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An adult male ebony leaf monkey (*Trachypithecus auratus auratus*) in the West Bali National Park (photo by N. Gunst)

PEMIMPIN UMUM : I WAYAN BATAN **DEWAN REDAKSI** : NYOMAN MANTIK ASTAWA (KETUA), IDA BAGUS ARKA, NYOMAN SADRA DHARMAWAN, IWAN H. UTAMA, I GUSTI NGURAH KADE MAHARDIKA, I KETUT PUJA, I KETUT SUATHA, TJOK GDE OKA PEMAYUN, ROOSTITA L. BALIA, I KETUT BERATA, **REDAKTUR PELAKSANA** : I NYOMAN SUARTHA & I G M KRISNA ERAWAN, **SEKRETARIS REDAKSI** : I NYOMAN SUARSANA, **STAF REDAKSI** : I WAYAN SUARDANA, I GUSTI NGURAH SUDISMA, AIDA LOUISE TENDEN ROMPIS, NI GUSTI AGUNG AYU SUARTINI, I MADE SUKADA, ANAK AGUNG SAGUNG KENDRAN, ANAK AGUNG AYU MIRAH ADI, I MADE KARDENA, YANA QOMARIANA. **TATA USAHA** : PUDJI RAHARDJO, WERDI SUSARI. **KANTOR REDAKSI & ALAMAT SURAT** : KLINIK HEWAN FKH UNUD, Jl. Raya Sesetan Gg. Markisa 6 Banjar Gaduh, Denpasar - Bali. **PENERBIT** : FAKULTAS KEDOKTERAN HEWAN, UNIVERSITAS UDAYANA BEKERJA SAMA DENGAN PERHIMPUNAN DOKTER HEWAN INDONESIA. **REKENING** : NOMOR 0118628705 A/N drh. I NYOMAN SUARTHA, M.Si BNI CABANG DENPASAR **DICETAK OLEH**: PERCETAKAN PELAWA SARI, JL. ANTOSURA 33 DENPASAR

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Enzyme Linked Immunosorbent Assay Test for Antibody of Classical Swine Fever Virus In Timor-Leste

UJI ENZYME LINKED IMMUNOSORBENT ASSAY TERHADAP ANTIBODI DI VIRUS CLASSICAL SWINE FEVER DI TIMOR-LESTE

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ABSTRAK

Telah dilakukan penelitian yang bertujuan untuk mengevaluasi pelaksanaan vaksinasi penyakit Classical Swine Fever (CSF) pada babi di Timor-Leste. Penelitian dilakukan dengan menganalisis persentase antibodi CSF pada serum babi yang diperoleh dari babi-babi di empat distrik yang terletak di bukit dan pesisir Timor-Leste. Evaluasi pelaksanaan vaksinasi juga dilakukan dengan mengamati faktor yang dominan yang berpengaruh terhadap peningkatan persentase antibodi pada serum. Sebanyak 240 babi diambil serum sebelum dan setelah vaksinasi diperiksa terhadap adanya antibodi CSF menggunakan PrioCheck CSFV Ab ELISA kit (Prionics Ag). Serum yang diperiksa terdiri atas 240 serum dari babi yang tidak divaksinasi dan 240 serum dari babi yang sama setelah diberi vaksinasi, menggunakan vaksin CSF. Interval pengambilan serum pertama dengan pengambilan serum kedua minimal 14 hari pasca vaksinasi. Hasil penelitian menunjukkan adanya perbedaan yang sangat nyata ($P < 0,01$) terhadap adanya antibodi pada babi yang divaksinasi dengan yang tidak divaksinasi. Sebanyak 75% serum babi dengan status divaksinasi, terdeteksi antibodi positif CSF, sementara hanya 16,7% serum babi dengan status tidak divaksinasi terdeteksi positif antibodi. Dari uji odd ratio, diketahui bahwa di antara faktor umur babi, jenis kelamin babi, letak geografis, dan status vaksinasi, ternyata yang paling berpengaruh terhadap peningkatan persentase antibodi CSF adalah faktor status vaksinasi.

