



The Essential Contribution of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park for Wildlife Genetics Conservation

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Abstract :

Way Kambas National Park (WKNP) is home of five protected big mammals including sumatran elephants. It shares its border with 22 of 37 villages surrounding the national park. Understanding their existence in the wild is a priority, and wildlife genetics is a crucially needed. Besides poaching and habitat fragmentation, wildlife-human conflict is one big issue. Elephant Training Center (ETC) in WKNP is built for semi in-situ conservation effort on captive sumatran elephants that mainly have conflict histories with local people. Participative observation and bio-molecular analysis were conducted to learn the importance of captive Sumatran elephant for conservation effort. Through captive sumatran elephants, database and applicable methods are expected to be developed supporting the conservation of their population in the wild. Participative observation and molecular identification was carried on captive sumatran elephants in ETC, WKNP under multiple year Terapan grant of Ministry of Research and Technology Higher Education, Indonesia. Gene sequence and cytological analyses showed that the captive sumatran elephants are closely related and tend to be domesticated. Translocation among ETC to avoid inbreeding, and maintaining the captive sumatran elephant as natural as possible are highly recommended. Developing genetic database can be a reference for both captive and wild sumatran elephants.

Keywords: captive Sumatran elephant, Elephant Training Center, Way Kambas National Park, genetic conservation, wildlife

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1. Introduction

Way Kambas National Park (WKNP) is the natural habitat of the Sumatran elephant (*Elephas maximus sumatranus*). Since 2011 the presence of Sumatran elephants has been recorded in critical status in the International Union for Conservation of Nature and Natural Resources (IUCN) [1]. This is caused by the pressure of habitat fragmentation, illegal hunting and conflicts with humans [2].

Efforts to maintain the existence and continued preservation of Sumatran elephants, such as monitoring, mapping Sumatran elephant range areas and capacity analysis Sumatran elephant habitat in order to survive in

the area adjacent to the human activity. The Elephant Training Center (ETC), WKNP was established with the aim of rescuing Sumatran elephants and supporting the efforts of semi-situ conservation of Sumatran elephants that are in conflict with humans. Currently there are 69 Sumatran elephants built with 39 individual males, 26 females and 4 tillers individual. The existence of the Sumatran elephant built in ETC, WKNP raises the question of being limited population movements, their role in conservation efforts and the possibility of release as well as wildlife attractions.

The presence of elephant populations in ETC, closed WKNP is very vulnerable to the threat of deep cross-breeding. In addition to information from the location, bio-

logical information of Sumatran elephants fostered in ETC, WKNP is not widely known including information on molecular biology. Kinship analysis is needed to prevent the decline of genetic diversity in the population and prevent the occurrence of deep intermarriage. This paper was compiled to see the important role of the built Sumatran elephants in ETC, WKNP in supporting the efforts of Sumatran elephant conservation in natural habitats.

2. Materials and Methods

DNA isolation has been carried out as the beginning of the process of phylogenetic map construction of Sumatran elephants from Sumatran elephants fostered in ETC, WKNP [3,4]. The process of taking blood samples in the Sumatran elephants fostered was carried out in collaboration with the medical team of the elephant hospital Rubini Atmawidjaja, ETC, WKNP. Method optimization has been done both through simple and molecular methods, and comparing the two [5]. Molecular analysis was continued to the level of DNA sequencing carried out to see genetic variation and phylogenetic map construction of elephants [6, 7, 8]. Phylogenetic maps of Sumatran elephants were analyzed from the results of DNA sequencing (702 base pairs) [9] 65 individual Sumatran elephants fostered. Phylogenetic map of individual Sumatran elephants fostered from 65 sequenced DNA profiles and cytological profiles of 21 Sumatran elephants under the guidance of ETC WKNP were built [9, 10].

3. Results And Discussion

Conservation efforts require molecular information as a basis for determining the appropriate policies and management, including the Sumatran elephant rescue. For this reason, it is necessary to sample the source of genetic material such as blood and feces as basic data material. In compiling biomolecular information on Sumatran elephants, Sumatran elephants fostered in ETC, WKNP can be the best source of genetic material. This is because the Sumatran elephant fostered is already familiar with human existence, located in a closed location so that it is easily accessible. Blood samples are possible to be taken because of the routine examination of Sumatran elephants fostered in ETC, WKNP.

Every Sumatran elephant is guided in ETC, WKNP is accompanied by at least one mahout who is very understanding of the characteristics and behavior of each of the Sumatran elephants fostered [9]. This is very helpful and provides convenience in handling blood sampling for molecular analysis without going through anesthesia and arrest (Figure 1).



Figure 1. Handling and blood sampling in Sumatran elephants fostered in ETC, WKNP by a medical team accompanied by mahout

With the assistance of mahout and the medical team that are well known it can be carried out invasive and participatory blood sampling. Further sampling can be done by ensuring the individual, sex and age both invasive (blood samples) and non-invasive (fecal samples) [9]. From the results of molecular analysis genetic information can be obtained from fostered Sumatran elephants in the form of phylogenetic maps of fostered Sumatran elephants from 65 sequenced DNA profiles (Figure 2-3) [9] and cytological profiles of 21 individual Sumatran elephants guided by ETC WKNP (Figure 4) [10] has been awakened.

Sumatran elephants fostered in ETC, WKNP come from the same large group, and are feared to have a close kinship. Further analysis of kinship is needed. If an individual exchange from another ETC is not carried out in the long term, it is feared that deep cross marriages will occur. Cytologically shows that Sumatran elephants fostered in ETC WKNP tend to be domesticated. This is indicated by 3 forms of nonsymmetric chromosomes, namely at number 12, 24 and 27.

Sumatran elephants fostered in ETC, WKNP in general are a group of relatives of Sumatran elephants from a large population and have a tendency to have experienced domestication, which is already accustomed to human presence and intervention. Therefore, the release of fostered Sumatran elephants is not recommended. In connection with these results, the identification and marking of individual molecular Sumatran elephants fostered in ETC WKNP and other locations is needed, so that exchange of fostered Sumatran elephants can be carried out between captive breeding groups that have different target Sumatran elephant populations, and adjusting the management of the built Sumatran elephant, and ultimately building a data center for Sumatran elephants to be guided by wild Sumatran elephants in their natural habitat. Although already in coaching, Sumatran elephants in ETC, WKNP have an important contribution as a source of genetic material in formulating policy on the conservation of Sumatran elephants in Indonesia.

4. Conclusions

Sumatran elephants that have been fostered can make a significant contribution as a source of genetic material to the world of conservation both to determine the form of efforts and management policies

5. Acknowledgement

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Molecular Detection of *Jembrana* Disease in Balinese Cattle (*Bos sondaicus*) Samples at the Lampung Veterinary Center

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Abstract. *Jembrana* Disease is an infectious disease in Balinese cattle, which is characterized by fever and inflammation of the oral mucous membranes (stomatitis, enlarged lymph nodes of the prescapularis, prefemolaric, and parotid, sometimes accompanied by blood sweating. This disease reaches a mortality rate of no more than 20% caused by the *Jembrana* Disease Virus (JDV). According to data from the Directorate of Animal Health, Jembrana Disease cases continue to increase from year to year, with as many as 398 cases in 2016 and increased to 4,797 cases in 2018. The presence of the *Jembrana* virus can be detected through tests. One of them is by identifying and isolating the pathogen. Technological breakthroughs in DNA-based testing systems are becoming an available alternative. Viruses can be detected through commonly used molecular analysis such as *Polymerase Chain reaction* (PCR). The *Jembrana* Disease test was carried out at the Biotechnology Laboratory of the Lampung Veterinary Center using PCR and then visualized by agarose gel electrophoresis. The test results of 53 samples consisting of 50 types of *buffycoat* samples and 3 types of organ samples showed that 1 type of organ sample was detected with JDV

Keywords: jembrana disease; balinese cow; polymerase chain reaction; buffycoat; organ.

INTRODUCTION

The development of the livestock sub-sector is part of the development of the agricultural sector and national development and makes a very important contribution to supporting the provision of nutritious food and animal protein and increasing national income (Fatimah and Rahim, 2019). The development of cattle farming or better known as cattle in Indonesia, has great potential to improve genetic quality in preserve it in the future. Sukarno and Setyawan (2015) stated that one of the cows in Indonesia that has great potential to be developed is the

Balinese cow. Balinese cattle are developed as a natural livestock resource that has distinctive characteristics and has good reproductive abilities in various environments in Indonesia

The problem that occurs in Balinese cattle breeders is diseases that attack their livestock, such as *Jembrana Disease*. *Jembrana Disease* is an infectious disease in Balinese cattle, which is characterized by fever, inflammation of the oral mucous membranes (stomatitis), enlarged lymph nodes of prescapularis, prefemoral and parotid, can be accompanied by blood sweating (Mardiatmi, 2015). According to Su *et al.* (2018), the mortality rate of this disease reaches 20% caused by the *Jembrana Disease Virus* (JDV). The Directorate of Animal Health stated that *Jembrana Disease* cases continue to increase from year to year, as many as 398 cases in 2016 and increased to 4,797 cases in 2018 (Firison *et al.*, 2022).

Jembrana virus is an RNA virus that infects target cells by attaching to their surface and inserting RNA genetic material into the target cell. The viral RNA then undergoes reverse transcription into cDNA. Viral DNA moves from the cytoplasm to the nucleus, where the integrase enzyme integrates viral DNA into the DNA of the host cell, thus forming a provirus (Krisnayanti *et al.*, 2020). Proviruses (DNA) are found in the blood of infected target cells, for example Balinese cattle. The presence of the Jembrana virus can be detected through the test process. One of them is the diagnosis of the disease, which can be identified by isolating the pathogen. Technological breakthroughs in DNA-based testing systems are becoming an available alternative. Indriawati *et al.*, 2013, stated that this virus can be detected at the DNA level through molecular-based techniques. A commonly used molecular-based technique is the *Polymerase Chain Reaction* (PCR) method.

Research to identify the existence of the jembrana virus molecularly by the *Polymerase Chain Reaction* (PCR) method under the Lampung Veterinary Center program.

METHOD

Tools and Materials

The tools and materials used are Thermo Cycler, PCR Work Station, biosafety cabinet (BSC) class II, laminar air flow, waterbath, centrifuge, vortex, micropipette and filter tip, 0.2 ml optical tube, optical plate, chamber, agarose gel electrophoresis, TAE buffer, RNA isolation kit from QIAGEN, RNA carrier, AVL buffer, AW1 buffer, AW2 buffer, and AE buffer.

How it works

The samples used were *buffycoat samples* and organ samples. Extraction was carried out using an RNA extraction kit inside a biosafety cabinet. RNA extraction was performed according to the protocol of the QIAamp® RNA Mini Kit (250). The extraction process aims to take RNA from the sample through four stages, namely lysis, *binding*, *washing* or purification, and *elution* using a DNA extraction reagent kit derived from QIAGEN (QIAamp® DNA Mini Kit (250) cat. no. 51306) carried out in a *biosafety cabinet* by following the predetermined protocol. The next stage is amplification which has the goal of multiplying the target DNA through several cycles that have been programmed on the ABI 7500 Real-Time PCR Thermo Cycler. The amplified product is further electrophoresed in 2% agarose gel in TAE buffer (Tris-acetate-EDTA), and added with ethidium bromide for visualization of results. The electrophoresis process lasts for 30 minutes at a voltage of 100V and the agarose gel is then visualized with UV light inside the GelDoc machine.

