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Research Articles

Herpesviridae identification method on Ruminants through Molecular Method in Lampung Disease Investigation Center

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ABSTRACT: Farming is one sector that has an important role in building Indonesia's economic growth, especially for daily food. Farm production is facing challenges, include the low production yield, traditional management, and problems related to animal health. Infectious Bovine Rhinotracheitis (IBR) and Malignant Catarrhal Fever (MCF) are diseases caused by Herpesviridae. These are high mortality rate disease cause huge economic loss. Farms located nearby deer captivity increases the risk of deer to be infected by the same diseases. Surveillance is needed to discover the number of infected animals and their effect on the environment. Herpesviridae identification in surveillance was done through an accurate method, e.g. a molecular method. This research is done by Lampung Disease Investigation Center and funded by the Food Agriculture Organization (FAO). Herpesviridae identification was done in three steps, DNA isolation, Polymerase Chain Reaction (PCR), electrophoresis, and visualization. @2020 Published by UP2M, Faculty of Mathematics and Natural Sciences, Sriwijaya University

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INTRODUCTION

The farming sector is one of many sectors that has an important role in economic growth because the demand of livestock increases along with an increase in population income. It means food consumed by people can be determined by their income. People with lower incomes tend to consume more carbohydrate whilst people with higher incomes tend to consume more protein. Therefore, troubles in the farming sector are not to be trifled with [1].

There are some problems related to livestock production in Indonesia, such as the long production period and the use of traditional management systems [2]. Besides, health-related problems are also becoming obstacles in the farming sector [3].

Herpesviridae is a family of viruses that causes various disease [4]. Infectious Bovine Rhinotracheitis (IBR) is one of the diseases caused by Herpesviridae in ruminants. That disease is a deadly disease due to its morbidity rate that can reach 100% and its mortality rate can reach 10% [5]. Besides IBR, Malignant Catarrhal Fever (MCF) is also another deadly disease. The mortality rate can reach 95% in a clinically infected animal [6].

Surveillance has become one of the important technical aspects in making efforts to prevent and control diseases of animal origin. Surveillance is done to detect the existence of a disease in a certain area and following up on the result [7]. It is stated that surveillance result is important for knowing the number of infected animals and its risk towards human and wildlife [8].

Lampung, as the connector of Sumatera and Java, has a high level of urbanization and human mobility. Dense population settlement and the existence of farms nearby deer captivity rise the potential of the virus transmission from livestock to wildlife. Therefore, this surveillance was done in ruminants, including cows, buffalos, goats, sheep, and deer [9].

Since 1970, disease identification in organisms can be done through the molecular method which is based on molecular biology. Identification can be done on a particular gene or protein, giving results with higher accuracy. Thus, virus identification through the molecular method becomes the best method used [10].

Herpesviridae identification method in Lampung Disease Investigation Center is based on predict protocol made by the Food and Agriculture Organization (FAO). This research was done to monitor interactions between livestock and wildlife. It is aimed to find out earlier the virus that can become an emerging infectious disease (EID) that can potentially affect wildlife.

METHODS

Herpesviridae identification is a research held by the Lampung Disease Investigation Center, funded by the Food Agricultural Organization (FAO), as a form of surveillance. The identification was done through three steps, DNA isolation, Polymerase Chain Reaction (PCR), and electrophoresis and visualization. The identification method is based on the Laboratory Protocols for PREDICT II Surveillance Version 1 by United States Agency International Development (USAID).

RESULTS AND DISCUSSION

DNA isolation is done inside the biosafety cabinet (Figure 1). The procedure was based on QIAamp® DNEasy Blood and Tissue Kit catalog number 69504. Conceptually, DNA isolation is done through four steps, namely lysis, binding, washing, and elution [11].

Figure 1. DNA isolation in Biotechnology Laboratory, Disease Investigation Center, Lampung.

Lysis is done by inserting 20 µl proteinase K and 200 µl buffer AL into the microtube. Then,

200 µl of the sample is added. The solution is homogenized through vortexing and incubated for 10 minutes at 56°C. Incubation is done to denature proteins, catalyzed by proteinase K.

Binding is done by adding 200 µl absolute alcohol. The solution is then homogenized through vortexing for 1 minute and transferred into the spin column. The solution is centrifuged at 8000 rpm for 1 minute.

Washing or precipitation is done twice by using buffer wash buffers, mentioned as buffer AW1 dan buffer AW2. First precipitation is done by inserting 500 µl buffer AW1 into the spin column. The solution is centrifuged for 1 minute at 8000 rpm. Collection tubes are replaced with the new ones after the process is done. Second precipitation is done with the help of 500 µl buffer AW2 and centrifuge is done for 3 minutes at 14000 rpm. Then, collection tubes are replaced with microtubes.

The last step, elution or purification, is done by pipetting 100 µl buffer AE into the spin column. The solution is then centrifuged for 1 minute at 8000 rpm. The spin column is removed and the microtube containing DNA from the sample is saved in the freezer at -20°C.

Polymerase Chain Reaction (PCR) is the process aimed to amplify a certain sequence of DNA. The DNA needs to be amplified to increase the quantity to detectable levels. Amplification is done through three steps, namely master mix composing, template addition, dan running PCR.

Master mix composing is done in the PCR work station (Figure 2). It is the process of preparing materials needed for PCR, consist of:

1) Amplification kit

The amplification kit used in this research is made by bioline (MyTaq™ HS Red Mix catalog number BIO-25047). It consists of an enzyme named Taq Polymerase that has a role in amplifying DNA, magnesium chloride ($MgCl_2$) as the cofactor of Taq Polymerase, and deoxynucleotide triphosphate (dNTP), which acts as a building block for newly synthesized DNA molecules.

