Jurnal Veteriner pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional Peringkat 2, Dirjen Riset dan Pengembangan,
Kemendiktisaintek RI SK No 10/C/C3/DT.05.00/2025, 21 Mar.2025

Juni 2025 Vol. 26 No.2: 210-220
DOI: 10.19087/jveteriner.2025.26.2.210
online pada http://ojs.unud.ac.id/index.php/jvet

Inactive Rabies Vaccine Stored at Room Temperature for 10 Hours Loses Its Ability to Stimulate Antibody Response in BALB/c Mice

(VAKSIN RABIES INAKTIF YANG DISIMPAN PADA SUHU KAMAR SELAMA 10 JAM KEHILANGAN KEMAMPUANNYA MERANGSANG RESPONS ANTIBODI MENCIT BALB/c)

Grace Yureka Aurel Siahaan[1], I Gusti Ayu Agung Suartini[2], Tri Komala Sari[3]

[1]Undergraduate Veterinary Medicine Study Program,
[2] Department of Veterinary Biochemistry,
[3]Department of Veterinary Virology
Faculty of Veterinary Medicine, Udayana University,
Jl. Sudirman, Sanglah Denpasar, Bali, Indonesia, 80234
Telp/Fax: (0361) 223791
Email: yurekagrace@gmail.com

ABSTRACT

Rabies vaccines are generally stored at 2-8°C to maintain antigen stability. However, most of Bali's mountainous and hilly areas often cause vaccines to be stored at room temperature or higher, which potentially damage the vaccine. One of the rabies vaccines used by the government is the inactivated rabies vaccine. This study was aimed to determine the effect of inactivated rabies vaccine storage time at room temperature to the immune response of BALB/c mice. This study used nine BALB/c mice that were divided into three different groups. Each group was induced with vaccines strored at room temperature (25-30°C) for 0 hours (P1), 6 hours (P2), or 10 hours (P3). Serum were collected before vaccination; and 2- and 4-weeks post vaccination. Antibodies were tested using an Enzyme-linked immunosorbent assay (ELISA) kit, and spleen samples were collected at week four for histological analysis with Hematoxylin Eosin staining. The results were analyzed using One-Way Analysis of Variance followed by Post Hoc-Bonferroni test. The histopathological examination of the spleen showed no differences between the three treatment groups. Our ELISA test showed that inactivated rabies vaccine stored for 10 hours at room temperature (25-30℃) was not able to induce antibody response.

Keywords**:** BALB/c mice**;** immune response; immunology; rabies vaccine; vaccine storage time

ABSTRAK

Vaksin rabies pada umumnya disimpan pada suhu 2-8°C untuk menjaga kestabilan antigen. Namun, sebagian besar wilayah pegunungan dan perbukitan Bali sering menyebabkan vaksin disimpan pada suhu ruang atau lebih tinggi, yang berpotensi merusak

vaksin. Salah satu vaksin rabies yang digunakan pemerintah adalah vaksin rabies inaktif. Tujuan penelitian ini adalah untuk mengetahui pengaruh lama penyimpanan vaksin rabies inaktif pada suhu ruang terhadap respons imun mencit BALB/c. Penelitian ini menggunakan sembilan ekor mencit BALB/c yang dibagi menjadi 3 kelompok berbeda. Setiap kelompok diinduksi dengan vaksin yang disimpan pada suhu ruangan (25-30°C) selama 0 jam (P1), 6 jam (P2), atau 10 jam (P3). Serum diambil sebelum vaksinasi; dan 2 dan 4 minggu pascavaksinasi. Antibodi diuji menggunakan kit *Enzyme-linked Immunosorbent Assay* (ELISA), dan sampel limpa diambil pada minggu ke-4 untuk analisis histologis dengan pewarnaan Hematoxylin Eosin. Hasilnya dianalisis menggunakan uji sidik ragam satu arah (*One-Way Analysis of Variance*) diikuti dengan uji Post Hoc-Bonferroni. Pemeriksaan histopatologi limpa tidak menunjukkan perbedaan antar ketiga kelompok perlakuan. Uji ELISA menunjukkan bahwa vaksin rabies inaktif yang disimpan selama 10 jam pada suhu ruang (25-30°C) tidak mampu menginduksi respons antibodi mencit BALB/c.