DISCUSSION

The samples tested were *buffy coat*, *blood with anticoagulant (EDTA-Ethylene diamine tetra-acetic acid)*, and organs (Figure 1). The anticoagulant blood is processed by the centrifuge and washing method to obtain a buffy coat. Blood plasma is also collected if the cattle are known to be in the phase of showing clinical symptoms such as high fever characterized by diarrhea. Organs, especially the spleen, are scraped with mortar, and PBS is added. This sample will later be tested using the PCR method to detect the presence of genetic material for the jembrana virus. All samples tested showed that there was 1 sample infected with *Jembrana Disease*, namely in the sample with agenda number AR 0908, while the other samples showed negative results for *Jembrana Disease*.

Fifty-three samples were obtained from survey activities on December 27, 2023 - February 4, 2024 to be tested for jembrana disease (Table 1) with 52 samples tested showing negative for *jembrana disease virus*. This test is intended for the identification of *jembrana disease virus* in Balinese cattle both symptomatic and asymptomatic.

Table 1. Results of JD sample test on pig blood at the Biotechnology Laboratory of the Lampung Veterinary Center on December 27, 2023 – February 4, 2024

No. Agenda	Number of samples	Sample type		Test results	
		Buffycoat	Organ	Positive	Negative
AR 0864, 0907, 0908	3	1	2	1	2
PR 1856	2	2	-	-	2
PR 1858, 1859	29	29	-	-	29
PR 0067, 0069, 0079	14	14	-	-	14
PR 0101	4	4	-	-	4
AR 0050	1	-	1	-	1
Sum	53	50	3	1	52

In the PCR process, negative controls are included in the form of DNA templates that are negative *for the disease virus* and positive controls in the form of DNA templates that are positive *for the virus and the virus is positive*. The results were considered valid if DNA bands appeared in the positive control with a DNA molecular length of 360 base pairs, and conversely, no DNA bands appeared in the negative control, indicating the absence of contamination. In sample wells 1 and 2, there are no DNA bands as in the negative control well, meaning that samples 1 and 2 are considered negative (Figure 1). The result is considered positive when DNA bands appear from the sample well with the same molecular length as the positive control. In the well, sample 1 and the negative control do not show DNA bands. Therefore, sample 1 is considered negative. Meanwhile, sample well 2 shows the presence of the same DNA bands with positive control, meaning that sample 2 is positive *for Jembrana Disease* (Figure 2).

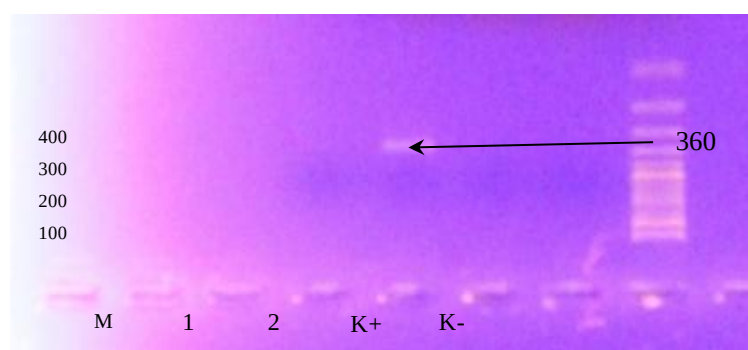


Figure 1. PCR results of Balinese cattle samples negative *for Jembrana Disease*

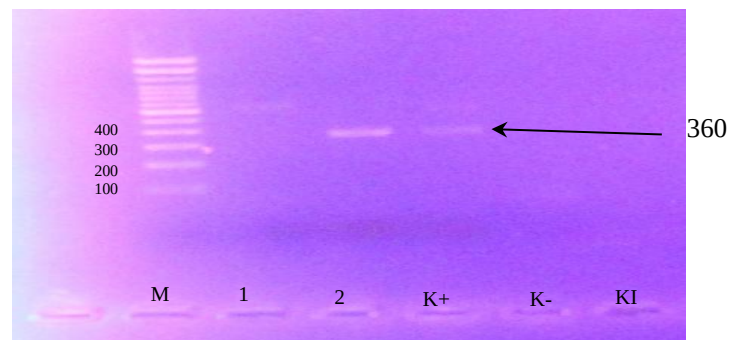


Figure 2. PCR results of Balinese cattle samples positive for *Jembrana Disease*

Monitoring by the PCR method was also carried out in 2013 by taking samples from all over Bali and the Bali Superior Cattle Farm Center (BPTU) Pulukan, showing that the presence of the jembrana virus was not detected (N=4,276) (Agustini et al., 2014). Using the same technique in 2015, the results of surveillance on samples from all over Bali also showed negative results for the presence of the jembrana virus (Agustini et al., 2016).

A number of samples that have been amplified in the PCR test but are below the detection limit, but can be clearly distinguished from the negative DNA control of *Jembrana Disease Virus* (JDV), were re-analyzed using conventional PCR. A JDV 1 *primary forward* pair with a sequence (5-GCAGCGGAGGTGGCAATTTTGATAGGA-3) and a JDV 3 *reverse primer* with a sequence (5-CGGGTGGTGGTGTCCACCCCATG-3) were used to amplify the 360 bp fragment within the open reading frame (Lewis et al., 2009). The number of these base pairs is referred to as the length of the DNA on the chromatin, abbreviated with bp (base pair). DNA bands of the same length were obtained at positive controls.

Testing for *Jembrana Disease* in Balinese cattle in Indonesia is important to consider because 27% of the total cattle population from Indonesia are Balinese cattle, making a major contribution to beef production in Indonesia (Desport and Lewis, 2010). The Directorate General of Livestock and Health (2007) stated that the strengthening of the Animal Disease Detection and Control System was carried out as an effort to deal with the threat of the entry of infectious animal diseases that have emerged and have the potential to cause losses to the Indonesian community and livestock. Molecular testing of *Jembrana Disease* with the *Polymerase Chain Reaction* method is important to ensure that the beef to be consumed is free of *Jembrana Disease*.

The ability of PCR in identifying the presence of the jembrana virus can be used to check health to speed up the identification process. *Polymerase Chain Reaction* (PCR) is one of the methods that is relatively often used in testing the identification of the presence of the jembrana virus. Testing for the detection of Jembrana disease can also use other methods such as *Indirect*

Enzyme Linked immunosorbent Assay (ELISA), Western Immunoblotting (WIB), and Immunohistochemistry (CPI) (Directorate of Animal Health, 2015).

CONCLUSION

The molecular detection technique of *Jembrana disease* in 53 samples was identified in 1 sample, which can be seen through the results of sample visualization, which showed the presence of DNA bands aligned with the positive control.

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The tools and materials used are Thermo Cycler, PCR Work Station, biosafety cabinet (BSC) class II, laminar air flow, waterbath, centrifuge, vortex, micropipette and filter tip, 0.2 ml optical tube, optical plate, chamber, agarose gel electrophoresis, TAE buffer, RNA isolation kit from QIAGEN, RNA carrier, AVL buffer, AW1 buffer, AW2 buffer, and AE buffer.

How it works

The samples used were *buffycoat samples* and organ samples. Extraction was carried out using an RNA extraction kit inside a biosafety cabinet. RNA extraction was performed according to the protocol of the QIAamp® RNA Mini Kit (250). The extraction process aims to take RNA from the sample through four stages, namely lysis, *binding*, *washing* or purification, and *elution* using a DNA extraction reagent kit derived from QIAGEN (QIAamp® DNA Mini Kit (250) cat. no. 51306) carried out in a *biosafety cabinet* by following the predetermined protocol. The next stage is amplification which has the goal of multiplying the target DNA through several cycles that have been programmed on the ABI 7500 Real-Time PCR Thermo Cycler. The amplified product is further electrophoresized in 2% agarose gel in TAE buffer (Tris-acetate-EDTA), and added with ethidium bromide for visualization of results. The electrophoresis process lasts for 30 minutes at a voltage of 100V and the agarose gel is then visualized with UV light inside the GelDoc machine.

DISCUSSION

The samples tested were *buffy coat*, *blood with anticoagulant (EDTA-Ethylene diamine tetra-acetic acid)*, and organs (Figure 1). The anticoagulant blood is processed by the centrifuge and washing method to obtain a buffy coat. Blood plasma is also collected if the cattle are known to be in the phase of showing clinical symptoms such as high fever characterized by diarrhea. Organs, especially the spleen, are scraped with mortar, and PBS is added. This sample will later be tested using the PCR method to detect the presence of genetic material for the jembrana virus. All samples tested showed that there was 1 sample infected with *Jembrana Disease*, namely in the sample with agenda number AR 0908, while the other samples showed negative results for *Jembrana Disease*.

Fifty-three samples were obtained from survey activities on December 27, 2023 - February 4, 2024 to be tested for jembrana disease (Table 1) with 52 samples tested showing negative for *jembrana disease virus*. This test is intended for the identification of *jembrana disease virus* in Balinese cattle both symptomatic and asymptomatic.

Table 1. Results of JD sample test on pig blood at the Biotechnology Laboratory of the Lampung Veterinary Center on December 27, 2023 – February 4, 2024

No. Agenda	Number of samples	Sample type		Test results	
		Buffycoat	Organ	Positive	Negative
AR 0864, 0907, 0908	3	1	2	1	2
PR 1856	2	2	-	-	2
PR 1858, 1859	29	29	-	-	29
PR 0067, 0069, 0079	14	14	-	-	14
PR 0101	4	4	-	-	4
AR 0050	1	-	1	-	1
Sum	53	50	3	1	52

In the PCR process, negative controls are included in the form of DNA templates that are negative *for the disease virus* and positive controls in the form of DNA templates that are positive *for the virus and the virus is positive*. The results were considered valid if DNA bands appeared in the positive control with a DNA molecular length of 360 base pairs, and conversely, no DNA bands appeared in the negative control, indicating the absence of contamination. In sample wells 1 and 2, there are no DNA bands as in the negative control well, meaning that samples 1 and 2 are considered negative (Figure 1). The result is considered positive when DNA bands appear from the sample well with the same molecular length as the positive control. In the well, sample 1 and the negative control do not show DNA bands. Therefore, sample 1 is considered negative. Meanwhile, sample well 2 shows the presence of the same DNA bands with positive control, meaning that sample 2 is positive *for Jembrana Disease* (Figure 2).

