have early, higher, and more sustained levels of protection probably due to the nature of their work, which has strongly primed their immune response.

The results demonstrated that the vaccine series can provide protection that can last for more than six years. This finding is consistent with several studies showing that virus-neutralizing antibodies are still detectable among vaccine recipients 5 to 14 years after the last dose, with or without a booster dose (Brown et al., 2008; Strady et al., 1998; Thraenhart et al., 1994). The protection appears to be sustained in the long-term for PreP prophylaxis, but a decreasing trend across time was observed for PEP prophylaxis. Mansfield et al. (2016) showed that standard PreP primes the immune system in both young and older people and can be boosted decades after the primary vaccine course. De Pijper et al. (2021) also demonstrated that rabies immunological memory is

reactivated within 7 days after the single intramuscular booster immunization, even when administered 10 – 24 years after PreP.

The results of this study also emphasized the importance of completing the recommended 3-dose series, which resulted in protective antibody levels in the majority of the participants. It is demonstrated that dose-compliant individuals are five times more likely to develop sufficient levels of protection against rabies. Strady et al. (1998) confirmed the superior long-term immunogenicity of the three-injection over the two-injection rabies vaccine protocol. This is in contrast to a study conducted by Baysal et al. (2009), which showed a low rate of protective antibody positivity even though participants were completely vaccinated. Almost half of the participants, however, were not able to comply with the complete vaccine dose series. Some factors that are attributed to noncompliance with vaccination include the animal involved testing negative for the virus, the patients having prior rabies treatment, the patients deciding not to return for treatment, the lack of awareness of the full vaccination regimen, and the patient declining initiation of rabies vaccination (Shi, 2020).

Limitations of this study include the non-identification of the vaccine brand, the intradermal vaccine administration, the 3-dose series vaccine schedule, and the use of 0.5 IU/mL as a critical threshold reference for the protective antibody level. When records were taken from the Davao City Health Office Animal Bite Treatment Center (DCHO-ABTC), which acquires its resources from a specific supplier, one specific vaccine brand can most likely be deduced, with the vaccine given via intradermal route of administration, the study results may not be applicable to antibody response towards vaccination given through the intramuscular (IM) route. While the antibody responses recorded were from the three-dose vaccine series, antibody responses after booster doses were not accounted for. The study results were analyzed based on the World Health Organization (WHO) recommended threshold value (0.5 IU/mL) using a standard ELISA assay which may not be representative of rabies virus-neutralizing antibodies.

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