Kata-kata kunci: BALB/c; respons imun; imunologi; vaksin rabies; waktu penyimpanan vaksin

INTRODUCTION

Rabies is one of the diseases that is a national and even international priority in the public health sector in several Asian countries (Ministry of Agriculture, 2019). Of the 38 provinces in Indonesia, 26 are rabies-endemic areas, and Bali is one of them (World Health Organization, 2023).

To control rabies in Bali, cross-sectoral coordination has been implemented at both the central and regional levels. Some control and prevention efforts include dog vaccination, sterilization, surveillance, depopulation or culling of stray dogs, and increasing public awareness (Nugroho *et al*., 2013). The World Health Organization (WHO) recommends that the most cost-effective way to overcome the rabies epidemic is to carry out a rabies vaccination program for rabies-transmitting animals (RTAs), such as dogs (Wirawan, 2018; World Health Organization, 2018).

The rabies vaccine is a vaccine used to prevent rabies, especially in animals. This vaccine works by stimulating the body's immune system to produce antibodies that can fight the rabies virus. The rabies vaccine is effective in preventing rabies if given correctly. Errors that can occur in the implementation of vaccination include preparation, administration, monitoring, recording, and transportation and storage (Poiraud *et al*., 2023).

Vaccines, like all biological substan-ces, degrade over time. Exposure to high temperatures, low temperatures, sunlight, or fluorescent light can accelerate this degra-dation process further, which can reduce the vaccine's ability to stimulate the immune system and protect individuals from rabies infection (World Health Organization, 2006).

Previous studies have shown that the length of time the vaccine is stored and the storage temperature can affect the quality of the rabies vaccine (Lankester *et al*., 2016; Lugelo *et al*., 2021). In general, rabies vaccines are stored at 2-8°C to maintain antigen stability (Centers for Disease Control and Prevention, 2021; World Health Organization, 2006). All vaccines will be damaged if exposed to temperatures above +8°C, either if exposed to high temperatures for a short duration or due to slight increases in temperature over a long period (e.g. due to frequent opening of the refrigerator door) (Alders, 2015). In field situations or in areas with limited infrastructure, rabies vaccines often have to be stored at room temperature or even above room temperature. This can potentially damage the rabies vaccine and reduce its effectiveness.

Rabies control programs generally require significant costs in management,

including cold chain logistics. The topography of Bali Province is mostly mountainous and hilly areas covering almost 85% of the area (Handayani *et al*., 2021). The average temperature in 2023 in Bali Province in several regencies according to the Bali Province Central Statistics Agency (2024_{a-d}) is 27ºC - 28ºC. This can limit the use of vaccines in hard-to-reach areas and limit vaccination application strategies in the field. The most commonly used cold chain logistics, such as coolboxes (Alders, 2015) cannot be relied on to store vaccines when the ice pack melts because the coolbox is often opened and closed. Vaccines that are resistant to high temperatures for a limited period of time can reduce this constraint, so that within these limitations, vaccines can be used safely and do not need to be destroyed or discarded.

In implementing the rabies control program, the inactivated rabies vaccine is one of the rabies vaccines used by the government which has a 44% adjuvant and a higher virus concentration than the previously produced vaccine (Daulay *et al*., 2019). The aim of this research is to determine the effect of room temperature storage time of inactivated rabies vaccine on the immune response of BALB/c mice. BALB/c mice were chosen because they are commonly used in immunological studies due to their strong systemic immune response and genetic uniformity (Zhang *et al*., 2023). The authors define the immune response as the antibody titer and the histology of the spleen after vaccination (Wardani *et al*., 2022).

RESEARCH METHODS

Ethical Clearance

This research was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia (Animal Ethics Approval Number: B/109/UN14.2.9/PT.01.04/2024).

Research Object

This study used nine female BALB/c mice aged 20 weeks and weighing around 20-25 g. The mice were kept in three cages (3 mice/cage) with dimensions of 47x33x15 cm. Standard feed and drink were provided *ad libitum*. The choice of using female mice in this study is because female mice are more sensitive to infection than male mice (Klein and Flanagan, 2016). The research samples were divided into three experimental treatment groups with three mice each in each group. The number of samples in this study was based on the number of ELISA kits available in the laboratory.