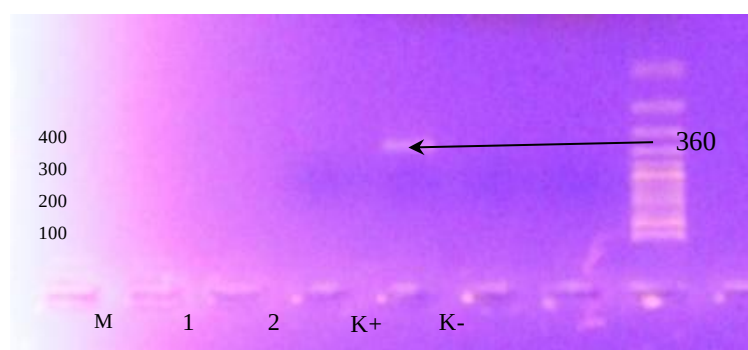


Figure 1. PCR results of Balinese cattle samples negative *for Jembrana Disease*

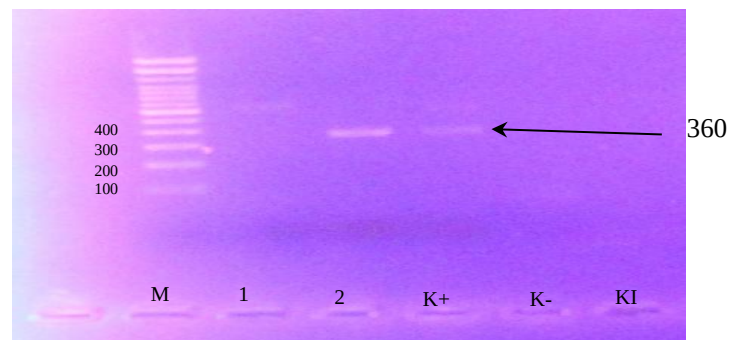


Figure 2. PCR results of Balinese cattle samples positive for *Jembrana Disease*

Monitoring by the PCR method was also carried out in 2013 by taking samples from all over Bali and the Bali Superior Cattle Farm Center (BPTU) Pulukan, showing that the presence of the jembrana virus was not detected (N=4,276) (Agustini et al., 2014). Using the same technique in 2015, the results of surveillance on samples from all over Bali also showed negative results for the presence of the jembrana virus (Agustini et al., 2016).

A number of samples that have been amplified in the PCR test but are below the detection limit, but can be clearly distinguished from the negative DNA control of *Jembrana Disease Virus* (JDV), were re-analyzed using conventional PCR. A JDV 1 *primary forward* pair with a sequence (5-GCAGCGGAGGTGGCAATTTTGATAGGA-3) and a JDV 3 *reverse primer* with a sequence (5-CGGGTGGTGGTGTCCACCCCATG-3) were used to amplify the 360 bp fragment within the open reading frame (Lewis et al., 2009). The number of these base pairs is referred to as the length of the DNA on the chromatin, abbreviated with bp (base pair). DNA bands of the same length were obtained at positive controls.

Testing for *Jembrana Disease* in Balinese cattle in Indonesia is important to consider because 27% of the total cattle population from Indonesia are Balinese cattle, making a major contribution to beef production in Indonesia (Desport and Lewis, 2010). The Directorate General of Livestock and Health (2007) stated that the strengthening of the Animal Disease Detection and Control System was carried out as an effort to deal with the threat of the entry of infectious animal diseases that have emerged and have the potential to cause losses to the Indonesian community and livestock. Molecular testing of *Jembrana Disease* with the *Polymerase Chain Reaction* method is important to ensure that the beef to be consumed is free of *Jembrana Disease*.

The ability of PCR in identifying the presence of the jembrana virus can be used to check health to speed up the identification process. *Polymerase Chain Reaction* (PCR) is one of the methods that is relatively often used in testing the identification of the presence of the jembrana virus. Testing for the detection of Jembrana disease can also use other methods such as *Indirect*

Enzyme Linked immunosorbent Assay (ELISA), Western Immunoblotting (WIB), and Immunohistochemistry (CPI) (Directorate of Animal Health, 2015).

CONCLUSION

The molecular detection technique of *Jembrana disease* in 53 samples was identified in 1 sample, which can be seen through the results of sample visualization, which showed the presence of DNA bands aligned with the positive control.

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Molecular Detection of Lumpy Skin Disease in Cattle Samples (*Bos taurus*) at Lampung Disease Investigation Center

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Abstract. *Lumpy Skin Disease*, LSD, was first discovered in Indonesia in Riau Province in February 2022. Although LSD is not a zoonotic disease, its infection can affect the production and reproductive performance of cattle, resulting in various impacts including reduced milk production, weight loss, miscarriages, and infertility as well as significant economic losses. It is a vector-borne smallpox disease of cattle and buffalo. Characterized by the appearance of pustules on the skin. Identification of LSDV can be carried out by three methods, namely *Polymerase Chain Reaction* (PCR), virus isolation, and electron microscopy. PCR is the cheapest and fastest method to detect LSDV. LSD disease testing at the Biotechnology Laboratory, Lampung Disease Investigation Center using the RT-PCR method. No LSD was found over 13 blood samples and 2 nasal swab samples from January 2024 sampling showed that all of them were negative to LSD.

Keywords: lumpy skin disease; polymerase chain reaction; cattle; farm; molecular analysis

INTRODUCTION

Animal husbandry is a business activity of a person or group of people who maintain and breed livestock with the aim of obtaining profit from the results of these activities (Rasyaf, 1994). One of the controls that a farmer needs to consider and manage is disease control. Disease control is an effort to prevent the spread of disease and treat disease (Yustendi et al., 2022), including in oxen or often called cattle.

Lumpy Skin Disease (LSD) is a disease that often affects cattle, first discovered in Indonesia in Riau Province in February 2022. Not recognized as a zoonotic disease, LSD infection can affect production and reproductive performance of cows and causes various impacts including reduced milk production, weight loss, miscarriage, and infertility as well as significant economic losses (Abutarbush et al., 2015).

A vector-borne pox disease of Asian cattle and buffalo, LSD is characterized by the appearance of pustules on the skin (Tuppurainen, 2017). The disease is caused by Lumpy Skin Disease Virus (LSDV), a virus from the Family Poxviridae, Genus Capripoxvirus along with two other viruses, goat pox and sheep pox (OIE, 2017). Identification of LSDV can be done by three methods, namely Polymerase Chain Reaction (PCR), virus isolation, and by electron microscopy. The PCR method is the cheapest and fastest method to detect LSDV. Skin nodules, crusts, saliva, nasal secretions and blood are suitable samples for detecting LSDV using PCR. Virus isolation followed by PCR to confirm virus identity is time-consuming and expensive, but has the advantage of detecting the presence of live virus in the sample. Although identifiable using electron microscopy, classic poxvirus virions cannot be distinguished at the genus or species level (OIE, 2017). Rapid, accurate diagnosis of bovine disease is important for proper farm management. Cattle diseases can have a serious impact on cattle production and welfare (Hilmi and Latifah, 2023). Sample testing in cattle (*Bos taurus*) with the aim of identifying LSD disease in blood samples and nasal swabs of cattle has been conducted at the Biotechnology Laboratory, Lampung Veterinary Center. The purpose of sample testing is to identify cattle samples from farmers infected with LSD, both symptomatic and asymptomatic.

METHOD

Tools and Materials

Tools and materials used were ABI 7500 Real-Time PCR Thermo Cycler, PCR Work Station, biosafety cabinet (BSC) class II, laminar air flow, waterbath, centrifuge, vortex, micropipette with filter tip, 0.2 ml optical tube, optical plate, DNA isolation kit from QIAGEN (QIAamp® DNA Mini Kit (250) cat. no. 51306) consisting of buffer AL, proteinase K, buffer AW1, buffer AW2, buffer AE, and 2 ml collection tube, amplification kit from bioline SensiFAST Probe Lo-ROX Kit (Lot No. SFPL 222110A), forward primer, reverse primer, probe, and Nuclease Free Water (NFW).

How it Works

The initial stage of sample testing was carried out by the extraction process using a DNA extraction reagent kit from QIAGEN (QIAamp® DNA Mini Kit) with stages, namely lysis, binding, washing or purification, and elution.carried out in a biosafety cabinet so that DNA derived from chromosomal DNA is obtained. The next step is to add a template with the aim of inserting the extracted DNA into a microtube that has been filled with master mix reagents. Amplification is done to multiply the target DNA through cycles that have been programmed on the ABI 7500 Real-Time PCR Thermo Cycler. RT-PCR results were analyzed qualitatively (positive/negative) with a descriptive analysis method, including the proportion of positive and negative results in the total sample.

Using the ABI 7500 Real-Time Polymerase Chain Reaction Thermo Cycler, LSD test results can be viewed on the amplification plot screen. As the instrument collects luminescence data during a run, the screen will display the sample amplification process. On the plate display layout tab, the amplification plot screen displays data to the well-selected when the continuous data collection method is set. Each cycle is marked with a normalized color luminescence graph of the amplification plot.

Results and Discussion

Samples obtained from survey and passive sampling activities in January 2024 and tested for LSD at the Biotechnology Laboratory, Lampung Veterinary Center totaled 15 samples (Table 1, Figure 1). Specimen types for LSD detection were 13 blood specimens and 2 swabs. The proportion of samples tested was 100% negative.

Table 1. LSD Sample Test Results in Cattle at the Biotechnology Laboratory of Lampung Veterinary Center in January 2024

No. Agenda	Total Sample	Test results	
		Positive	Negative
PR 0002, 0004, 0005, 0006	4	-	4
PR 0008, 0010	2	-	2
PR 0009	1	-	1
PR 0031, 0032, 0033, 0034	4	-	4
PR 0056, 0057	2	-	2
PR 0059	2	-	2
Sum	15	0	15

RT-PCR is able to detect and present PCR reaction results in real time on the screen of a connected computer. This makes it possible to quantitatively count the number of DNA initial reactions and has time efficiency in virus detection speed.

