Jurnal Veteriner Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional, Dirjen Penguatan Riset dan Pengembangan, Kemenristek Dikti RI S.K. No. 36a/E/KPT/2016

Juni 2019 Vol. 20 No. 2 : 264-268
DOI: 10.19087/jveteriner.2019.20.2.264
online pada http://ojs.unud.ac.id/index.php/jvet

Protective Immune Response of Post Rabies Vaccinated Dogs in Buduk Village, Mengwi, Badung, Bali

(RESPONS IMUN PROTEKTIF PADA ANJING PASCAVAKSINASI RABIES DI DESA BUDUK, MENGWI, BADUNG, BALI)

Ida Bagus Kade Suardana[1]*, Ida Bagus Oka Winaya[2], Desak Wiga Puspita Dewi[3], I Nengah Sudarmayasa[3], Kadek Karang Agustina[4]

[1]Veterinary Virology Laboratory, [2]Veterinary Pathology Laboratory,
[3]Veterinarian Education Program, [4]Veterinary Public Health Laboratory,
Faculty of Veterinary Medicine, Udayana University,
Jln. Sudirman, Sanglah, Denpasar, Bali, Indonesia 80234
Phone 0361 223791; *Email: idasuardana@unud.ac.id

Abstrak

In efforts to eradicate rabies disease, vaccinations have been carried out targeting so-called rabies carrier animal such as dog andcat. Mass rabies vaccination has been done by the government of Bali annualy, in every sub-village at vaccination post or at every household. Minimum vaccination coverage to protect the threatened dog population from rabies is 70%. To determine whether the vaccinated dogs have protective antibody against rabies, this study have been done to find out the immune response of dogs after rabies vaccination in Buduk village. Sera were collected using random sampling in each subvillage, a total of 30 serum sample were examined, and the antibody was tested using kit ELISA Rabies®. Elisa test resulted in 90% of dogs have protective antibody titers (OD>0.5 EU). It concluded that dogs in Buduk village have a good immune response against rabies vaccine.

Keywords: rabies; vaccination; ELISA test; protective antibody; immune response

Abstrak

Dalam upaya memberantas penyakit rabies, vaksinasi telah dilakukan pada hewan pembawa rabies (HPR) seperti anjing dan kucing. Vaksinasi massal pada anjing telah dilakukan oleh Pemerintah melalui Dinas Peternakan dan Kesehatan Hewan setiap tahun, yang berlokasi di setiap Banjar secara door to door. Cakupan vaksinasi minimum untuk melindungi populasi anjing yang terancam dari rabies adalah 70%. Untuk menentukan apakah anjing yang divaksinasi memiliki antibodi terhadap rabies, telah dilakukan penelitian terhadap respon imun anjing setelah vaksinasi rabies di desa Buduk, Mengwi Badung Bali menggunakan metode observasi deskriptif. Sebanyak 30 sampel serum anjing post-vaksinasi rabies diuji menggunakan kit ELISA Rabies®. Tes Elisa menghasilkan 90% anjing memiliki titer antibodi pelindung (OD>0,5 EU). Dapat disimpulkan bahwa anjing di desa Buduk memiliki respon imun yang baik terhadap vaksin rabies.

Kata-kata kunci: rabies; vaksinasi; tes ELISA; antibodi respons imun

INTRODUCTION

Rabies is a zoonotic disease with a high mortality rate both in animal and in human (Jemberu *et al.,* 2013). According to WHO (2005), more than three million people in the world are at risk for rabies. The disease is thought to kill around 50,000-60,000 people in 85 rabies-endemic countries (Knobel *et al.,* 2005; WHO, 2005). Rabies disease is caused by the genus Lyssavirus of the family *Rhabdoviridae* and attacks warm-blooded animals (Krebs *et al.,* 1995; Baloul and Lafon, 2003; Astawa *et al.,* 2016). Various efforts have been made to overcome rabies disease in Bali, but until now it cannot be completely eradicated. The eradication of rabies disease is carried out by mass vaccination, control of animal populations of rabies transmitters and some other measures such as impounding and binding rabies-carrying animals (Kamil *et al.,* 2004; Dietzshold *et al.,* 2005; Dibia, 2007; Keuster and Butcher, 2008; Agustina *et al.,* 2018; Hiby *et al.,* 2018).