Research Design

This study was conducted using a completely randomized design (CRD) with a hierarchical pattern, employing BALB/c mice as experimental subjects divided into three treatment groups, each consisting of three mice.

Research Variables

In this study, the variables used are as follows: (i) the independent variable is the storage time of the vaccine at room temperature (25-30℃), which is divided into three treatments: 0 hours (applied directly), 6 hours, and 10 hours; (ii) the dependent variable is the immune response of BALB/c mice, specifically antibody titers and histological features of the spleen; and (iii) the control variables are mice body weight, age, sex (female), feed and water, management of care and environmental conditions.

Data Collection

Vaccines preparation and vaccination. The vaccine used in this research was the inactivated rabies vaccine with three different storage time that were chosen to suit field conditions within a day. The vaccine storage times were as follows: Group P1, vaccine stored at room temperature (25–30℃) for 0 hour; Group P2, vaccine stored at room temperature (25–30℃) for 6 hours; Group P3, vaccine stored at room temperature (25–30℃) for 10 hours.

The 0 hour time is defined as storing the vaccine at the optimal temperature (2-8℃) then applied directly. Before vaccination was given, a serum sample was taken

from each group which was used as a sero-negative control. The mice (BALB/c) were vaccinated after pre-vaccination sampling. Vaccination was applied intramuscularly at a dose of 0.1 mg/10 g body weight.

Blood Sampling Procedure. As much as 0.5–1.0 mL blood samples were taken serially through the orbital sinus before vaccination, and at 2- and 4-weeks after vaccination. Blood were withdrawn after anesthetizing the mice with a mixture of ketamine (Vetalar®, Bohringer Ingelhim, Ingelheim, Germany) xylazine (Xyla®, Interchemia, Castenray, The Netherlands) acepromazine (PromAce®, Bohringer Ingelhim, Ingelheim, Germany) anesthesia at a dose of 0.1 mL/10 g body weight.

Enzyme-linked Immunosorbent Assay (ELISA). The blood sample was centrifuged until the serum was completely separated. The ELISA test procedure (Kit Elisa Rabies Pusvetma™, Balai Besar Veteriner Farma /Pusvetma, Surabaya, Indonesia) involved the addition of 100 µL (in duplicate) positive control serum, ST 1 EU control serum, negative control serum, and diluted serum sample to the ELISA microplate followed by incubation at 37℃ for one hour. After incubation, the microplate was washed with 200 µL PBST 4-5 times. 100 µL of Protein A Conjugate at a dilution of 1:16,000 were then added to the microplate followed by incubation at 37℃ for one hour. Microplate was then washed and 100 µL of substrate solution was added to each well. Microplate was placed in a dark container. After a specific color change occured (± 10 minutes) 100 µL stop solution was added then signal was read using an ELISA reader at a wave length of 405 nm.

Histological examination

In the fourth week of post-vaccination, organ samples were taken from only one mouse in each treatment group. Mice were physically euthanized by cervical dislocation while the mice were still under the influence of anesthesia after blood was drawn. The sacrificed mice were necropsied to take the spleen as an organ sample for histology preparations. The sample is then soaked in 10% Neutral Buffer Formalin (NBF) solution to stop biological processes. The organ samples were made into thin slices to be stored in tissue cassettes and fixed in 10% NBF solution for 24 hours to ensure that the samples were well preserved. After the organ sample was fixed, the next process was dehydration into a series of graded alcohol concentrations (70%, 80%, 90%, 96%, and 100%) for several hours to remove water from the tissue. Tissue sample was then soaked in xylol to remove alcohol from the tissue, then blocked in liquid paraffin. The preparation blocks were then cut using a microtome with a thickness of 3.5 microns. The sample sections are attached to object glasses using a water bath, then incubated for one day at 50℃ to dry the tissue. Tissue preparations were stained with Hematoxylin Eosin (HE) dye to visualize the tissue structure under a binocular light microscope.

Data Analysis

Data for antibody titer were analysed using One-Way Analysis of Variance (ANOVA) followed by Post Hoc-Bonferroni test. Histological analysis of the spleen included examination of lymphoid cells and its follicles proliferation.