2) Nuclease free water

The water used to dilute master mix concentration and prevent DNA denaturation by nuclease. Even though it is mentioned separately, it is included in the amplification kit.

3) Primer

The sequence of nucleotides used for initiating the amplification process. There are two primers used, forward and reverse primer.

Figure 2. Master mix composing in Biotechnology Laboratory, Disease Investigation Center, Lampung.

Nested PCR technique is used in Herpesviridae identification. It is an amplification technique that uses two sets of primers. The first set, called an outer primer, will anneal out of the DNA target, amplifying a longer fragment. Unlike the first set, the second set, called an inner primer, will anneal in the DNA target. Nested PCR is done to decrease nonspecific PCR products. This method is done through the two-phase of amplification [12].

Due to the use of nested PCR technique in Herpesviridae identification, there are differences in master mix composition and primer sequences. Master mix composition in the first phase consists of 12.5 µl amplification kit, 1 µl forward primer, 1 µl reverse primer, and 5.5 µl nuclease-free water in each tube. The sequence of nitrogen bases of the first set of primers is as follows (Table 1).

Table 1. Nitrogen base sequence in the first set of primer for herpesviridae.

Forward	5'-TTGTGGACGAGRSIMAYTTYAT-3'
Reverse	5'-ACAGCCACGCCNGTICCIGATGC-3'

Master mix composition in the first phase consists of 20 µl amplification kit, 1.5 µl forward primer, 1.5 µl reverse primer, dan 12 µl nuclease-free water in each tube. The sequence of nitrogen base of the second set of primers is as follows (Table 2).

Table 2. Nitrogen base sequence in the second set primer for Herpesviridae.

Forward	5'-GCAAGATCATNTTYRTITCITC-3'
Reverse	5'-TGTTGGTCGTRWAIGCIGGRT-3'

The amount of master mix composed is usually not the same as the amount of the sample. The excess of the master mix is aimed as control. The control has an important role in herpesviridae identification. There are three controls used in PCR, those are:

1. Positive control that contains the genetic material of the identified organism (positive sample).
2. A negative control that doesn't contain the genetic material of the identified organism (negative sample).
3. Internal control contains the master mix only.

The composed master mix will be mixed with the sample through add template process. This process is done in a different room with master mix composing. Next, running the PCR is done. It is the process of running the PCR in a thermal cycler. This process is begun by setting the PCR cycle for Herpesviridae, which is Cycle 1 (1x): 94°C 2 mins, Cycle 2 (45x): Step 1: 94°C 30 sec, Step 2: 48°C 1 min, Step 3: 72°C 1 min, Cycle 3 (1x): 72°C 7 mins, Cycle 4 (1x): 4°C. These settings are used for both amplification phases. This amplification program is based on predict protocol by Food and Agriculture Organization (FAO).

Electrophoresis and visualization are done after running the PCR. Electrophoresis is the process of separating a compound based on its size with the help of electric current. To separate the DNA with gel electrophoresis, the DNA is inserted into the well in the gel and electrified [13].

Electrophoresis is begun by making 1% agarose gel. 1.5 mg of agarose gel powder is dissolved in 150 ml TAE, while heated inside the microwave for three minutes. Then, 12 µl SYBR® safe DNA gel stain is added. The solution is shaken to be homogenized. Once homogenized, the solution is poured into a casting tray that has a comb. The solution is cooled down to harden. The hardened agar will have wells made by the comb.

A hardened agar is put into the chamber. Then, 6 µl sample is inserted into the well in the agar. Next, the camber is connected to a power supply. Electrophoresis is run for 30 minutes with the voltage of 100 V and the current of 300 A. The result of electrophoresis will be visualized under UV light and captured with a camera that has been connected to a computer through an application called EOS Utility (Figure 3).

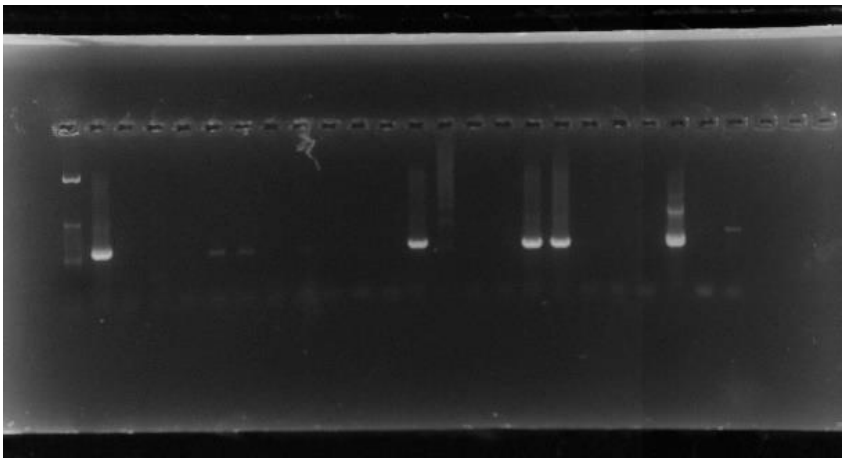

Figure 3. Electrophoresis result of Herpesviridae.

After the result is visualized, the bands in the image are observed. A sample is stated presumptive positive if the band is located in the same line compared to the positive control. The negative sample will not have the band in the same line compared to the positive control. If a band exists in internal control, it is concluded that there is contamination during PCR.

CONCLUSIONS

Identification of Herpesviridae using the molecular method is done through three steps, namely DNA isolation, PCR, and electrophoresis, and visualization. The existence of band on the gel after electrophoresis shows that this method is effective to identify herpesviridae virus from the samples.

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