In Indonesia, rabies disease has been found since the Dutch colonial government and until now cannot be solved completely. Currently, rabies is still endemic in several areas such as Flores, East Nusa Tenggara, Sulawesi, Kalimantan, West Sumatra, and Bali (Susetya *et al.,* 2008). The emergence of rabies cases in humans and animals in Bali in November 2008 proves that the spread of rabies in Indonesia tends to be widespread and difficult to overcome. Since the rabies case first appeared in Kuta District Badung in November 2008, rabies infection quickly spread to other districts, therefore in 2009 Bali was declared to have a rabies endemic (Dibia *et al.,* 2015).

The eradication of rabies in Bali through a mass vaccination program against all rabies-sensitive animals has been done (Hiby *et al.,* 2018). A good immunity occurs when the antibody titer is formed reaches a value of 0.5 IU/ml Optical Density (OD) (Dartini *et al.,* 2012). In Badung Bali, rabies vaccination in dogs has been done since December 4th, 2008, followed by mass vaccination in all regencies/cities in Bali Province. Although rabies vaccination has been done routinely, the incidence of rabies in dogs still persists. This study is carried out a dog serum examination to determine whether the vaccination has been done already produces protective antibody titer.

RESEARCH METHODS

The data collection and analysis were conducted descriptively on the immune response of dogs post-rabies vaccination. Sampling was done three months after rabies vaccination. Measurement of serum antibody titers was using the ELISA test (KIT ELISA Rabies, Deptan RI No. D. 09123751 VKCD-KIT). The sample is declared protective against rabies when it reaches a titer of 0.5 IU/mL or more.

The research stages included: Rabies vaccination performed simultaneously with mass vaccination programs conducted by the Department of Animal Husbandry, Fisheries, and Marine Affairs of Badung Regency. Blood sample collection was done three months after the rabies vaccination. As many as 30 dogs blood sample from Buduk Village has been collected. Blood was left at room temperature until the serum goes out. The serum was separated into an Eppendorf tube and stored at -20°C until an ELISA test is performed.

Serum Examination

The procedure of serum examination was done in accordance with the guidance of ELISA Kit ® (Pusvetma, Surabaya, Indonesia). The serum samples were inactivated at 56°C for 30 min, then diluted 1:50 by adding 5 ìL serum samples with 245 µL of solvent. The positive control serum (25 times dosage) and the negative control serum were diluted 50 times, 100 times, 200 times and 400 times. Serum samples and controls were put into each microplate well 100 µL, two wells were left without serum as a conjugate control. The microplate is covered with a plastic cover and incubated at 37°C for 45 to 60 minutes. The serum fluid on the test microplate was discarded, washed five times and added with a diluted conjugate of 100 ìL each well. The microplate was re-closed and incubated at 37°C for 45 to 60 minutes. The liquid was removed and the plate was washed five times and 100 µL of 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid/ABTS) substrate was added to each well. The microplate was incubated at room temperature in dark condition for 30 minutes. At last 100 ìL stop solution was added to each well. The 405 nm wavelength was used in Optical Density (OD) reader. The Equivalent Unit (EU) of each OD samples was calculated using the formula already provided by the KIT. Serum titers of 0.5

EU or more are considered protective. The ELISA process was done in Biotechnology Laboratory of Balai Besar Veteriner Denpasar, Bali.

RESULT AND DISSCUSSION

The results showed 27 (90%) of 30 samples had protective (seropositive) antibodies with an average OD value of 0.9 EU, and 3 samples (10%) had a non-protective (seronegative) antibody titer with OD values of less than 0.4 EU (Table 1).

From the results of the study, 90% (27/30) dogs have a seropositive (protective) antibody titer, this means that rabies vaccination has provided immunity that is able to cope with rabies infection. The implementation of vaccination has provided a good immune response. According to WHO (2005), dog population with a protective antibody titer of 70% can prevent the emergence of rabies disease. The vaccine used was Rabisin® (Merial/Romindo Primavetcom) vaccine form of liquid preparation conatining inactive rabies virus for subcutaneous (SC) or intramuscular (IM) usage.

Seropositive occurs both in dogs that were first vaccinated and in dogs that have been vaccinated more than once (Tab 2). Three months after the first vaccination (primary immune response) has been found to be seropositive, which means there has been a good immune response from stimulation of vaccine antigens. The immune system is a very complex system with various roles in the effort to maintain body balance (Nicholson, 2016). To perform the function of immunity, in the body there is a system called the lymphoreticular system (Rankin and Artis, 2018). This system is a network or collection of cells scattered throughout the body e.g. in bone marrow, thymus, lymph nodes, lymphatic system, gastrointestinal tract, and several other organs (Liao and Padera, 2013).