RESULTS AND DISCUSSIONS

Antibody Titer

The pre-vaccination antibody titer of mice in all treatment groups was between 0.152-0.281 EU, which was a non-protective antibody titer (Table 1). The antibody titer induced by the vaccine stored at room temperature (25-30℃) for 0 hours (P1) increased in the 2nd and 4th week post-vaccination, although the increase in antibody titer was not significant (p>0.05; One-Way ANOVA) (Figure 1). The antibody titer of mice induced by the vaccine stored at room temperature (25-30℃) for 6 hours

(P2) increased in the 2nd week post-vaccination, although not significant (p>0.05; One-Way ANOVA) (Figure 1). In the 4th week post-vaccination, the antibody titer of P2 treatment group decreased but was not significant (p>0.05; One-Way ANOVA) (Figure 1). In the P3 treatment group (induction by vaccine stored at room temperature (25-30℃) for 10 hours), there was no increase in antibody titer in the 2nd week post-vaccination (Figure 1). Furthermore, antibody titer in the 4th week post-vaccination, although they appeared to increase from the 2nd week, remained below the pre-vaccination antibody titer (Figure 1).

In Table 2, based on the results of the One-Way ANOVA test, the antibody titer data of group P1 (vaccine stored for 0 hours at room temperature) showed a p value of 0.085 (p>0.05; One-Way ANOVA). While the results of the One-Way ANOVA test for the antibody titer of group P2 (vaccine stored for 6 hours at room temperature) showed a p value of 0.141 (p>0.05; One-Way ANOVA). These results indicate that the average antibody titer of mice in group P1 (0 hours) and P2 (6 hours) were not significantly different. On the other hand, the results of the One-Way ANOVA test for the antibody titer data of group P3 (vaccine stored for 10 hours at room temperature) showed a p value of 0.001 (p<0.05; One-Way ANOVA). These results indicate that the average antibody titer of mice in the P3 group was significantly different.

Results of the Post Hoc-Bonferroni test analysis in Table 3 revealed that the trend of antibody titers in P1 (0 hours) and P2 (6 hours) showed no significant changes across all serum collection periods, from week 0 (pre-vaccination) through week four post-vaccination (p>0.05). The antibody titer of week 0 vs. week 2; week 0 vs. week 4; and week 2 vs week 4 in both group P1 and P2 were not significantly different (p>0.05). In contrast, P3 treatment group showed a significant difference in antibody titers from week 0 to week 2 (p<0.05), and from week 2 to week 4 (p<0.05). However, no significant difference was observed between week 0 and week 4 (p>0.05).

Pre-vaccination antibody titer data were used as baseline values to compare changes in antibody titer that occurred after vaccine induction. The minimum protective antibody titer for rabies virus or RABV is 0.5 EU (Cahyana *et al.*, 2021). The pre-vaccination antibody titer analysis data showed that the antibody titer in each treatment group were not significantly different and were non-protective against rabies. This indicates that all mice were in the same conditions and never exposed to rabies antigen. In the 2nd week for treatment group P1 (0 hours) and P2 (6 hours), there was an increase in antibody titer as much as 1.375 times and 1.142 times, respectively (Figure 1). Although the increase in antibody titer is non significant (p>0.05, One-Way ANOVA), it indicates induction of an immune response. Meanwhile, for P3 (10 hours), there was no increase in antibody titer at all, even the value was significantly below the pre-vaccination antibody titer value (p<0.05; One-Way ANOVA) (Figure 1). This indicates that storing the vaccine at room temperature (25-30℃) for 10 hours has the potential to damage the antigenic properties of the rabies vaccine. Temperatures above the cold chain temperature have been shown to affect vaccine potency (Dumpa *et al*., 2019; Lankester *et al*., 2016). Vaccine damage can be caused by storing the vaccine for too long at room temperature or outside the recommended temperature range, so that its potency or efficacy is reduced (Pambudi *et al*., 2022). Vaccine potency will decrease more quickly when exposed to temperatures above the recommended storage range (2-8℃) for too long (Hikmarida, 2014; Kusumadewi and Lestari, 2020).

