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The Titer Testing in Post-Vaccination Rabies Immunoglobulin G (IgG) with the Administration of Wild Horse Milk

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Abstract

The occurrence of human rabies in Indonesia approximately amounted to 168 cases. The countermeasures taken were vaccination, quick treatment for individuals, KIE (*Komunikasi, Informasi, dan Edukasi*; Communication, Information, and Education), surveillance, selective dog elimination and post-exposure management. Vaccination was one of the effective countermeasures against the distribution of rabies. It might be conducted either before or after the virus exposure. Despite its effectiveness, it could end in failure due to several factors, one of which was the nutritional status of patient. However, the failure could be overcome by enhancing the immune system (immunostimulator) with the administration of wild horse milk. The milk contained lactoferrin known as protein inducing antibody. Sample consisted of 15 rabbits. They were divided into three groups. Each group consisted of five rabbits. Group t1 for titer testing in immunoglobulin after anti-rabies vaccination; while Group t2 and t0 for titer testing in immunoglobulin after rabies-vaccination and administration of wild horse milk and for the control group without any treatment respectively. The results showed a titer increase in Ig G after vaccination by 40% in Group t2 and t2 ($p>0.05$). Besides, the administration of wild horse milk could increase titer in IgG after vaccination for two weeks ($p<0.05$). In Conclusion, Administration of wild horse milk could increase titer in IgG after vaccination.

1. Introduction

Rabies is caused by the rabies virus, a virus species of *Lyssavirus* genus in the family *Rhabdoviridae*. The neurotropic virus can develop in the nervous tissue. Human can be infected by the virus if bitten by rabid dogs, cats, monkeys or bats **(Johnson, Cunningham and Fooks, 2010; Shi *et al.*, 2018)**. Rabies has spread all over the continents and annually caused 59,000 deaths in over 150 countries. There were 95% of rabies cases in both Africa and Asia **(World Health Organization, 2018)**.

The annual occurrence of human rabies in Asia was as follows: 20,000 cases in India; 2,500 cases in China; 20,000 cases in the Philippines; 9,000 cases in Vietnam and 168 cases in Indonesia. 24 of 34 provinces in Indonesia was endemic; while the ten others were considered rabies-free. According to the data of GHPR (Animal Transmitter Rabies Bite) cases issued by the Ministry of Health, GHPR had increased by 86.3; from 45,466 (2009) to 84,750 cases (2012). The increase was due to KLB (Extraordinary Condition) in Bali in 2009-2012.

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Furthermore, the KLB was a trending topic again in 2019. Rabies endemically occurred in West Nusa Tenggara, specifically in Dompu and Sumbawa. The first occurrence was in Dompu then spread to Sumbawa. According to the findings of epidemiologic investigation by an integrated team from Ditjen P2P Ministry of Health and Ditjen PKH Ministry of Agriculture, there were 192 cases of rabid animal bite and two death cases in human bitten by rabid animals. Meanwhile, according to weekly reports from Health Office and Livestock Service of Dompu, there were 735 cases of rabid animal bite and six death cases in human bitten by rabid animals until the third week in February 2019 **(Indonesian Health Ministry, 2014; Nadine-Davis, 2015; Agustina *et al.*, 2018)**.

Human can be infected by rabies through various ways, particularly when their exposed skin or mucous membrane having contacted with blood contaminated by saliva of individuals bitten by rabid animals **(Zhu *et al.*, 2015)**. Ministry of Health, with the Governments of Dompu and Sumbawa collaborated to overcome KLB of rabies in West Nusa Tenggara. The countermeasures were vaccination, quick treatment for individuals bitten by rabid animals (washing the bitten area using a soap and running water for 15 minutes), KIE, surveillance, selective dog elimination, and post-exposure management for human **(Reni *et al.*, 2010; Agustina *et al.*, 2018)**.

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Vaccination is an effective countermeasure against the spread of rabies. It may be administered either before or after the virus exposure. Rabies vaccine contains inactive virus derived from the continuous cell pathway. The virus is administered in intramuscular or intradermal way in accordance with the protocol recommended by WHO. Recommended pre-exposure vaccination should be administered to health workers, laboratory workers and travelers in endemic areas. Moreover, post-exposure treatment or post-exposure prophylaxis (PEP) is an effective treatment against rabies. PEP contains of vaccination and rabies immunoglobin (RIG) effective to prevent diseases. A successful rabies vaccination indicates antigen and antibody titer formed by a certain product of vaccine induced after inoculation **(Faisal *et* al., 2010; WHO, 2018).**

One of the efforts to minimize any failure in post-exposure vaccination is to administer substances that may increase body immune response, or immunostimulator. We can use healthy food products derived from either plants or animals. For example, milk, having an active component can be regarded healthy due to its nutrition content that prevents and cures diarrhea, impaired mineral adsorption and immunodeficiency. Furthermore, protein in Sumbawa horse milk contains lactoferrin, lactoperoxidase, lysosome and immunoglobin known as antimicrobial protein. Several research found that Sumbawa wild horse milk can serve as an immunomodulator. The milk contains lactobacillus and destroys bacteria cells, enhances immune responses (increasing the IgA and IgG production) and activates macrophage and specific antibody response against foreign antigens **(Faisal *et al*., 2010; Reni *et al*., 2010)**.