Kata-kata kunci: CSF, Vaksinasi, ELISA

ABSTRACT

The objective of this study was to evaluate the implementation of Classical Swine Fever (CSF) vaccination on pigs in Timor-Leste. The study was conducted by analyzing the percentage of CSF antibody in pigs sera that obtained from pigs in four districts which were located in the hills and coast of Timor-Leste. Evaluation was also carried out by observing the dominant factor that affecting the increase of antibody titers in the sera. A total of 240 pigs sera were taken before and after vaccination and then checked for antibodies against of CSF virus by using PrioCheck CSFV Ab ELISA kits (Prionics Ag). Two hundred and forty serums obtained from non-vaccinated pigs and 240 other serum obtained from the same pigs, after being vaccinated with CSF vaccine. Time interval from the first and the second serum collection was at least 14 days post-vaccination. The results showed there was a significant difference ($P < 0.01$) for the presence of antibody in vaccinated pigs compared with the unvaccinated. A total of 75% serum from vaccinated pigs was found positive for the antibody containing, while only 16.7% of serum from non-vaccinated pigs was positive. The odd ratio analysis showed that the most influential factor for the increase of antibody titer against CSF virus was vaccination status. among the other factors of age, sex and geographical study.

Keywords: ELISA, Classical Swine Fever Virus, Antibody

INTRODUCTION

Pigs have a high significance and benefits for Timorese people in terms of cultural values. Actually people using pigs as a symbol of cultural function in the society including ceremonial events, such as: weddings, funerals, birthdays and holiday celebration. Pigs population in Timor-Leste was reported as much as 330 455 heads, spread across the district (NSD and UNFPA, 2011). The population of pigs will continue to improve, so it can be help to increase the income of Timorese people in terms of tackling protein deficiency and food insecurity.

One of the diseases in Timor-Leste often caused large losses is Classical Swine Fever (CSF). Classical Swine Fever vaccination program in Timor-Leste conducted annually and needs to observe or evaluate regularly in order to determinate the effectiveness of vaccine that used to increase protective antibodies against CSF disease. To make sure the type of vaccine used is suitable or unsuitable with subtype of CSF virus in the field, therefore it needs to observe the antibody through ELISA test.

The success of vaccination program is determined by formation of protective antibody in pigs (Ratundima *et al.*, 2012). The percentage of the protective antibody influenced by many factors, such as: the type of antigen in the vaccine (Guo *et al.*, 2011), integrity of the vaccine, age of pigs when vaccinated (associated with maternal antibody), application of vaccination by officers, environmental conditions and pig health condition (Moening, 2000).

Type of CSF vaccine currently used in Timor-Leste is SF Swine Fever Live Vaccine

Strain GPE Negative (Malaysia Vaccines and Pharmaceuticals (MVP)). Detection of antibodies in serum can be used in various ways, such as: polymerase chain reaction (PCR) (Paton *et al.*, 2000); virus neutralization (VN) (Sarosa *et al.*, 2004); fluorescent antibody test (FAT), and enzyme-linked immune sorbent assay (ELISA).

RESEARCH METHOD

Sampling

Serum samples of 240 pigs were collected two times, before and after CSF vaccination. In total 480 serum samples were evaluated. Time interval of samples collection between first and the second time serum collection done at least 14 days. The geographic of pig sera samples was located in coastal and hill, spread over at four districts, namely: Aileu, Baucau, Liquica and Manatuto District. These locations are located in the territory of Timor-Leste. The number of pig's serum samples were taken based on vaccination status, sex, age and geographical location (Table 1).

Purposively, the serum samples were taken from pigs that kept free and allowed by the owners to take their pigs' blood. The blood was taken from jugular vein by using syringe sized 5 mL, before it then was prepared for serum processing. The samples of blood were inserted into the tube of reaction and stored at room temperature during one hour with the horizontal position. Next, blood incubation performed during 24 hours at 4°C of temperature until its formed serum.

The period for serum sample collection was done in May to June 2013. Each of the

Table 1 Distribution of unvaccinated and vaccinated of pig's serum samples located in the coast and hills of Aileu, Baucau, Liquica and Manatuto Districts, Timor-Leste.

Type of Pigs	Sex	Level of Age	Coast		Hills	
			Non vaccination	Vaccination	Non-vaccinatttion	Vaccination
Local	Male	0-6 months	30	30	30	30
		> 6 months	30	30	30	30
	Female	0-6 months	30	30	30	30
		>6 months	30	30	30	30
number			120	120	210	120
Total						480

sera was subsequently kept by putting them into a micro tube sized 1.5 mL. Furthermore, the sera were stored at -18°C of temperature before they were ready to be ELISA tested for antibody against Classical Swine Fever virus.

The PrioCHECK® CSFV Ab was used as the material kit for detection of antibody against the high, medium and low strains virulence of CSFV infection. Detection of virulent strains for CSFV antibodies is very important to evaluate in order to trace the subclinical infection of CSFV. The reagents used in PrioCHECK® CSFV Ab, was a monoclonal antibody (mAb's) against different epitopes on the E2 envelope protein (GP-55) of CSFV.