In week 4, for the P1 treatment group (0 hours), an increasing trend in antibody titer (Figure 1) was still observed although the increase was not significant (p>0.05; One-Way ANOVA). Meanwhile, in the P2 treatment group (6 hours) there was a decreasing trend (Figure 1) in antibody titer and in the P3 treatment group (10 hours) the antibody titer in the 4th week post-vaccination remained below the pre-vaccination antibody titer. Based on the

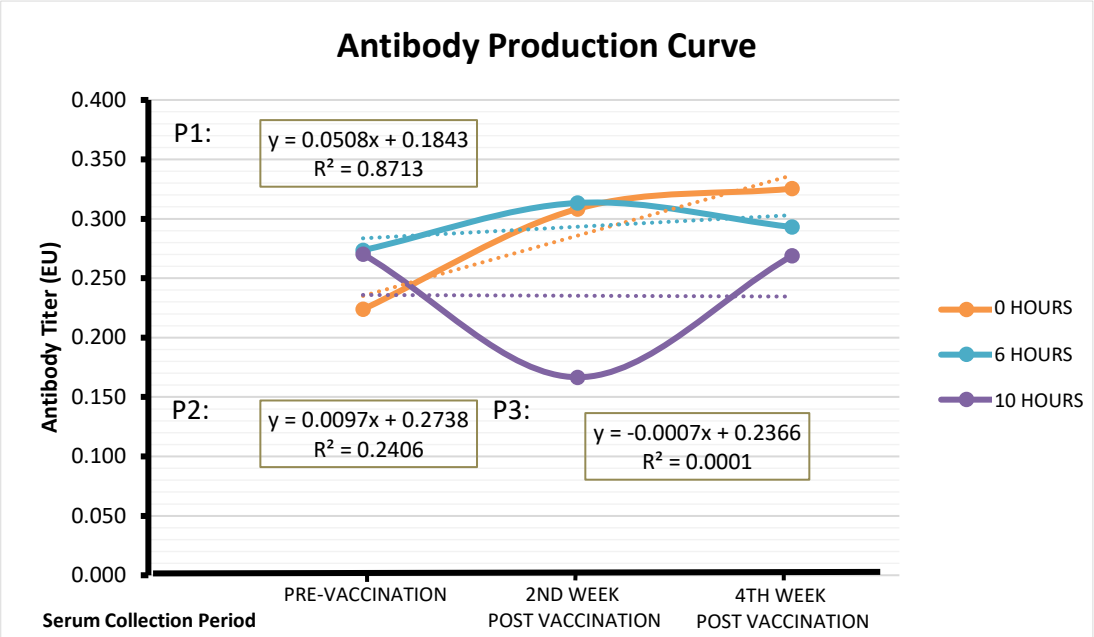

Figure 1. Time course of antibody production

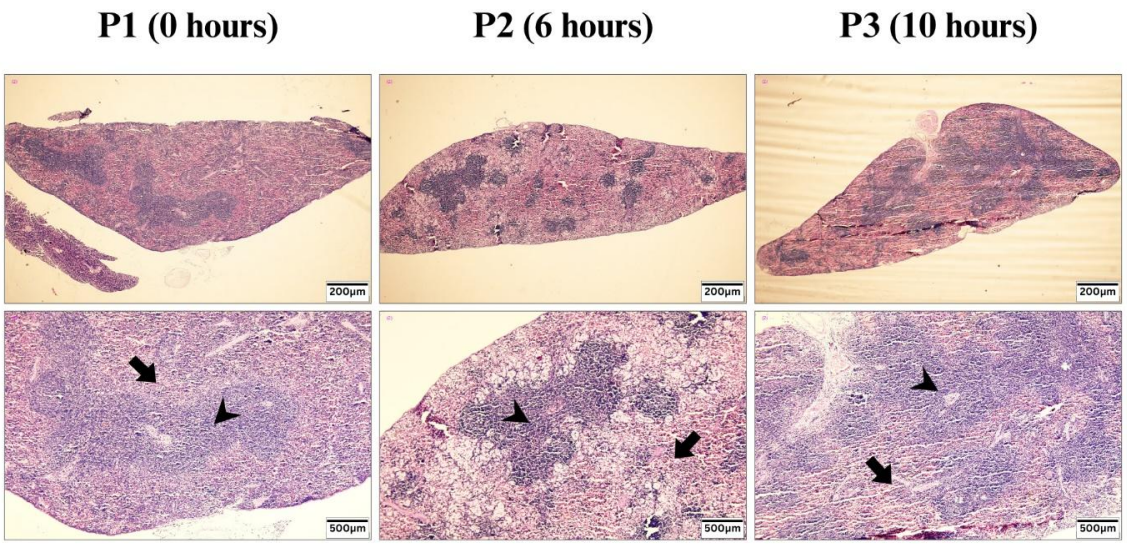

Figure 2. Histological examination of mice spleen in P1, P2, and P3. Spleen tissues were stained with H&E. Upper line displayed at 40× magnification. Bottom line was viewed at 100× magnification. Arrow: red pulp. Arrow head: white pulp and lymphoid follicle.

Table 1. Antibody Titers to Rabies Virus Antigen

Treatment	Mice Samples	Pre-vaccination (EU)	2nd Week Post-vaccination (EU)	4th Week Post-vaccination (EU)
P1 (0 hour)	P1.1	0.152	0.309	0.339
	P1.2	0.253	0.305	0.372
	P1.3	0.267	0.311	0.265
	Mean±SD	0.224±0.063	0.308±0.003	0.325±0.055
P2 (6 hours)	P2.1	0.267	0.314	0.331
	P2.2	0.273	0.323	0.281
	P2.3	0.281	0.303	0.267
	Mean±SD	0.274±0.007	0.313±0.010	0.293±0.034
P3 (10 hours)	P3.1	0.277	0.165	0.269
	P3.2	0.265	0.171	0.271
	P3.3	0.269	0.164	0.267
	Mean±SD	0.270±0.006	0.167±0.004	0.269±0.002

Note: The mean difference is significant at the 0.05 level

Table 2. One-Way Analysis of Variance Test Results

Treatment (Storage time)		Analysis of variance				
		Sum of Squares	df	Mean Square	F	Sig.
0 Hours	Between Groups	.014	6	.002		
	Within Groups	.032	8			
	Total	.002	2	.001	2.763	.141
6 Hours	Between Groups	.003	6	.000		
	Within Groups	.005	8			
	Total	.021	2	.011	571.814	.000