Mass vaccination as a method for Rabies control has been known since the 1920s (Knobel *et al.,* 2005). Vaccination is the most effective approach in controlling rabies in both animals and humans (Lembo *et al.,* 2010; Moore and Hanlon, 2010). Antibodies play a central role in the prevention of rabies infection. According to Moore and Hanlon (2010), antibodies formed by rabies vaccination are in fact very effective in preventing infection because rabies vaccine is able to stimulate high levels of neutralization antibodies. Faizah *et al.* (2012) prove that the vaccine used in rabies control in Bali is effective in forming humoral and cellular immunity with protective duration of immunity (e" 0.5 IU) to five months post-vaccination. While Dartini *et al.* (2012) reported the results of vaccination studies in field conditions with the same type of vaccine having a protective duration of immunity up to nine months post-vaccination.

Table 1. The average of anti-rabies virus antibody titer of rabies three months post vaccination in Buduk Village in Badung, Bali.

Sample	Average of Antibody titer (±SD)	Percentage (%)
Seropositive		
27 (27/30)	1.49 EU ± 1.72	90
Seronegative		
3 (3/30)	0.33 EU± 0.12	10

Table 2. Distribution of antibody data from rabies vaccination in sample dogs

Sample size	Rabies Vaccination (%)	Antibody	
		Seropositif (%)	Seronegative
16	One time	87.5	12.5
8	Two times	87.5	12.5
5	Three times	100	0
1	Four times	100	0

The humoral immune response begins with B lymphocyte differentiation into a population (clone) of plasma cells that release specific antibodies into the blood. In the humoral immune response, it also applies the primary immune response that forms the memory B cell. Each lymphocyte clone is programmed to form one specific antibody type against a specific antigen (Clonal selection) (LeBien and Tedder, 2008). These antibodies will bind to the antigen to form antigen-antibodies complexes that can activate complement and result in the destruction of the antigen. In order for B-lymphocytes to differentiate and to form antibodies, the help of T-helper lymphocytes (T-helper), which, on certain signals either via MHC or signals released by macrophages, stimulates the production of antibodies (Lederman *et al.,* 1992; Broek *et al.,* 2018). In addition to T-helper cells, the production of antibodies is also regulated by suppressor T cells (T-suppressors), so that the production of antibodies is balanced and in accordance with the required (Consales and Bolzan, 2007).

Dogs that have vaccinated more than once time also produce protective antibody titers, meaning secondary immune responses are well underway. Although the antigen in the first contact (primary response) can be destroyed and then the immune system cells involved, the primary immune response could lead to the formation of clones or groups of cells called memory cells that can recognize the antigen concerned (Charlton *et al.,* 2016). If in the future the same antigen enters the body, it will proliferate and produce a specific secondary response that progresses faster and more intensively than the primary immune response (Faber *et al.*, 2002; Astawa *et al.,* 2016).

CONCLUSION

To conclude, 90% of 3-month post vaccinated dogs in Buduk Village Badung Bali have a protective antibody titer against rabies.

SUGGESTION

The following quarterly rabies post vaccination studies need to be carried out within a period of one year to see the development of antibodies, using a larger number of samples and a wider study location.

ACKNOWLEDGMENT

The authors would like to thank The Rector of Udayana University and Dean of the Faculty of Veterinary Medicine that funded this research through Awarded Grant of Study Program with contract No. 1670/UN14.2.9/LT/2017. Thanks to the Department of Animal Husbandry, Fisheries, and Marine Affairs of Badung Regency for support this research.

REFERENCES

Agustina KK, Cahyani PSD, Suardana IBK. 2018. Dog demography and level of knowledge against rabies in positive and negative case of rabies areas in Mendoyo-Jembrana, Bali, Indonesia. *J Veteriner* 18(4): 642-548.

Astawa NM, Kencana GAY, Suardana IBK. 2016. Monoclonal antibodies as ligands for purification of rabies virus proteins from the brain tissues of infected dogs and mice. *J Veteriner.* 17(4): 556-570.

Baloul L, Lafon M. 2003. Apoptosis and rabies virus neuroinvasion. *Biochem.* 85: 777-788.

Broek TVD, Borghans JAM, Wijk FV. 2018. The full spectrum of human naive T cells. *Nat Rev Immunol* 18(6): 363-373.

Charlton CL, Lai FY, Dover DC. 2016. How to determine protective immunity in the post-vaccine era. *Hum Vaccin Immunother* 12(4): 903-906.

Consales CA, Bolzan VL. 2007. Rabies Review: Immunopathology, clinical aspects and treatment. *J Venom Dis* 13(1): 1-33.