The PrioCHECK® CSFV Ab test procedure is catagorised with high sensitivity and specificity. The ELISA kits consisted of a microplate, conjugate, dilution buffer, antigens, demineralized water, washing fluid, serum reference 1, reference serum 3, chromogram TMB substrate, stop solution, and distilled water. The ELISA test also equipped with shaker, micro pipettes, tips, container / trough Elisa, Elisa reader and Falcon tube.

Sample Testing Procedure

As much as 20 µL sampel solvent was added into wells of the microplate. The negative control serum 80 µL was added into A1 and B1 wells of the microplate. Similarly, 80 µL of the low positive control serum was added into wells C1 and D1 as well as the positive control serum with the sam volume was also added into E1 and F1 wells. Then, the serum samples was added into other wells with 80 µL volume for each. The Microplate was covered and stirred slowly to mix the solution in the wells, then incubated for 60 min at 37°C of temperature. After that the microplate was washed with washer buffer six times. Followed by adding the conjugate 100 µl into all of the wells. The microplate then was incubated for 30 minutes at 37°C of temperature. Next, 100 µL of chromogen (TMB) was poured

into the microplate wells before it incubated again for 20 min at room temperature. A 100 µl of stop solution was then added into the wells and sifted until they evenly mixed. The optical density (OD) of each wells was measured at a wavelength of 450 nm on a maximum period of 15 minutes after adding the stop solution. The average value of OD450 of the negative control serum (OD max) was counted. The percentage of inhibitor (PI) of the weak positive control serum positive control serum and serum samples was counted by using the following formula:

$$PI = 100 - \frac{[OD_{450} \text{ sample test}]}{OD_{450} \text{ max}} \times 100$$

Test Validation :

1. Average OD 450 value of the negative control serum should be > 1.0
2. Percentage of weak positive serum control inhibition should be > 50%
3. Percentage of positive serum control inhibition must be > 80%

Interpretation of result : If the percentage inhibition of serum Elisa test was less than to 40%, then the result was catagorised negative. However, if the percentage inhibition of serum Elisa test was greater than or equal to 40%, then the result was positive contained the antibody.

RESULTS AND DISCUSSION

Based on the results of Elisa test for pigs sera, the percentage inhibition of CSF antibody of unvaccinated and vaccinated pig sera is shown on the comparison results of PI values (Table 2). The percentage of inhibition of unvaccinated pigs sera antibody against CSF virus showed the PI value was <15.47%. While the percentage of CSF antibody inhibition in the sera of vaccinated pigs was > 60.96% of PI value. Thus, the PI value between the groups of

Table 2 The Percentage Inhibition PI value between groups of non vaccination and vaccination pigs.

Pig group	Mean (%) / PI	Sig (2 –headed)
PI non vaccination	15.47	
PI vaccination	60.96*	0.000

Explanation: Signal * means the relationship between two variables significantly different (P<0.01).

vaccination and unvaccination pigs, showed a significant difference .

The status of vaccination was defined which was unvaccinated or vaccinated group in the sample pigs. A chi-square test was used to statistically analyze the percentage of CSF antibodies on unvaccinated and vaccinated pigs (Table 3).

The pig group was vaccinated using SF Swine Fever Vaccine Live GPE Negative Strain vaccine in the research. The Elisa test showed that the percentage of protective antibody was 75%. Vaccination is given to stimulate the immune system to produce antibody against CSF virus, The antibody can be detected in the vaccinated pigs. Vaccination is employed in many countries to prevent outbreaks and has been used effectively in systematic and consistent programs to reduce disease outbreaks to a point where eradication measures are feasible (Douglas, 2002). Although the epidemic situation can be effectively controlled by vaccination, it is difficult to eradicate it by using live vaccine due to multiple factors, such as: proper implementation of vaccination regimens, interference of maternal antibody and virus persistence (Sheu et al., 2006; Chen et al., 2012). The effectiveness of vaccination can be determined from the existence of antibody detection against CSF virus in serum (Ratundima et al., 2012).

The group of unvaccinated pigs showed 16.7% percentage of protective antibody. This indicates that, there was a natural immune on the pigs due to natural infection or pigs had maternal antibody (Van-Oirschot, 2003). Antibody cannot be detected in certain titer because of in beginning incubation period infection or due to immunosuppressive effect of

the CSFV. However, it will surely detected in at least 21 days post-infection. Antibodies may not be detected in natural infection occurred, and possibility caused by a low virulence virus (Saroso et al., 2004).