10 Hours	Between Groups	.000	6	.000		
	Within Groups	.021	8			
	Total	.021	8			

Table 3. Post Hoc-Bonferroni Test Analysis Results

Dependent variable Treatment (Storage time)		Multiple comparison (I) Serum collection period (weeks)	(J) Serum collection period (weeks)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
0 hour	Bonferroni		2	-.084333	.039296	.227	-.21352	.04485
			4	-.101333	.039296	.126	-.23052	.02785
			0	.084333	.039296	.227	-.04485	.21352
			4	-.017000	.039296	1.000	-.14618	.11218
			0	.101333	.039296	.126	-.02785	.23052
			2	.017000	.039296	1.000	-.11218	.14618
6 hours	Bonferroni		2	-.039667	.016876	.171	-.09515	.01581
			4	-.019333	.016876	.887	-.07481	.03615
			0	.039667	.016876	.171	-.01581	.09515
			4	.020333	.016876	.821	-.03515	.07581
			0	.019333	.016876	.887	-.03615	.07481
			2	-.020333	.016876	.821	-.07581	.03515
10 hours	Bonferroni		2	.103667*	.003517	.000	.09210	.11523
			4	.001333	.003517	1.000	-.01023	.01290
			0	-.103667*	.003517	.000	-.11523	-.09210
			4	-.102333*	.003517	.000	-.11390	-.09077
			0	-.001333	.003517	1.000	-.01290	.01023
			2	.102333*	.003517	.000	.09077	.11390

Note: The mean difference is significant at the 0.05 level

data from the antibody titer analysis, it was shown that each vaccine treatment in the 2nd and 4th week post-vaccination did not reach a protective titer of 0.5 EU.

In several previous studies, a booster was needed in the 2nd week post-vaccination to increase the titer (Astawa *et al*., 2018).

Histological observations

The spleen is a secondary lymphoid organ that is responsive to antigen stimulation (Arfanda *et al*., 2019). The white pulp in the spleen is one of the parameters for the

formation of an immune response, while the red pulp functions as a filter for the circulation of erythrocyte cells.