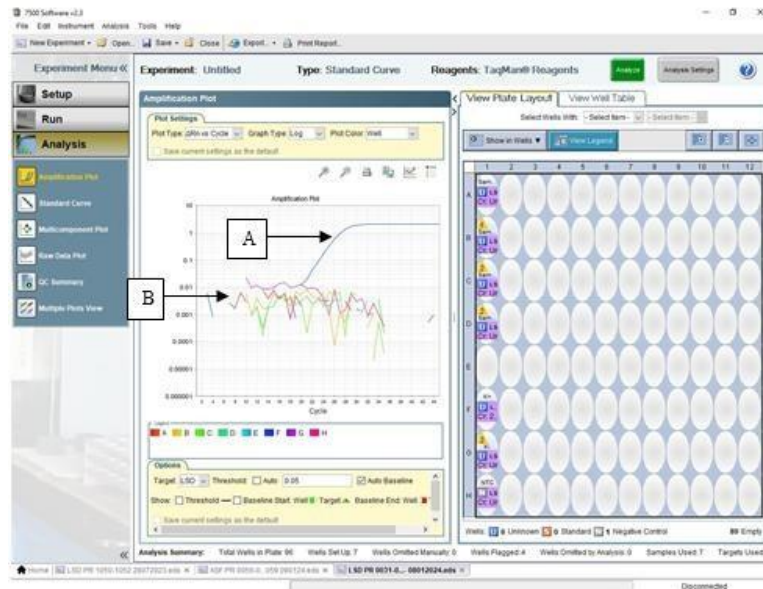


Figure 1. Visualization of LSD Negative Test Computer Results
(Source: Biotechnology Laboratory, Lampung Disease Investigation Center)

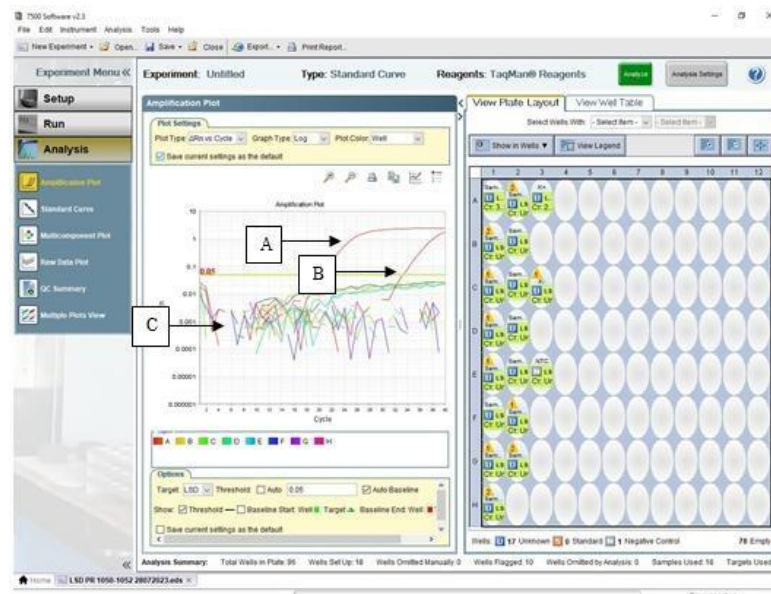


Figure 2. Visualization of LSD Positive Test Computer Results
(Source: Biotechnology Laboratory, Lampung Disease Investigation Center)

Based on the predetermined range, the interpretation of CT values is divided into high (positive and negative) and low (negative) values. A low CT count indicates a high concentration of viral genetic material in the sample, while a high CT count indicates a low concentration of viral genetic material. Selection of wells on the plate layout tab to view the high and low CT values on the amplification plot screen. The amplification plot is set with a range of 0.05 and a luminescence graph with various colors.

The blue color as a positive control indicates a graph that crosses the range, while the color The other tested samples show a graph that rises and falls and is below the range line, which means that the sample test results are negative (Figure 1). In the positive RT-PCR test results, the graph for the negative control in red and the graph for test sample number 8 in magenta show a graph that crosses the range line so that it can be said that the sample is positive for LSD (Figure 2). The graph line below the light green range line is the negative control, while graph below the other range line is the sample, so it can be said that the sample is LSD negative.

Clinical symptoms LSDV infection include fever up to 41.5°C, anorexia, decreased milk production, runny nose, conjunctivitis, hypersalivation, depression, and swollen lymph nodes. Generally, nodules/bumps are found on the head, neck, back, abdomen, tail, and genitals. These nodules become necrotic, causing a mass that leaves a deep hole (Sendow et al., 2021). LSD disease can also cause permanent or temporary sterility in bulls. Affected cattle generally have difficulty making a full recovery. Secondary infections, especially pneumonia and fly pustules, are common and cause deep wounds. Some animals do not show clinical symptoms even if antibodies are detected (Issamoy et al., 2020).

Transmission of the virus is through various vectors, namely flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti*). The virus can be transmitted to susceptible animals through direct contact with infected secretions or through indirect contact, such as contaminants from animal owners or objects (Ratyoha et al., 2022). In addition, LSD infection can also occur in utero. The disease is transmitted from infected mothers to calves through milk or injured skin. Apart from vectors, LSD transmission can occur through the consumption of contaminated food or water, direct contact, natural mating, and artificial insemination. Mass vaccination is the most effective way to control the spread of this disease (Tuppurainen, 2017).

CONCLUSION

The proportion of LSDV test results in 15 cattle samples in January 2024 at the Biotechnology Laboratory of Lampung Veterinary Center showed 100% negative results.

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Oral Swab-Based DNA Extraction Of *Cynopterus Brachyotis*: An Initial Step For Species Confirmation

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Abstract

Background. Identifying bat species based on morphological characteristics often faces challenges due to character overlap among species, especially within cryptic species groups. *Cynopterus brachyotis* is one of the fruit bats with high genetic diversity but relatively uniform morphology.

Aims. Therefore, a molecular approach is needed to support accurate species confirmation. Under the HETI Research Grant of Innovation and Collaboration Batch 3- II Year 2025, this study aims to obtain genomic DNA from *C. brachyotis* bats caught at the Lampung Disease Investigation Center as the initial step of molecular species confirmation.

Methods. Samples were obtained by taking oral swabs on bat individuals caught using mist nets. DNA extraction is performed using silica membrane-based methods with commercial kits. Quantitative evaluation of DNA was performed using a Qubit Fluorometer, while qualitative evaluation was performed by agarose gel electrophoresis.

Conclusion. The results showed that the DNA concentration was in the low range (0.1–0.7 ng/μL), with a DNA band appearing very thin on electrophoresis.

Implementation. Nevertheless, the quantification results confirm that DNA was successfully extracted and remains suitable for advanced molecular analysis, with optimization at the DNA amplification stage.

Keywords: *Cynopterus brachyotis*, DNA extraction, Lampung Disease Investigation Center, oral swabs,



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INTRODUCTION

Bats are a group of mammals with high diversity and have an important ecological role, especially as pollinators and seed dispersers (Marlinda *et al.*, 2021; Schultes, 2003). One of the species of fruit bats that is commonly found in Indonesia is *Cynopterus brachyotis*. However,

morphologically, the identification of this species is often limited by the similarity of physical characteristics between species in the genus *Cynopterus*, even including the *cryptic species* group (Campbell *et al.*, 2004; Chattopadhyay *et al.*, 2016).

DNA-based molecular approaches have been widely used to overcome the limitations of morphological identification because they provide more objective and accurate genetic information (Hebert *et al.*, 2003). DNA analysis allows the identification of species based on differences in genetic sequences that are not always reflected in morphological characters, especially in groups of organisms with a high degree of phenotypic similarity. The molecular approach is considered a reliable method for more precise species confirmation. One of the genetic markers commonly used in taxonomic and phylogenetic studies is the cytochrome b gene (Farag *et al.*, 2020). The cytochrome b gene shows a balanced degree of conservation and variation, making it effective for distinguishing closely related species (Kamagi *et al.*, 2021).

The quality and quantity of the extracted DNA largely determine the success of molecular analysis. The DNA extraction is a crucial step in species confirmation research. This study is focused on DNA extraction from *Cynopterus*. *Brachyotis* bats were found in the Lampung Disease Investigation Center area using oral swab samples. The Lampung Disease Investigation Center is located in a semi-urban environment that supports wildlife, including bats. This habitat condition makes it a relevant research location, especially for studying the existence and molecular identification of *Cynopterus brachyotis* to complement morphological identification in groups of species with similar physical characteristics.

Identifying bat species, especially in the fruit bat group, remains challenging due to morphological similarities and the presence of *cryptic species*. *Cynopterus brachyotis* is known to have high genetic diversity but relatively uniform morphological variation, so morphological approaches often lead to identification uncertainty. To overcome these limitations, DNA-based molecular approaches have advanced significantly. They are now standard in modern taxonomy, primarily through the use of mitochondrial gene markers such as *cytochrome b* and DNA barcoding.

Several previous studies have shown that the quality and quantity of the extracted DNA mainly determine the success of molecular analysis. Generally, invasive tissue samples (e.g. wing or muscle tissue) are used to obtain high-quality DNA. However, this approach has ethical and conservation limitations, especially in wildlife. Therefore, non-invasive methods such as oral swabs are gaining attention, although they often yield DNA at low concentrations. This

research is in the early stages of developing non-invasive methods to support the molecular identification of bats in Indonesia, particularly in semi-urban areas and for disease surveillance.

METHODS

Research under the Research Grant of Innovation and Collaboration Batch 3- II Year 2025, carried out in December 2025, in collaboration with the Lampung Disease Investigation Center. Bat life sampling using the mist-net method was conducted in the Lampung Disease Investigation Center area, Bandar Lampung. An oral swab sample was collected with a cotton swab, and DNA extraction was performed at the Biotechnology Laboratory of the Lampung Disease Investigation Center.

Oral swab samples are vortexed to ensure homogenization and release of biological material from the cotton swab into the medium. The sample is then prepared for DNA extraction.

DNA extraction was performed using the PureLink™ Viral DNA Mini Kit according to the manufacturer's silica membrane-based protocol. The sample was mixed with lysis buffers and proteinase K, then incubated to lyse the cells. After ethanol addition, the suspension is transferred to the spin column to bind the DNA to the silica membrane. The washing stage is performed to remove contaminants, followed by DNA elution with *RNase-free water*. The extracted DNA is stored at -20°C until further analysis.

DNA evaluation was performed quantitatively using a Qubit™ Fluorometer and qualitatively by agarose gel electrophoresis. DNA quantity was measured using 10 μL of extracted DNA mixed with 190 μL qubit™ reagent (199 μL dsDNA BR Buffer + 1 μL Qubit™ Reagent), homogenized slowly, incubated for 2 min at room temperature, and read using a Qubit™ Fluorometer ($\text{ng}/\mu\text{L}$). Quality analysis was performed using a 1% agarose gel prepared from 1 g of agarose in 100 mL of 1× TAE buffer, with the addition of SYBR® Safe DNA Gel Stain, and loaded into a chamber containing 1× TAE buffer. The DNA sample and DNA marker (6 μL) were inserted into the gel well, then electrophoresed for 35 minutes at 100 V and 300 mA. The results of electrophoresis were visualized under bluelight and documented using a camera connected to a computer via the EOS Utility app to capture DNA band images. Although molecular approaches to bat identification have been widely developed, some research gaps remain unanswered:

1. There is a lack of studies that specifically evaluate the effectiveness of oral swabs as a source of DNA in *Cynopterus brachyotis* in Indonesia.

2. Lack of empirical data on the quantity and quality of DNA from oral swab extraction and its implications for advanced stages, such as PCR and sequencing.
3. There have not been many studies that link the local ecological context (semi-urban, disease investigation centers) to the success of non-invasive DNA extraction in bats.
4. Previous research has focused more on the final results of molecular identification, rather than on the early stages of DNA extraction as the foundation of successful follow-up analysis.

DISCUSSION

The main novelty of this research lies in:

1. **The use of oral swabs as a non-invasive method** for DNA extraction of *Cynopterus brachyotis* in Indonesia, which is still rarely reported.
2. **Focus on the early stages of molecular identification**, i.e. quantitative and qualitative evaluation of extracted DNA, as an important basis before PCR analysis and species confirmation.
3. **The context of the research location** at the Lampung Disease Investigation Center, which is relevant for the integration of biodiversity studies with disease surveillance and environmental health.
4. Provision of **baseline data** on the range of oral swab DNA concentration (0.1–0.7 ng/μL) which can be used as a reference for the optimization of DNA amplification methods in future studies.

