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Anti-rabies in the serum of laying hens after single dose rabies vaccination: An initial observation

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Abstract

Rabies is a strategic infectious animal disease that attacks the central nervous system. It is a serious public health problem in developing countries, especially in Asia. Approximately 59,000 human deaths occur due to rabies annually. In the addition of vaccines availability, development of practical, accurate and affordable rabies diagnostics is also urgently needed. One of them is immunological-based assay, such as ELISA and lateral flow assay, which requires the readiness of rabies antibodies. Recently, IgY produced from laying hens is quite promising. In this study, before producing rabies IgY, we observed the immune response in laying hens that received a single vaccination dose of rabies vaccine. The results of analysis using in-house ELISA showed the presence of antibodies to the rabies vaccine. Whether the antibodies obtained can be used for diagnostic development still needs to be elucidated since the data available in this study is still limited.

Keywords: Antibody; Chicken; ELISA; IgY; LFA; Rabies

1. Introduction

Rabies is a strategic infectious animal disease that affects the central nervous system. The disease is caused by an RNA (ribonucleic acid) highly neurotropic virus in the family *Rhabdoviridae*, genus *Lyssavirus* [1]. It is a serious public health problem in developing countries, especially in Asia. Approximately 59,000 human deaths occur due to rabies annually [2]. Vaccination is the most effective approach to rabies control for both animals and humans. Currently, rabies vaccines are widely available for both animals and humans with adequate quality [3]. However, rabies cases in some countries are still occurring and even increasing. Rabies cases in Indonesia are increasing from a low rabies endemic country to a moderate rabies endemic country. [4]. In cases of human infection, dog bites account for 95% of the virus spread through saliva from dog bites or scratches [5]. The mortality rate can be 100% as soon as clinical symptoms appear [6]. If a practical, accurate and affordable rapid detection kit is available, it is possible to reduce the mortality rate.

Antigen-antibody reaction-based pathogen detection methods such as enzyme-linked immunosorbent assays (ELISA) and lateral flow assay (LFA) are examples that are quite commonly used by the public. These methods are still being developed, including to diagnose the presence or absence of rabies antigens in animal fluids or tissues. These methods require the availability of sufficient rabies antibodies. Generally, the antibodies used are polyclonal and monoclonal antibodies, which are usually produced using laboratory animals such as goats, rabbits, and mice. The use of these animals has raised concerns about the invasive nature of antibody harvesting. For this reason, antibody production using laying hens has become a promising alternative because the antibody is in the form of yolk immunoglobulin (IgY), which in addition to the relatively high concentration of antibodies obtained, it does not require blood collection as in mammals, so it is noninvasive [7], [8].

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In this study, the production of IgY against rabies is reported. The chickens were immunized only once, thereby reducing the suffering of the animals. Before getting to the harvesting of IgY, we analyzed the presence of rabies antibodies in the serum of the hens, four weeks post immunization.

2. Materials and Methods

This study was conducted in accordance with the provisions of the Faculty of Animal Science, University of Mataram in applying experimental animals. Two laying hens of the Hy-Line Brown breed were used. The chickens were vaccinated using one of the commercially available rabies vaccines, with a single dose injected in the neck area.

Four weeks post-vaccination, chicken serum was collected from the wing veins (vena pectorales). The serum obtained was then analyzed using an inhouse ELISA. ELISA plate wells were filled with 100 µl vaccine at three dilutions i.e. 1000-,100-, and10 times dilution in PBS at pH 7.4. Then the ELISA plate containing the diluted vaccine was incubated at 4 °C for 12 hours.

After washing, each well was blocked using 3% skimmed milk in PBS pH 7.4 containing 0.05% tween 20, for 60 minutes at 37 °C. The plate was then washed using PBS pH 7.4 containing 0.05% tween 20 for four washes. After washing, pre- and post-vaccination chicken serum was added to the wells (50 µl/well) according to the treatment. Chicken serum was diluted using sample diluent i.e. PBS pH 7.4 containing 1% BSA for 100 times dilution. After incubation at 37 °C for 60 minutes, each well was washed four times, then 50 µl of secondary antibody (HRP conjugated Goat Anti-Chicken IgY) was added per well. The secondary antibody was diluted 20,000 times according to the manufacturer (Invitrogen, USA). After incubating for 60 min at 37 °C, each well was washed again four times, then 50 µl of TMB substrate per well was added, and incubated in a dark room at 37 °C for 10-15 min. The reaction was stopped using 50µl solution of H2SO4 1N and finally the reaction results were read at 450 nm.