The high intensity purple color of white pulp is caused by an increase in the number of lymphoid cells. Lymphoid cells, such as lymphocytes, have nuclei that absorb basophilic dyes very well (Gurina and Simms, 2023). This basophilic dye usually interacts with nucleic acids in the cell nucleus, giving a strong purple color to tissues that have many cell nuclei. The intensity of the purple color in the white pulp indicates the proliferation of lymphoid cells which is a characteristic of an active immune response or increased cellular activity in the area. When lymphoid cell proliferation occurs, the number and size of lymphoid follicles increase causing an increase in the amount of visible white pulp. The results of histopathological analysis in Figure 2 also show that proliferation of lymphoid follicles can be seen from the large number of white pulps.

The results of the histological examination of the spleen organ in Figure 1 show no difference between the three spleens in the treatment groups. Differences in immune response induction cannot be detected or are difficult to see from the HE examination of the spleen organ due to the slight difference in antibody titers.

CONCLUSION

Inactivated rabies vaccine that was stored at room temperature (25-30℃) for 10 hours was not able to induce antibody response in BALB/c mice. Histology examination of the spleen organ was not sufficient to detect any differences in immune responses.

SUGGESTION

By implementing strict cold chain protocols, stabilizers, and real-time monitoring, the efficacy of rabies vaccines can be preserved even in resource-limited settings. The authors recommend avoiding storing the vaccine at room temperature (25-30℃) for a long period of time (> 6 hours). Insulated vaccine containers lined with coolant packs offer portable cooling solutions for field use, effectively maintaining vaccines at 2–8°C during transportation and/or short-term storage to ensure short-term stability.

ACKNOWLEDGEMENT

This research is supported by 2022 International Research Collaboration Funding Scheme Provided by Udayana University Institute of Research and Community Services (Contract Number: B/78. 598/UN14.4.A/PT.01.03/2022 with Dr. Tri Komala Sari as Principal Investigator). We thanked Disease Investigation Center (DIC/ BBVet) Denpasar for histology analysis.

REFERENCES

Alders R. 2015. *Veterinary Cold Chain Manual: Ensuring effective vaccines*. Canberra. Australian Center for International Agricultural Research

Arfanda AI, Suprijatna E, Isroli I. 2019. Effect of Frequencyand Feeding Period of Crossbred Native Chicken on Relative Weight of Limfoid Organs. *Jurnal Sain Peternakan Indonesia* 14(3): 306–311. https://doi.org/10.31186/jspi.id.14.3.306-311

Astawa INM, Agustini NLP, Masa IWT, Aryawiguna IPGW. 2018. Protective antibody response of Balb/c mice to Bali rabies virus isolate propagated in BHK-21 cells. *Journal of Veterinary Medical Science*, *80*(10), 1596–1603. https://doi.org/10.1292/jvms.17-0385

Central Statistics Agency of Bali Province. 2024a-d. Observation of Climate Elements at the I Gusti Ngurah Rai Meteorological Station, Badung Regency. *2022-2023*. BPS Bali. https://bali.bps.go.id/. [25 April 2024]

Cahyana IPAT, Pharmawati M, Narayani I. 2021. Detection of Antibody Levels in Blood Sera of Kintamani Dog

Post Vaccination of Rabies With Direct ELISA. *Metamorfosa* 8(1): 172–177. https://doi.org/10.24843/metamorfosa.2020.v08.i01.p019.

Centers for Disease Control and Prevention. 2021. *Vaccine Storage and Handling.* Atlanta Georgia. CDC US.

Daulay RSD, Pancawidyana D, Aulanni'am. 2019. *Study of Duration of Immunity of Neo Rabivet Vaccine Pusvetma*. Jakarta. Directorate of Animal Health, Indonesian Ministry of Agriculture. Pp. 317–322. https://repository.pertanian.go.id/handle/123456789/8999

Dumpa N, Goel K, Guo Y, McFall H, Pillai AR, Shukla A, Repka MA, Murthy SN. 2019. Stability of Vaccines. *American Association of Pharmaceutical Scientists* (AAPS) *Journal of Pharmaceutical Science and Technology* 20(2): 42. https://doi.org/10.1208/s12249-018-1254-2

Gurina TS, Simms L. 2023. *Histology, Staining*. St. Petersburg, Florida. Stat Pearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557663/?report=classic

Handayani CIM, Rahmaeni ND, Mustafa F, Billah M. 2021.*Food Supporting Capacity Status of Bali Island.* http://ppebalinusra.menlhk.go.id/wp-content/uploads/2022/11/Dokumen-Status-Pangan-Pulau-Bali-2021.pdf

Hikmarida, F. 2014. Relationship Between Storage and Recording with Quality of DPT Vaccine Cold Chain in Puskesmas. *Jurnal Berkala Epidemiologi* 2(3): 380–391.