The results of DNA quantification using the Qubit Fluorometer showed that the DNA concentration in the oral swab of *Cynopterus brachyotis* ranged from 0.1 to 0.7 ng/μL and remained detectable in all samples. This is consistent with the principle of Qubit fluorometry, which uses a specific dye for double-stranded DNA (dsDNA), resulting in high sensitivity for detecting DNA even at low concentrations and greater accuracy than conventional spectrophotometry methods (Nakayama *et al.*, 2016).

Qualitative analysis through agarose gel electrophoresis showed thin DNA bands (faint bands) (Figure 1). This thin band reflects a low amount of DNA, so the fluorescent intensity of the intercalated dye is not strong enough to be clearly visualized. The intensity of the bands in the electrophoresis gel is affected by the amount of DNA and the integrity of the fragments; samples with little or fragmented DNA produce faint bands (Wittmeier & Hummel, 2022).

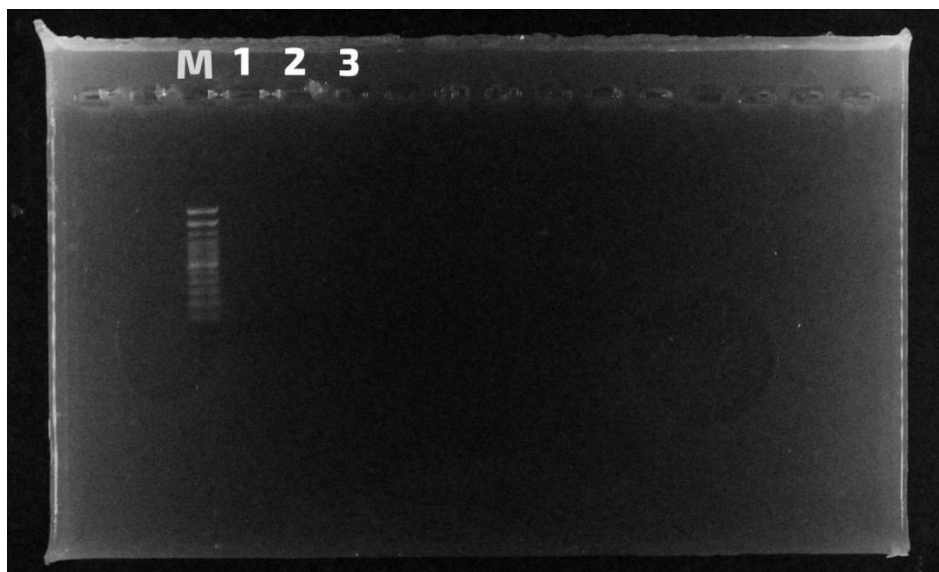


Figure 1. Results of electrophoresis of *C. brachyotis* oral swab samples

Fluorometry can detect small amounts of DNA despite its high fragmentation, while visualization of bands on gels requires larger amounts of DNA to produce clear signals. The two methods complement each other in assessing the success of DNA extraction (Cotes-Perdomo *et al.*, 2025). Although the electrophoresis band is thin, the presence of DNA is still quantitatively confirmed. This suggests that the extracted DNA could still potentially be used in advanced molecular analysis, such as PCR for species confirmation. The success of PCR amplification in low-concentration samples depends on proper primer design, polymerase enzyme sensitivity, and optimal reaction conditions. Optimizing the number of PCR cycles, annealing temperature, or DNA concentration can increase the likelihood of amplifying the target DNA from small initial samples (Cotes-Perdomo *et al.*, 2025).

CONCLUSION

DNA extraction from *Cynopterus brachyotis* oral swab samples obtained at the Lampung Disease Investigation Center was successful; the DNA concentration was relatively low (0.1–0.7 ng/ μ L), and the DNA band on electrophoresis appeared to be very thin. These results suggest that DNA from oral swab samples can still be obtained and potentially used for advanced molecular analysis, including species confirmation. This study confirms the importance of DNA extraction as the first step in molecular species identification, especially for species with similar morphologies.

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Amplification of The GAPDH Gene from The Urine eDNA of Sumatran Rhino in Sumatran Rhino Sanctuary, Way Kambas National Park

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Abstract: Profiling the genetic character of each individual sumatran rhino is important in order to maintain individual viability and genetic variability of Sumatran rhinos. Non-invasive sampling is needed to minimize the disturbance of individual sumatran rhinos, this is due to the solitary character of sumatran rhinos and tend to be afraid to interact with humans. Until now, non-invasive sampling of sumatran rhinos in Way Kambas National Park (WKNP) has only been done through the source of the puddle. This study aims to amplify the GAPDH gene from sumatran rhino environmental DNA (eDNA) sourced from urine. Urine sampling was carried out on four of the eight sumatran rhino individuals in WKNP. DNA extraction of four sumatran rhino urine samples was carried out with reference to the DNeasy® Blood & tissue kit extraction protocol. Amplification of eDNA extraction results was carried out by polymerase chain reaction (PCR) using GAPDH primers. Visualization of extraction and amplification results from four individual sumatran rhinos at TNWK was tested qualitatively with 1% agarose gel electrophoresis. Electrophoresis results showed positive results after amplification of four individual sumatran rhino urine samples at sizes between 100-200bp. This study successfully amplified the GAPDH gene from four sumatran rhino individuals in WKNP based on qualitative tests. In further conservation efforts, it is necessary to explore eDNA extraction from other potential sources.

Keywords: eDNA, GAPDH gene, Sumatran rhino.

Pendahuluan

Taman Nasional Way Kambas (TNWK) merupakan salah satu dari dua taman nasional yang berada di Provinsi Lampung dan terletak di Kecamatan Labuhan Ratu, Kabupaten Lampung Timur yang telah ditetapkan sebagai ASEAN *Heritage Parks* pada 27 Juli 2016 (Balai TNWK, 2018). Kawasan TNWK memiliki potensi sumber daya alam baik flora maupun fauna dan merupakan habitat bagi

satwa endemik khas sumatera meliputi gajah sumatera (*Elephas maximus sumatranus*), harimau sumatera (*Panthera tigris sumatrae*), tapir (*Tapirus indicus*), beruang madu (*Helarctos malayanus*) dan badak sumatera (*Dicerorhinus sumatrensis*) (Balai TNWK, 2017).

Secara khusus, pemerintah membangun Suaka Rhino Sumatera (SRS) di TNWK sebagai upaya pelestarian badak sumatera secara semi-insitu di bawah pengelolaan

Yayasan Badak Indonesia (YABI) (Priyambodo *et al.*, 2022). Anggara & Setiawan (2019) meneliti daya dukung SRS terhadap upaya pelestarian badak sumatera di TNWK dari berbagai hal, termasuk potensi tumbuhan pakan, analisis palatabilitas, ketersediaan air, dan perlindungan habitat.

Badak sumatera mempunyai karakter individualis yang cenderung soliter (Awaliah *et al.*, 2019). Hal ini berimplikasi pada laju reproduksi badak sumatera yang rendah. Berbagai upaya telah dilaksanakan oleh pemerintah dalam rangka peningkatan jumlah individu badak sumatera di SRS. Upaya yang dilakukan oleh pemerintah dengan bekerja sama dengan berbagai pihak ini telah berhasil ditandai dengan lahirnya badak sumatera Andatu pada tahun 2012, badak sumatera Delilah pada tahun 2016, dan anak dari badak sumatera Rosa dan badak sumatera Andatu pada tahun 2022 (Anugrah, 2022), sehingga saat ini, terdapat delapan individu badak sumatera yang menghuni SRS TNWK.

Upaya penambahan jumlah individu badak sumatera di SRS TNWK yang merupakan bagian dari upaya konservasi massif yang dilakukan oleh kolaborasi berbagai pihak telah mulai menampakkan hasil. Di sisi lain, upaya penambahan jumlah individu badak sumatera dengan cara mengawinkan dengan kerabat dekat individu badak sumatera mempunyai risiko akan turunnya keragaman genetik pada populasi yang ada (Leroy, 2014). Oleh karena itu, perlu dilakukan *profiling* karakter genetik masing-masing individu badak sumatera dalam rangka menjaga viabilitas individu dan variabilitas genetik dari badak sumatera.

Tantangan karakterisasi profil genetik badak sumatera selain merupakan hewan yang dilindungi karena berstatus kritis, juga karena perilaku yang soliter dan cenderung takut untuk berinteraksi dengan manusia. Hal ini menyebabkan perlu dilakukan pengambilan sampel secara noninvasif untuk meminimalisasi terganggunya individu badak sumatera. Hasil penelitian Priyambodo *et al.*, (2022) telah berhasil melakukan pengambilan sampel DNA dari lingkungan pada kubangan aktif dan pasif badak sumatera di SRS TNWK. Namun belum melakukannya pada sumber *environmental DNA* (eDNA) lain.

Urine dapat menjadi sumber eDNA karena merupakan cairan sisa metabolisme hasil ekskresi ginjal, dan dimungkinkan mengandung sel darah merah, sel darah putih, dan sel epitel. Saat urinasi dapat ditemukan sel epitel yang berasal dari kandung kemih (Hidayati & Tahrir, 2016; Yudianto dan Sispirasari, 2016). *Profiling eDNA* dilaksanakan dengan mengamplifikasi gen penyandi Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) pada individu badak sumatera dari sampel urine. Gen GAPDH akan diekspresikan menjadi salah satu enzim glikolitik yang telah dikenal sebagai *housekeeping gene* dan telah digunakan secara luas dalam penelitian protein, mRNA, dan DNA (Zhang *et al.*, 2015).

Bahan dan Metode

Bahan dan alat

Bahan yang digunakan dalam penelitian ini meliputi urine dari empat individu badak sumatera di SRS TNWK, kit ekstraksi DNA dari QIAGEN seri *DNeasy® Blood & Tissue Kit*, *MyTaq™ HS Red Mix (Bioline)*, *nuclease-free water*, *forward* dan *reverse primer GAPDH*, *agarose*, *tris-acetate EDTA (TAE)*, dan pewarna *SYBR Safe*. Alat yang digunakan dalam penelitian ini meliputi tabung mikro, tip mikro, tabung *vacutainer* 10 ml, *micro-pipet*, alat vortex, *Laminar Air Flow*, *set alat elektroforesis horizontal*, dan *UV transluminator*.

Pengambilan sampel

Penelitian dilaksanakan secara eksploratif dan dianalisis secara deskriptif. Pengambilan sampel dilaksanakan setelah penerbitan surat izin Keputusan Direktur Jendral Konservasi Sumber Daya Alam dan Ekosistem Nomor 257/KSDAE/SET/KSA.2/6/2018 dan Surat Izin Masuk Kawasan Konservasi (SIMAKSI) dari Balai TNWK Nomor SI. 220/BTNWK-1/2020. Pengambilan sampel urine dilakukan pada empat dari delapan individu badak sumatera di SRS TNWK.