The administration of Sumbawa wild horse milk, according to some research *in vivo*, can enhance immunity in Hepatitis A vaccine given to Balb/c mice. It indicates that the milk can increase receptor capacity of macrophage and cytokine production that activates macrophage. In addition, the milk contains lactoferrin that induces the formation of antibody **(Yuki, 1998; Nadine-Davis, 2015)**. The research aims to analyze titer in IgG after the post-vaccination administration of wild horse milk. It also aims to investigate the variation of immune response given by experimental animals administered with anti-rabies vaccine.

2. Material and methods

2.1 Treatments in Experimental Animals

The experimental animals were healthy male rabbits aged seven-eight weeks old (weight of 700-800g). The research had been approved by the Ethic Commission of Faculty of Medicine, Universitas Mataram (Approval Number: 251/UN18.F7/ETIK/2019).

The rabbits were divided into three groups i.e. rabbits without treatment (t0), rabbits with vaccination (t1) and rabbits with vaccination and wild horse milk (t2). The research was performed in six treatment stages i.e.:

Stage 1: acclimatization process in rabbits adjusting to their food, water and laboratory condition for seven days.

Stage 2 : blood sample taking in rabbits before anti-rabies vaccination

Stage 3 : administration of anti-rabies vaccination in the treatment group

Stage 4 : blood sample taking in rabbits after anti-rabies vaccination

Stage 5 : administration of wild horse milk in the group administered with rabies vaccine

Stage 6 : final blood sample taking after the administration of rabies vaccine and wild horse milk

Sample blood taken in each treatment stage was investigated. We analyzed the titer in IgG using ELISA method.

2.2 Anti-rabies Vaccination and Administration of Wild Horse Milk

PVRV (Purified Vero Rabies Vaccine) consisted of dried vaccine in a vial and solvent of 0.5 mL in a syringe. Rabbits in the treatment groups were administered with PVRV of 0.5mL in an intraperitoneal way. Two weeks later, they were administered with wild horse milk. Meanwhile, rabbits in the control group were administered with aquadest and usual rabbit food. Wild horse milk was directly administered. We filled a drink container with the milk in such a way, preventing it to spill out. For the administration, the rabbits were individually caged. It eased our evaluation. They were administered milk of 300 mL/day/rabbit. We took the rabbits' blood through their cubital vain following the administration. The blood sample was frozen for two hours. It was centrifuged at 3000 rpm for ten minutes. The serum was separated for our investigation object. The bled rabbits were killed and buried to prevent the spread of rabies.

2.3 Statistical Analysis

The effect of the administration of wild horse milk on rabbits was analyzed using One Way Anova. The difference between two treatment groups was analyzed using Paired t-test ($p<0.05$).

3. Results

Rabies vaccine was administered to the rabbits in a subcutaneous way. Table 1 shows the result of titer testing in IgG two weeks before and after the administration.

Table 1. Titer Testing in IgG Before and After Anti-rabies Vaccination

Treatment Group	Titer in IgG (IU/mL)		P
	Before Vaccination	After Vaccination	
t0	0.26 ± 0.08	0.24 ± 0.05	0.374^{ns}
t1	0.22 ± 0.04	0.88 ± 0.79	0.128^{ns}
t2	0.20 ± 0.07	0.84 ± 0.89	0.127^{ns}
p	0.420^{ns}	0.279^{ns}	

Data were in forms of mean value and standard deviation.
p : significance value with a 95% confidence level
t0 : normal control group without any administration of vaccine and milk
t1 : vaccinated group
t2 : vaccinated group administered with milk

Table 2. The Result of Titer Testing in IgG After the Administration of Vaccine and Wild Horse Milk

Treatment Group	Titer in IgG (IU/mL)		P
	After Vaccination	Post-vaccination with Milk Administration	
t0	0.24 ± 0.05	0.20 ± 0.07	0.374^{ns}
t1	0.88 ± 0.79	0.90 ± 0.77	0.374^{ns}
t2	0.84 ± 0.89	1.24 ± 1.07	0.037^{*}
P	0.279^{ns}	0.134^{ns}	

Data were in forms of mean value and standard deviation
p : significance value with a 95% confidence level
t0 : normal control group without any administration of vaccine and milk
t1 : vaccinated group
t2 : vaccinated group administered with milk

Table 1 indicates an insignificantly increased titer in immunoglobin G (IgG) ($p>0.05$). The mean of titer in IgG after

vaccination <1.0 IU/mL indicated a negative zero. Group t2 was administered wild horse milk for two weeks. The post-vaccination titer in their IgG was then analyzed.
Group t2 indicated a significantly increased titer in IgG ($p = 0.037$); while Group t0 and t1 did not ($p>0.05$). The mean of titer in IgG after the administration of vaccine and wild horse milk 1.24 IU/mL indicated a positive zero.