The percentage of undetected antibody in unvaccinated pig sera was 83.3%. In free of CSF disease countries or in the area where the eradication is in progress, vaccination is normally prohibited. The serological diagnosis of CSF is important to be done for disease surveys and the detection of hidden clusters of CSF (Moenning, 2000). Detection of antibody against CSFV depends on two factors: the acute form of CSFV infection, which is characterized by short incubation period of infection and disease courses. In this condition the infected pigs may have no produced detectable serum antibody. Additionally, the low virulence strains generally produce long incubation period and diseases courses, in which the infected pigs tend to develop the specific serum antibody (Ayala et al., 2008).

The percentage of negative detected antibodies in vaccinated pig sera in which they had been vaccinated with SF Swine Fever Vaccine Live GPE Negative Strain, was observed 25%. The negative antibodies sera in vaccinated pigs can be influenced by various factors, such as: maternal antibodies, environmental stress, infectious diseases, age, and in condition of malnutrition (Moenning, 2000). On the other hand, since CSFV vaccination strains are harmless, the also the production of specific serum antibodies particularly without clinical sign (Ayala et al., 2008). Failure of vaccination program can also happen. The failure can be caused by the vaccine was not able to protective antibody. In some cases, the applied vaccines did not reach protective antibody level although its

Table 3 Elisa test results of percentage antibody inhibition against CSF on unvaccinated and vaccinated pigs' sera .

Pig Vaccination Status	Vaccination Status (% CSF antibody)		Sub Total Sample	Total Sample
	Negative Antibody	Positive Antibody		
Non Vaccination	83,3 ^a (200/240)	16,7 ^c (40/240)	240	480
Vaccination	25 ^b (60/240)	75,0 ^d (180/240)	240	

Explanation: Different letters between columns and rows indicate highly significant relationship (P< 0,01).

already used the recommended vaccine dose . Another cause of vaccination failure occur is depressed immune response in the animal when the vaccination applied, for example the vaccination is given when the animal is in a severe parasitic infestations, malnutrition or stress (Moenning, 2000).

Another factor that may cause is if the strain antigen in vaccine used, which results in specific antibody, but its not matched with the new infected antigen (Ratundima et al., 2012). The failure of vaccination can also be caused by improper dosing or partially spilled dose vaccine applied when the applicator injected the vaccine. This can result in lack of protective antibody release. The cold change problem in handling the vaccine, for example the temperature is fluctuated during vaccine storage and its transport can affect the antigen stabilization in the vaccine. Therefore, it may not trigger the specific antibody. CSF vaccine from MVP Malaysia requires optimum storage temperature ranged from 2- 6°C (RDPIII / MAF, 2013).

Most of the information about the immunological mechanisms that lead to protection against CSF infection comes from experiments by using the modified strain-C live vaccine from China. The strains-C live vaccine, in general is safely accepted in all age pigs in providing complete protection by protective antibody level in vaccinated pigs. The strain-C of live vaccine induces neutralizing antibody, which are generally detected in vaccinated pigs aged 2-3 weeks after primary vaccination (Suradhat et al., 2007). Vaccinated pigs with strain-C live vaccine, seems to be completely protected against virulent of CSFV by at least one week post vaccination challenge (Van Oirschot, 2003).

Timor-Leste is one of the developing countries in Asia, which relies on economic resilient community in raising pigs. The outbreak of Classical Swine Fever (CSF) disease, or known as Hog Cholera, can damage economic sector in a country. Pigs are the only species that is susceptible to CSF virus, and infected pigs are a source of transmission to the other healthy pigs (Wirata et al., 2010). Some actions need to be done on CSF endemic area to reduce the related losses. One program that is believed can minimized the risk of outbreak is conducting vaccination (Saatkamp et al., 2000; Edward et al., 2000). The purpose of the vaccination is not only to protect the animals from severe clinical

signs of disease, but also to prevent infection, multiplication, excretion of the virus and able to prevent viral replication in the tonsils or hole body of the swine (Moenning, 2000).

CONCLUSION

Comparison of PI values between unvaccinated and vaccinated pigs showed that the PI values of the protective antibody is $\geq 60.96\%$. Positive antibodies in unvaccinated pigs compared to vaccinated pigs shows highly significant difference. The prevalence of positive antibodies in the group of unvaccinated pigs was 16.7%; while in the vaccinated pig group was 75% ($P < 0.01$).

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