Penentuan empat individu dilaksanakan dengan *purposive sampling* untuk mewakili sebaran usia individu badak sumatera. Pengambilan urine didampingi oleh *keeper* masing-masing individu badak sumatera (badak sumatera Andatu, Rosa, Bina, Delilah) yang

dibantu oleh tim medis YABI TNWK. Pengambilan sampel urine dilakukan dengan koleksi urine badak sumatera di dalam kantung perawatan sebanyak 7 ml urine badak sumatera, selanjutnya dimasukkan ke dalam tabung *vacutainer* 10 ml. Seluruh sampel urine badak sumatera yang telah dimasukkan ke dalam tabung *vacutainer* 10 ml disimpan dalam lemari pendingin dengan suhu 4°C.

Ekstraksi DNA

Ekstraksi DNA sampel urine empat individu badak sumatera dilakukan dengan mengacu pada protokol ekstraksi *DNeasy® Blood & Tissue Kit* nomor katalog 69504 dari QIAGEN yang terdiri atas tiga tahap utama, yaitu lisis, ekstraksi, dan elusi.

Amplifikasi DNA

Penggandaan hasil ekstraksi DNA dilaksanakan dengan reaksi *polymerase chain reactin* (PCR) menggunakan primer GAPDH melalui proses *master mix* dan amplifikasi. Pembuatan *master mix* dicampurkan larutan yaitu *MyTaq™ HS Red Mix (Bioline)* nomor katalog BIO-25047, sebanyak 11 µl *nuclease-free water* dengan 2 µL, primer *forward* 3' TCACCAGGGCTGCTTTTAAC 5' dan primer *reverse* 5' TTGATTTTGGAGGGATCTCG 3' masing-masing sebanyak 2 µl. Larutan *master mix* yang telah homogen ditambahkan DNA *template* sebanyak 3 µL.

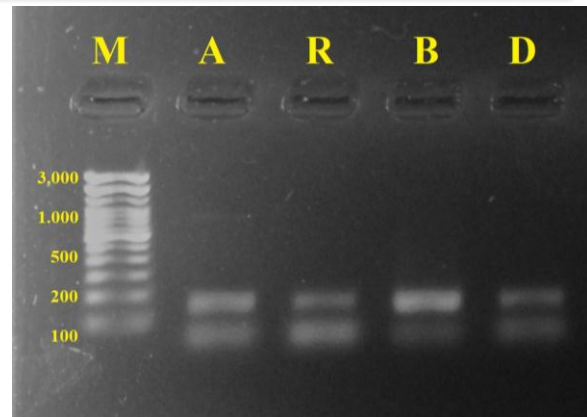
Visualisasi Hasil Amplifikasi

Visualisasi hasil ekstraksi dan amplifikasi dari empat individu badak sumatera di SRS TNWK diuji secara kualitatif dengan elektroforesis gel agarosa 1% dalam *buffer* TAE dengan pewarna *SYBR Safe*.

Hasil dan Pembahasan

Hasil amplifikasi

Hasil elektroforesis menunjukkan hasil positif setelah dilakukannya amplifikasi pada empat sampel urine individu badak Sumatera. Hasil uji elektroforesis atas empat amplicon eDNA sampel dapat dilihat pada Gambar 1.



Gambar 1. Hasil uji elektroforesis atas empat amplicon eDNA sampel (M: marker, A: Andatu, R: Rosa, B: Bina, D: Delilah)

Pembahasan

Hasil penelitian menemukan delapan individu badak yang mendiami SRS TNWK, yaitu Andalas. Andatu, Bina, Delilah, Harapan, Ratu, Rosa, dan satu individu yang dilahirkan dari pasangan Andatu dan Rosa pada tahun 2022 (Puspa, 2022). Pada penelitian ini diambil empat sampel dari delapan individu badak sumatera di SRS TNWK (50% dari total anggota populasi). Pengambilan sampel dilakukan secara *purposive sampling* agar mewakili semua rentang usia individu dan jenis kelamin badak sumatera. Empat individu yang menjadi sampel dalam penelitian ini adalah Andatu, Rosa, Bina, dan Delilah.

Andatu merupakan individu badak sumatera yang lahir dengan adanya campur tangan manusia. Andatu lahir pada 23 Juni 2012, yang merupakan badak sumatera jantan pertama yang lahir di habitat in-situ dalam 124 tahun terakhir di Asia (Rahmad, 2017). Rosa merupakan badak sumatera betina yang diperkirakan lahir pada tahun 2000 yang dipindahkan ke SRS TNWK sejak 25 November 2005 (Puspa, 2022). Bina merupakan badak sumatera betina yang berasal dari Bengkulu yang diperkirakan lahir pada 1979 dan merupakan individu paling tua di SRS TNWK (YABI, 2022). Delilah merupakan badak sumatera betina yang lahir di SRS TNWK pada 12 Mei 2016 hasil perkawinan dari Ratu dan Andalas (Nilam, 2019).

Pengambilan sampel dilakukan pada masing-masing *paddock* individu badak sumatera di SRS TNWK. Proses pengambilan sampel melibatkan *keeper* dan tim medis YABI di SRS TNWK. Pengambilan sampel dilaksanakan sebisa mungkin tidak mengganggu aktivitas badak sumatera, sehingga prinsip pengambilan sampel

secara non-invasif dapat terjaga. Metode non-invasif sangat disarankan dalam melakukan identifikasi keberadaan satwa terutama di habitat alamnya, tanpa harus menyentuh atau bertemu langsung dengan satwa. Hal ini sejalan dengan upaya konservasi genetik badak sumatera yang sumber DNA-nya bersumber pada sumber materi genetik satwa yang tertinggal pada lingkungannya yaitu jejak kaki, kotoran, bekas pakan, dan urine (Pusparini, 2006).

Berdasarkan hasil elektroforesis atas amplikon gen GAPDH dari empat individu badak sumatera (Gambar 1) menunjukkan hasil positif. Nampak amplikon gen GAPDH dari empat individu badak sumatera yang diambil eDNA-nya (Andatu, Rosa, Bina, dan Delilah) menunjukkan DNA pada ukuran antara 100-200bp. Hal ini selaras dengan hasil penelitian rentang ukuran gen GAPDH pada mamalia lain. Ukuran GAPDH tikus jantan adalah 114bp (Salehi *et al.*, 2017), sedangkan ukuran gen GAPDH pada kambing (*Capra hircus*) adalah 200bp (Tirumurugan *et al.*, 2010). Glyceraldehide-3-phosphate dehydrogenase (GAPDH) merupakan gen yang sering digunakan dalam proses penandaan individu, baik hewan maupun tumbuhan. Pada hewan, GAPDH berperan sebagai enzim glikolitik dalam mengkatalis konversi molekul glyceraldehide 3-phosphate to 1,3-diphosphoglycerate (Yogalingam, 2013).

GAPDH juga berperan penting dalam menginduksi eliminasi mitokondria saat terjadi stress oksidasi (Qvit, 2016). Keberhasilan amplifikasi gen GAPDH ini dapat dijadikan acuan dalam pelaksanaan pengambilan sampel dengan menggunakan metode non-invasif selanjutnya. Hasil uji kualitas atas amplifikasi eDNA urine badak sumatera dapat digunakan untuk mengetahui keberadaan badak sumatera di lokasi tersebut tanpa harus bertemu langsung dengan satwa. Penelitian ini dilakukan untuk mendukung Rencana Aksi Darurat (RAD) badak sumatera di TNWK. Hal ini sejalan dengan pernyataan Nagarajan *et al.* (2022) yang menyatakan bahwa metode deteksi Berdasarkan eDNA merupakan upaya komplemen untuk melengkapi biomonitoring secara tradisional dalam bidang ekosistem, baik terrestrial dan akuatik.

Kesimpulan

Berdasarkan uraian di atas, dapat disimpulkan bahwa gen GAPDH dari empat

individu badak sumatera di SRS TNWK telah berhasil didapatkan berdasarkan uji kualitatif. Hal ini dapat dijadikan landasan untuk melengkapi data dalam rangka upaya konservasi badak sumatera.

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An assessment of Sumatran elephant presences (*Elephas maximus sumatranus*) in Kotaagung Utara (Lampung, Indonesia) as a potential tourism attraction

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Abstract. Setiawan A, Monik DT, Charles Y, Rustiati EL, Ruciyanah Y, Anwar Z. 2021. An assessment of Sumatran elephant presences (*Elephas maximus sumatranus*) in Kotaagung Utara (Lampung, Indonesia) as a potential tourism attraction. *Biodiversitas* 22: 5397-5407. Kesatuan Pengelolaan Hutan (KPH) Kotaagung Utara is a region of approximately 56.02 ha, consisting of 8.82% forest cover and 91.18% non-forest cover. This region has a high diversity of flora and fauna and has potential as a tourist destination with various and unique biophysical attractions. One of the special features of this location is the presence of Sumatran elephants [*Elephas maximus sumatranus* (Temminck, 1847)]. Wild elephants in KPH are a tourism potential that can be developed to become a tourism attraction. The objectives of this research were to find out the elephant home ranges and the right window of time to observe the Sumatran elephants and assess safety, security, and convenience considerations for visitors to observe wild elephants. The method used in the research was an observation, and the results were qualitative data. The data collection's elephants movement through the use of GPS Collar from 2017-2020. The study identified that there are 16 wild elephants known as the Bunga group. The elephants can be observed from 7:30 to 12:00 am or 3:00 to 6:00 pm, with a duration of 3 hours per day. Steps can be taken to increase visitor's safety, security and convenience with the building of a treehouse, evacuation route, use of binoculars, and Personal Protective Equipment (PPE).

Keywords: Ecotourism, KPH Kotaagung Utara, tourism object, visitors

Abbreviation: Gapoktan: Gabungan Kelompok Tani Hutan; HKm: Hutan Kemasyarakatan; IUPA: Izin Usaha Pemanfaatan Alam; KHL: Kawasan Hutan Lindung; KPH: Kesatuan Pengelolaan Hutan; KPHL: Kesatuan Pengelolaan Hutan Lindung; PPE: Personal Protective Equipment; WWF: World Wide Fund for Nature

INTRODUCTION

Forest resources and forest areas must be managed and utilized sustainably to meet the social, economic, cultural and spiritual needs of present and future generations (Amenu 2017). One of the management can be used as nature tourism. Nature tourism is a form of tourism experience that promotes beauty, uniqueness, and value of biodiversity and artificial nature. According to Saat et al. (2015), ecotourism is a model of developing nature tourism that brings the joy of appreciating nature's beauty while sustaining the well-being of local communities and includes elements of education and conservation. With ecotourism, there's an emphasis on having interpretive and educational materials to provide information for potential tourists visiting the tourism place. Potential tourists are usually defined as people or individuals who will travel to areas with tourist attractions to get new experiences. The tourism potential is supported and facilitated by the

location of tourist destinations, which depends on the resources, facilities and services provided by local communities, businesses, and local government (Arida et al. 2017). Kesatuan Pengelolaan Hutan (KPH) Kotaagung Utara is a region that has a high diversity of flora and fauna and has potential as a tourist destination with various and unique biophysical attractions. It is the largest KPH in Lampung Province after KPH Batu Tegi. One of the unique aspects of this location is the Sumatran elephant's existence [*Elephas maximus sumatranus* (Temminck, 1847)].