4. Discussion

Rabies is a zoonosis disease due to RNA virus from the genus Lyssavirus, the family *Rhabdoviridae*. It attacks the central nervous system of both human and mammals. The primary reservoir of rabies is domestic dogs. Most cases (98%) were triggered by dogs' bite; while other cases were by monkeys and cats'. Unvaccinated patients may lead to death by 100% **(Krebs *et al*., 2003; Johnson *et al*., 2010)**. We used uninfected rabbits in this research. We vaccinated them and gave them milk after the vaccination. We intended to analyze the titer in their IgG based on the capability of the rabies vaccine used and the administration of wild horse milk.
Administration of rabies vaccine aimed to develop the active immune system of rabid patients through the humeral immune system and specific immune system. The first system manifested an antibody that would neutralize viruses existing outside the cell; while the later manifested CTL that would destroy rabid cells. Activation of humeral immune response was started by phagocytizing viruses by antigen presenting cells (APC). Following the process, antigens would be presented to helper T lymphocytes. The cells produced various mediators that would activate cell B to be plasma cells producing antibody. Besides, the mediators also activate other T cell sub-sets to be specific cytotoxic cells **(Aubert, 1992; Salimei and Fantuz, 2013)**. Referring to the findings, there was a titer increase in immunoglobulin G (IgG) two weeks after vaccination. However, statistically speaking, there was no significantly different titer increase before and after vaccination. Such difference was triggered by various factors; such as immune responses to vaccination process emerging seven-ten days after vaccination. The response might lead to the peak on the 28th day after vaccination **(Togawa *et al*., 2002; Salimei and Fantuz, 2013)**. Another factor was that animal condition. The animal condition would give different response to the vaccine. Furthermore, vaccination route, animal's age while being vaccinated, type of vaccine, vaccination schedule and animal's origin and health status were other factors. Unvaccinated normal group indicated a negative zero in all rabbits due to the absence of immune response **(Johnson *et al*., 2010; Shi *et al*., 2018).**
We also figured out that the administration of wild horse milk could increase titer in IgG of group t2 after vaccination significantly ($p<0.05$). The milk served as an immunomodulator due to its lactoferrin glycoprotein important for body immune system. Glycan bound in the milk lactoferrin was complex. We revealed the complexity after checking it in an immunoblotting way by Con-A and WGA lectin labelled using peroxidase. Con-A lectin was specifically bound to high-mannose glycan. Horse milk lactoferrin had a complex glycan. As a result, the lactoferrin could increase immune response. It stimulated the activity of peritoneal macrophage phagocytosis and increased cells producing IgA in the intestinal tissue **(Hurley *et al*., 1993; Kuwata *et al*., 2001; Togawa *et al*., 2002)**. Glycan in horse milk lactoferrin was similar to that in breast milk lactoferrin. The components of the glycan gave implications to the increase in brush border membrane. Due to fragment adsorption, lactoferrin in intestinal cells could pass the non-specific interaction with glycosaminoglycan and be specifically interacted through receptor **(Debbabi *et al*., 1998; Faisal *et al*., 2010; Blaise and Gautret, 2015)**.
Increased titer in IgG after the administration of vaccine and wild horse milk was also caused by protein in milk. The protein underwent an autofermentation process, increasing humeral immune responses. The increase activated specific immune responses. β-lactoglobulin and α-lactoalbumin in milk and lactoferrin served as an antivirus inhibiting virus replication through a special signal in virus cycles **(Bojsen *et al*., 2007; Banyard and Fooks, 2011).** Lactic acid bacteria produced by wild horse milk served as a probiotic, a strong activator for immune response due to its specific molecules on the cell wall surface. The bacteria could affect lymphocytes for immunoglobulin secretion **(Togawa *et al*., 2002; Tang *et al*., 2005; Fotschki *et al*., 2016).**
The results of titer increase in antibody of rabbits administered wild horse milk after vaccination was in line with the findings of other research. The research argued that fermented horse milk increased IgA concentration in the serum by 46.20% and lymphocytes by 95.47%. The horse milk was administered to mice's inmmunoglobuline (IgA) antibody after hepatitis A vaccination **(Kuwata *et al*., 2001; Faisal *et al*., 2010)**. Horse milk could also increase the activity of macrophage in cellular immune in mice against salmonella **(Hurley *et al*., 1993; Togawa *et al*., 2002)**. Furthermore, perioral administration of fermented Sumbawa horse milk could increase the potential of Vibrio cholera conjugated with cholera toxin in term of significant S-IgA immune response induction. The milk protectively prevented liquid secretion in the intestines of Balb/C mice. It indicated that components of Sumbawa wild horse milk could increase the capacity of receptor in macrophages. In addition, the components could also increasingly produce cytokines activating macrophages. Besides, the milk lactoferrin could induce the formation of antibody **(Rupprecht *et al*., 2007; Reni *et al*., 2010; Fotschki *et al*., 2016)**.

5. Conclusion

Administration of wild horse milk could increase titer in IgG after anti-rabies vaccination in rabbits.

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Declaration of interest

The authors report no conflicts of interest.

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