Ministry of Agriculture (Indonesia), [FAO] Food and Agriculture Organization, & [WAP] World Animal Protection. 2019. *National Masterplan for Rabies Eradication in Indonesia (first).* https://rr-asia.woah.org/wp-content/uploads/2020/03/roadmap-rabies-v05_indonesia.pdf

Klein SL, Flanagan KL. 2016. Sex differences in immune responses. *Nature Reviews Immunology* 16(10): 626–638. https://doi.org/10.1038/nri.2016. 90

Kusumadewi A, Lestari AD.2020. Overview of the Vaccine Cold Chain Management System in Several Subdistrict Health Centers in East Jakarta in 2019. *Medical Sains* 4(2): 153–162.

Lankester FJ, Wouters PAWM, Czupryna A, Palmer GH, Mzimbiri I, Cleaveland S, Francis MJ, Sutton DJ, Sonnemans DGP. 2016. Thermotolerance of an inactivated rabies vaccine for dogs. *Vaccine* 34(46): 5504-5511. https://doi.org/10.1016/j.vaccine.2016.10.015

Lugelo A, Hampson K, Czupryna A, Bigambo M, McElhinney LM, Marston DA, Kazwala R, Lankester F. 2021. Investigating the Efficacy of a Canine Rabies Vaccine Following Storage Outside of the Cold-Chain in a Passive Cooling Device. *Frontiers in Veterinary Science* 8: . 728271. https://doi.org/10.3389/fvets.2021.728271

Nugroho DK, Pudjiatmoko, Diarmitha I, Tum S, Schoonman L. 2013. Analysis of rabies surveillance data (2008-2011) in Bali Province, Indonesia. *Outbreak Surveillance and Investigation Reports* 6(2): 8–12. http://osirjournal.net/issue.php?id=41

Pambudi NA, Sarifudin A, Gandidi IM, Romadhon R. 2022. Vaccine cold chain management and cold storage technology to address the challenges of vaccination programs. *Energy Reports* 8: 955–972. https://doi.org/10.1016/j.egyr.2021.12.039

Poiraud C, Réthoré L, Bourdon O, Lorrot M, Prot-Labarthe S. 2023. Understanding and preventing vaccination errors. *Infectious Diseases Now* 53(2): 104641 https://doi.org/10.1016/j.idnow.2023.01.001

Wardani PIK, Kencana GAY, Winaya IBO, Supartika IKE. 2022. Histopathology of Spleen of Laying Hens Post-Vaccination with Avian Influenza H5N1 Isolates from Bali. *Buletin*

Veteriner Udayana 28. https://doi.org/10.24843/bulvet.2023.v01.i01.p04

Wirawan DN. 2018. Returning Bali Conditions To Rabies Free. *Public Health and Preventive Medicine Archive* 3(2): 90–91.

World Health Organization. 2006. *Temperature sensitivity of vaccines*. Geneva. WHO. www.who.int/vaccines-documents/

World Health Organization. 2018. WHO *Expert Consultation on Rabies, third report*. Geneva. WHO Technical Report Series, No. 1012. www.who.int/bookorders*Enhancing Multisectoral Collabora-tion to Combat Rabies in One Health approach in Bali*. Geneva. WHO. www.who.int. [26 April 2024]

Zhang Q, Zhu W, Zou Z, Yu W, Gao P, Wang Y, Chen J. 2023. A Preliminary Study in Immune Response of BALB/c and C57BL/6 Mice with a Locally Allergic Rhinitis Model. *American Journal of Rhinology & Allergy* 37(4): 410–418. https://doi.org/10.1177/19458924231157619