The Sumatran elephant is the largest land mammal in Sumatra under the Order Proboscidea. According to the International Union for Conservation of Nature (IUCN), the Sumatran elephant is listed in the Red List of Threatened Species with the critically endangered category (Critically Endangered). In Indonesia, the Sumatran elephant has been protected since 1931 by the Wild Animals Protection Ordinance No. 134 and 226 since 1931. Furthermore, the

protection of Sumatran elephants is strengthened by Government Regulation No. 7, established in 1999, under the Preservation of Plant and Animal Species and Regulation of the Minister of Environment and Forestry Number P.106/MenLHK/Setjen/Kum.1/12/2018. However, the elephant population continues to decrease.

In general, Sumatran elephants can be found at low swamps, tropical forests (open canopy), and up to an altitude of ± 1.000 meters above sea level (Sitompul et al. 2013). In line with Febryano and Rusita (2018), the Sumatran elephants tend to live near the water resources and areas with adequate forage supplies. The availability of food will affect the behavior of the elephant itself. Based on Sari et al. (2016) behavior of animals can be categorized based on their daily activities, namely, active time, movement area, food hunting, communication behavior, and reproduction. The elephants live in the group for specific activities such as food hunting (Kurniadu et al. 2020). Additionally, these animals will continue exploring and expanding their home range, especially for foraging (Bahar et al. 2018). One of the methods used to examine the roaming area of wild elephants in KPH Kotaagung Utara is by mapping and observing.

Factors such as safety, security and convenience are essential for tourist destinations. Every tourism attraction requires some form of security and accessibility, including protection from environmental conditions, activities, and road access to these attractions. Furthermore, to tolerate all forms of disturbance that will occur to tourists, like a risk of contracting a disease or accident while in a tourist attraction. This is

intended to avoid various possible accidents experienced by tourists, including preventing disease transmission from animals to tourists or vice versa. If a tourist attraction can provide a sense of security and comfort, tourists will not feel physically and financially disadvantaged. The existence of wild elephants in KPH Kotaagung Utara is a tourism potential that has not been utilized. The various effort is necessary to develop it as a tourism attraction. The area's development as a tourism attraction will help increase regional income, especially for the people around the room. Hence, this research aims to investigate the presence, roaming areas, and suitable time to search for wild elephants while and to acknowledge the need for safety, security, and convenience for visitors to observe the Sumatran elephants in KPH Kotaagung Utara, Lampung, Indonesia.

MATERIALS AND METHODS

Research location

The research was conducted in KPH Kotaagung Utara, Tanggamus Regency, Lampung Province, Indonesia which is in the protected area of Kawasan Hutan Lindung (KHL), especially on Register 31 Pematang Arahan (Figure 1). The research was conducted from February 2021 to March 2021. The equipment used was a camera and binoculars, ArcGIS app, and data were recorded via Microsoft Exel and questionnaire.

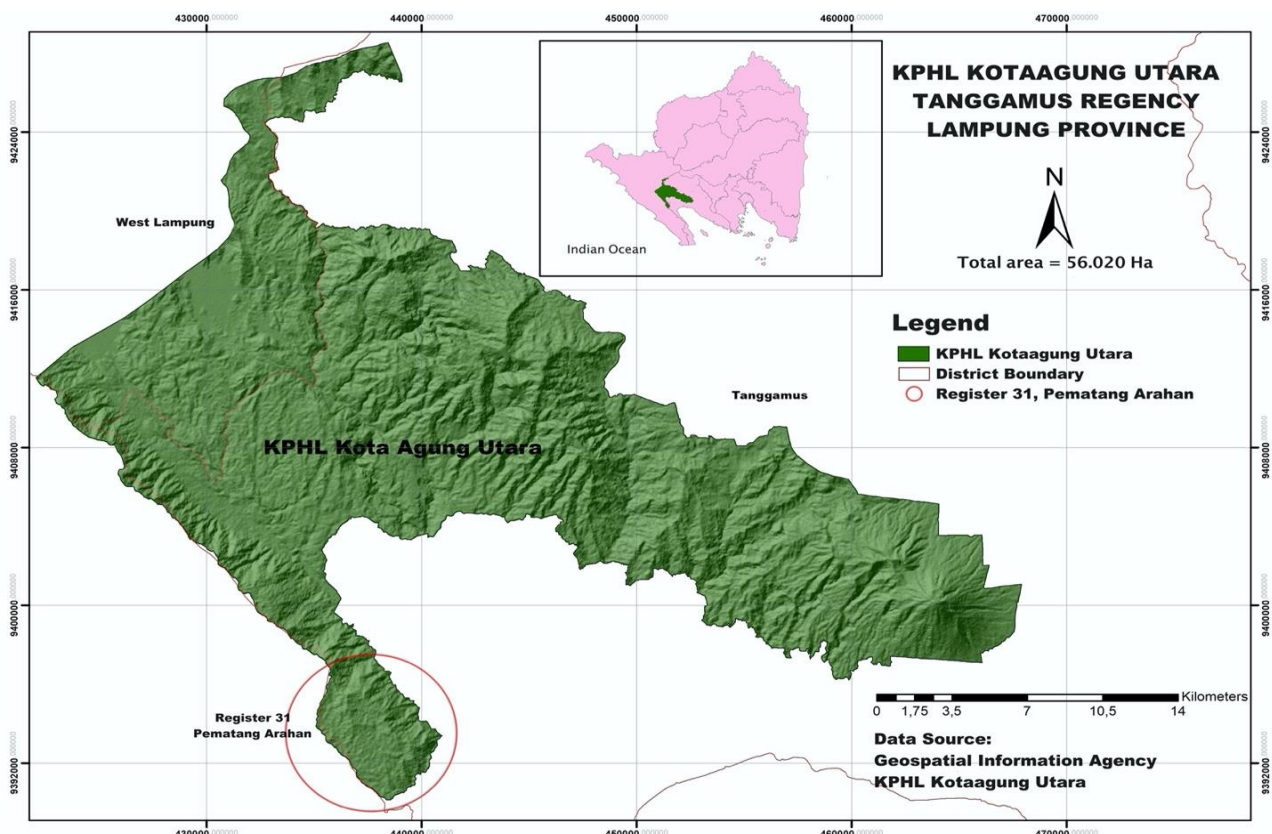


Figure 1. Research Location KPH Kotaagung Utara, Tanggamus Regency, Lampung Province, Indonesia

Procedures

Data types and sources

The data were collected from two sources: primary and secondary data. Preliminary data were obtained by direct observation, meanwhile, secondary data were obtained from published literature, company websites, newspapers, and journals (Cerar et al. 2017). The data used in this research are presented in (Table 1).

Data collection

Register 31 Pematang Arahan is located in KPH Kotaagung Utara, Tanggamus Regency with a total area of 1505 ha. The elephant roaming area was obtained by mapping the range of Sumatran elephants in the Register 31 Pematang Arahan by overlaying elephant location obtained from a 4 year Global Positioning System (GPS) elephant collaring project by World Wide Fund for Nature Indonesia Southern Sumatra Program (2017-2020). The GPS is a modern satellite-based navigation system used to track locations. The *GPS Collar* is shaped like a belt and can be secured to an animal. The *GPS Collar* was used to monitor technology in recent decades, can improve scientific tools (Moßbrucker et al. 2016). The installation of a *GPS Collar* is intended to monitor the movement patterns and animal positions so that it can be used to mitigate conflicts between humans and Sumatran elephant wildlife. Then, primary and secondary data suitability obtained by direct observation of the Sumatran elephant's gathering times and points from treehouses to observe wild elephant's activities more closely at Register 31 Pematang Arahan, KPH Kotaagung Utara. The purpose of this observation can be to determine the place (safe and comfortable) and the right time for tourists to see elephants.

In-depth interviews were conducted with the society around KPH Kotaagung Utara, and respondents were obtained using a Snowball approach. Additionally, a structured interview was conducted with the Head of KPH Kotaagung Utara. The result from the structured interviews was used to determine the society's and the manager of KPH Kotaagung Utara perceptions and opinions on the development planning of tourism attraction. Subsequently, another questionnaire was carried out on the society's perspective of elephants, the elephant's existence precisely,

and the potential of the elephant's as a tourism attraction in KPH Kotaagung Utara.

Data analysis

The data were processed and analyzed data qualitatively. Qualitative analysis is a method used to solve problems that are not limited to data collection and organization but includes analysis and explanation of research results (Lester et al. 2020). The analysis is a process where the data is generated, then defined by existing problems. The data were collected by getting information and the results are described in words or sentences. The preparation can be implemented accurately according to the facts in the field, therefore, it indicates that the results were obtained regarding the planning of tourism objects in KPH Kotaagung Utara well-executed.

RESULTS AND DISCUSSIONS

General condition of research field

The KPH Model Kotaagung Utara (Unit X) was formed based on the Forestry Minister's Decree Republic of Indonesia No. SK.379/Menhut-II/2011 on July 18th, 2011 on Determination of Protected Forest Management Unit Area Model Kotaagung Utara (Unit X), located in Tanggamus Regency, Lampung Province. KPH Kotaagung Utara was established by the District Regulation of Tanggamus Regency Number 21 of 2011 concerning the Establishment of the Organization and Work Procedure of the KPH in Tanggamus Regency, Lampung Province. Geographically, KPH Kotaagung Utara in Tanggamus Regency is located at 104017'BT-104042'BT and 5010'LS-5030'LS, Lampung. The KPH Kotaagung Utara are divided into 3 main registers, namely Register 30 Gunung Tanggamus, KPH Register 31 Pematang Arahan, and also KPH Register 39 Kotaagung Utara which is part of Tanggamus District Forestry Services Management Area. Management blocks in KPH Kotaagung Utara consist of Core Blocks and Utilization Blocks with a total area of ± 56.020 ha, divided into 217 plots consisting of 55 core plots and 162 utilization plots.

Tabel 1. Data types and sources

Data	Data sources
Tourist attraction coordinates	Observation
Landscape of the area	Observation
Potential tourist attraction	Observation and interview
The time and place elephants visit frequently	Observation, interview of Gapoktan, and <i>GPS Collar</i> is the equipment for monitoring the elephant's home range
Management plan for tourist attractions and tourism areas (safety, security and convenience) for visitors	Observation and interview

Tourists attraction

Areas that are unique, and value in the form of biodiversity, culture and man-made nature, which have the potential to be developed as an attraction for tourists. The unique attributes of the Kesatuan Pengelolaan Hutan Lindung (KPHL) Kotaagung Utara are Sumatran elephants, *Rafflesia arnoldii*, and waterfalls at the end of the water flow. In addition to seeing the Sumatran elephant, tourists can get to witness the *Rafflesia* sp. habitat. There are 13 types of *Rafflesia* flowers scattered on Sumatra, Java, and Kalimantan in Indonesia (Susatya 2011). Based on the observation done using GPS *Collar* since 2017-2020 in KPH Kotaagung Utara, the elephants are primarily present every year in the same month. By then, the opportunity to see the elephants in their nature is possible. Wild elephants are such a big attraction for tourists (Lo and Janta 2021; Miftahudin et al. 2021). Wildlife tourism provides an opportunity for tourists to interact with animals which will strengthen an emotional connection closely. It will provide the tourist with new experiences which will develop a pro-environmental behavior (Flower et al. 2021). In the research area, the elephants are periodically passed over the same path. There are 2 treehouses which are 120 m and 113 m apart from the elephant cross trail, which is being used to observe the elephants. The Royal Jozini website stated that the elephant must be followed at least 70-100 m away.