Dartini NL, Mahardika IGNK, Putra AAG, Scott H. 2012. Uji banding dua kit elisa untuk deteksi antibodi terhadap virus rabies pada anjing. *Bul Vet BBVet Denpasar* 24(80): 143-149.

Dibia IN. 2007. Evaluasi pemberantasan rabies di Pulau Flores Provinsi Nusa Tenggara Timur: Kajian Surveilans Tahun 2006. *Bul Vet BBVet Denpasar* 19 (70): 6-13.

Dibia IN, Sumiarto B, Susetya H, Putra AAG, Scott H. 2015. Analisis faktor resiko kasus rabies pada anjing di Bali. *Bul Vet BBVet Denpasar* 27 (86): 7-24.

Dietzshold B, Schnell M, Koprowski H. 2005. Pathogenesis of rabies. *Curr Trop Microbiol Immunol* 292: 45-56.

Faber M, Pulmanausahakul R, Hodawadekar SS, Spitsin S, Mc Gettigan JP, Schnell MJ, Dietzschold B. 2002. Overexpression of the rabies virus glycoprotein result in enhancement of apoptosis and antiviral immune response. *J Virol* 76: 3374-3381.

Faizah, Astawa, NM, Putra AAG, Suwarno. 2012. The humoral immunity response of dog vaccinated with oral SAG2 and parenteral Rabisin and Rabivet Supra 92. *Indo J Biomed Sci* 6(1): 26-29.

Hiby E, Agustina KK, Atema KN, Bagus GN, Girardi J, Harfoot M, Haryono Y, Hiby L, Irawan H, Kalalo L, Purnama SG, Subrata IM, Swacita IBN, Utami NWA, Januraga PP, Wirawan DP. 2018. Dog ecology and rabies knowledge of owners and non-owners in Sanur, a Sub-District of the Indonesian Island Province of Bali. *Animals* 8(112): 1-18.

Jemberu WT, Molla W, Almaw G, Alemu S. 2013. Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia. *PLoS Negl Trop Dis* 7(5): e2216.

Kamil M, Sumiarto B, Budhiarta S. 2004. Kajian kasuskontrol rabies pada anjing di Kabupaten Agam, Sumatera Barat. *Agrosains* 17(3): 313-320.

Keuster T, Butcher R. 2008. Preventing dog bites: Risk factors in different cultural settings. *Vet J* 177: 155-156.

Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda MEG, Shaw A, Zinsstag J, Meslin F. 2005. Reevaluating the burden of rabies in Africa and Asia. *Bul WHO* 83(5): 360-368.

Krebs JW, Wilson ML, Childs JE. 1995. Rabies-epidemiology, prevention, and future research. *J Mammalogy* 76: 681-694.

LeBien TW, Tedder TF. 2008. B lymphocytes: how they develop and function. *Blood* 112(5): 1570-1579.

Lederman S, Yellin MJ, Inghirami G, Lee JJ, Knowles DM, Chess L. 1992. Molecular interactions mediating T-B lymphocyte collaboration in human lymphoid follicles. Roles of T cell-B-cell-activating molecule (5c8 antigen) and CD40 in contact-dependent help. *J Immunol* 149(12): 3817–3826.

Lembo T, Hampson K, Kaare MT, Ernest E, Knobel D, Kazwala RR, Haydon DT, Cleaveland S. 2010. The Feasibility of Canine Rabies Elimination in Africa: Dispelling Doubts with Data. *PLOS Negl Trop Dis* 4(2): e626.

Liao S, Padera TP. 2013. Lymphatic function and immune regulation in health and disease. *Lymphat Res Biol* 11(3): 136-143.

Moore SM, and Hanlon CA. 2010. Rabies-specific antibodies: Measuring surrogates of protection against a fatal disease. *PLOS Negl Trop Dis* 4(3): e595.

Nicholson LB. 2016. The immune system. *Essays Biochem* 60(3): 275-301.

Rankin LC, Artis D. 2018. Beyond host defense: Emerging function of the immune system in regulating complex tissue physiology. *Cell* 174: 554-567.

Susetya H, Sugiyama M, Inagaki A, Ito N, Mudiarto G, Minamoto N. 2008. Molecular epidemiology of rabies in Indonesia. *Virus Res* 135: 144-149.

World Health Organization. 2005. *Report of WHO Expert Consultation on Rabies. First Report.* WHO Technical Report Series. Geneva 931.

Wunner WH, Briggs DJ. 2010. Rabies in the 21st century. *PLOS Negl Trop Dis* 4(3): e591.