3. Results and Discussion

The main objective of this research is to obtain rabies antibodies from chicken eggs (IgY). The antibodies obtained are planned to be used as material for further research in the development of kits for immunoassay-based rabies antigen detection. Before arriving at this main objective, we first analyzed whether one of the commercially available rabies vaccines, which has been recognized for its efficacy, can trigger an immune response in laying hens. Chickens were vaccinated with a single dose, according to the vaccine manufacturer's instructions. Vaccination was performed without the use of other adjuvants, such as oil adjuvants as is commonly used in vaccination studies using experimental animals. The results of the study are presented in Fig. 1.

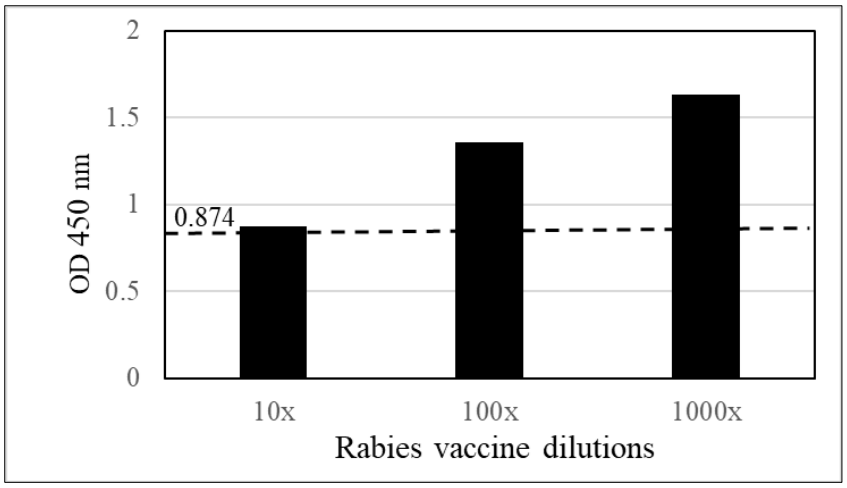

Figure 1 An overview of antibody titer (OD450), as the results of the immunological reaction between chicken serum (100 times dilution) of four weeks post single dose of rabies vaccine immunization and the rabies vaccine coated on the ELISA plate wells at three different dilutions (n=2). The dashed line shows the mean absorbance value of the non-vaccinated chicken serum.

Results presented ini Fig. 1 shows an interesting phenomenon that when the antigen (vaccine) coated on the ELISA plate at a high concentration (10 times dilution), its reaction to the serum of vaccinated chickens is the same as that of non-vaccinated chickens. Antibody titers then increased when the vaccine was diluted 100- and 1000-fold, while the serum was at the same dilution level. This phenomenon is commonly known as the prozone effect due to antigen excess [9]. In addition to the prozone effect, there may be an inhibitory factor derived from the adjuvant in the vaccine we used to make the inhouse ELISA in this study, which may contribute to the suppression of the reaction with the serum of the vaccinated chickens. However, further studies are needed to confirm this conjecture.

Furthermore, if the results in Fig. 1 is observed more closely, the antibody titer of the serum of vaccinated chickens in this study is still not optimal compared to the serum of unvaccinated chickens (calculated based on the cut off value). There are several possibilities that could explain this, including the relatively short period of time for blood sampling i.e. four weeks post-vaccination. It was reported in previous studies that at week fourth post-vaccination, the immune response started to increase [10]. Another possibility is that vaccination with a single dose still needs to be reconsidered, because other studies have reported that an increase in immune response occurs when a booster is given and the antibodies last up to 19 weeks or more [11].

From the foregoing results and discussion, it should be noted here that the present study is still being continued with the repeated immunization format. This will be carried out in parallel with the collection of eggs produced by the vaccinated layers. Immunoglobulin from the eggs (IgY) will be isolated, purified, and further analyzed for its feasibility to be used as raw material for the development of an immunoassay-based rabies diagnostic kit as targeted at the beginning of this study.

4. Conclusion

Antibodies to rabies were detected in the serum of laying hens at four weeks post single immunization using one commercially available rabies vaccine. Antibodies produced were still not optimal. Observations are continuing with a repeated vaccination format, with a harvest period of more than four weeks. The use of other rabies vaccines that commercially available should also be investigated.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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