Supporting factors

In general, a tourist destination will provide new experiences to tourists in attractions that can be enjoyed. An outdoor area is provided for tourists to play in, which also includes education. Supporting aspects can influence the factors that attract tourists to travel. For example, tourists may want to take a vacation to relax, enjoy, and relieve boredom (Kusumaningrum and Wachyuni 2020). But the decision for a location to visit may be influenced by the facilities and attractions facilities and attractions offered by tourist destinations, so tourists may find them to see the exact location frequently (Koranti et al. 2018). The cool air temperature combined with the beautiful scenery is a supporting factor in KPH Kotaagung Utara. In addition, adequate facilities and infrastructure and a relatively beautiful and well-maintained location make some rare plants such as *R. arnoldii* and Sumatran elephants alive and still looking for food around this location.

Behavior and roaming area (*home range*) of the Sumatran elephant (*Elephas maximus sumatranus*)

There are 24 Sumatran elephants in Tanggamus, consisting of two groups: the Bunga group (16 elephants) and the Citra group (8 elephants). In line with Alpiadi et al. (2019), who stated that elephants behave in a matriarchal pattern or live in groups. The roaming areas of the two groups include KPH Kotaagung Utara and Bukit Barisan Selatan National Park (TNBBS), based on movement observed over 1 to 2 years. However, the Bunga group dominates the Kotaagung Utara KPH area. Meanwhile, the Citra group dominates the Pemerihan area

(TNBBS). The movement of each group is influenced by the season and the condition of their habitat, especially food and home range. The number of wild elephants in a group ranges from 2 to 50 be solitary (Larisha et al. 2016). Each group is led by an adult female, while the adult males tend to stay for a short period only to mate with several females. Male elephants born in the group once reaching sexual maturity will be forced to leave their mother's group and join other male groups. Meanwhile, old elephants will live with the new social network and establish a social hierarchy (Evans 2019).

The population of Sumatran elephants is decreasing from year to year (Qomariah et al. 2019). Collins (2018) states the most significant factor affecting the elephant population is shifts and changes in a habitat like the large-scale conversion of forest to monoculture plantations. Habitat change in the transformation of primary forest land into agricultural and plantation areas is a fundamental problem for elephant-human conflicts (Mustafa et al. 2018). Ministry of Environment and Forestry in RTMPPGS recognized that to minimize elephant-human disputes, there is a need to optimize barrier management and encourage coexistence practices between humans and elephants. Local resources are mobilized to manage the conflict with elephants, including cooperation with village areas, such as education programs about elephants, capacity building of the team tasked with monitoring wild elephants, and monitoring programs for the sustainability of society's resources in the long term. The prevention executed by the community around the KPH Kotaagung Utara area does carry out mitigation activities to prevent wild elephants from entering their plantation areas by using batons. These efforts were quite successful in chasing away elephants and minimizing the occurrence of non-natural elephant deaths and human deaths due to conflict. However, knowledge of elephant roaming is essential for humans. The range of humans is undoubtedly higher than elephants globally, and spatial planning is vital to reduce conflicts between humans and elephants (Sukmantoro et al. 2019).

Another prevention way to minimize conflicts with elephants was to observe the roaming area, pathways, and distribution of elephants in a location. World Wide Fund for Nature (WWF) and KPH Kotaagung Utara were tracking elephants using GPS *Collar* to monitor the location of the elephant groups and studied the movement patterns and the habitat range of elephants (Matthews et al. 2013). Traditionally, the home range is defined as the total area used by animals group to carry out their activities for a specific period (Fauvelle et al. 2017).

By looking at the path each group chooses during roaming, the study can identify home range restrictions. For example, based on the results of observations in 2017-2020 conducted by WWF and KPH Kotaagung Utara using a GPS *Collar*, the home range of two groups of wild elephants in Tanggamus covers the area of Register 31, Pematang Arah (Figure 2). However, the main home range is in the Sido Makmur III, Murah Rezeki I, Mandiri Jaya, and Block Lindung II.

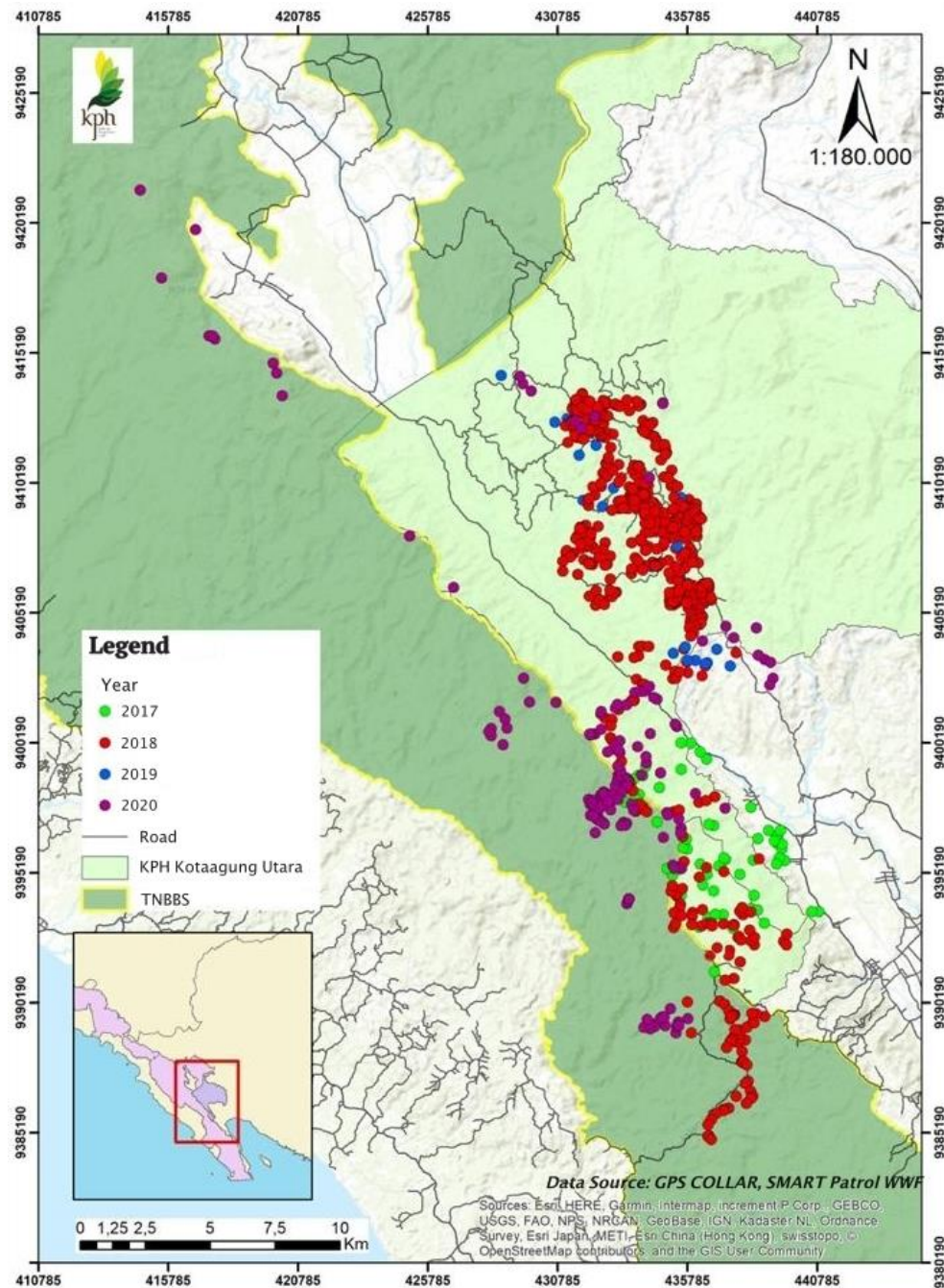


Figure 2. Distribution map of wild elephants (Bunga group) in Tanggamus Regency, Lampung Province, Indonesia for 2017-2020

The elephant food sources in each area are the main factor influencing the roaming activity of the wild elephant groups in KPH Kotaagung Utara. The number of plant species that grow under trees is few because forest vegetation composition has reached a climax. Thus the elephants rarely pass through such areas. This observation is supported by Dormann et al. (2020), who asserted that if the primary forest has reached the climax stage of succession, plant species diversity and abundance at the forest floor are relatively low. Another study speculates that high canopy forests may protect them from sunbeam (Mills et al. 2018). Furthermore, according to Songer et al.

(2012), the elephant's carrying capacity and food supply are reduced due to forest conversion effects. As a result, Sumatran elephants have to move away from forest areas to meet their needs. Other factors that may influence elephant movement, such as the need for safe locations to carry out daily activities: rest, communicating, and dressing up, are elephant behaviors to defend themselves from threats. The gift of nature in the forest is deserved to be guarded, and mother nature needs you to keep an eye out for her (Basappanavar 2021). If the environment and surroundings are not safe, the elephant will sleep standing up (Gravett et al. 2017).

According to Posta (2011) elephants spend approximately 16 hours a day eating and are more active at night. Elephants were supplied as much as 250kg of food per day (Berliani et al. 2018). The dominant plant species in the Sido Makmur III, Murah Rezeki I, Mandiri Jaya, and Block Lindung II areas are kans grass (*Saccharum spontaneum*), weeds (*Imperata cylindrica*), robusta coffee (*Coffea canephora*), Arabian coffee (*Coffea arabica*), gamal (*Gliricidia maculata*), dadap (*Erythrina variegata*), Java palisander (*Dalbergia latifolia*), fern palm (*Cycas rumphii*), sengan buto (*Enterolobium cyclocarpum*), rambutan (*Nephelium lappaceum*), and mangosteen (*Garcinia mangostana*). Clegg and O'Connor (2017) found that food chosen by elephants consists of grass, shrubs, foliage, bark, as well as hydrophyte, and fruit. The profitability of available food is determined by environment and time too. Sugiyanto et al. (2017) also revealed that elephants have foraging strategies when consuming grass and leaves in the dry and wet seasons. Elephants will choose fresh grass in the wet season because it contains carbohydrates and low fiber content (lignohemicellulose). Meanwhile, in the dry season, elephants prefer leaves containing high protein content, like (8-10% Malvaceae and 10 to 20% Leguminosae) (Singh 2019). The images were taken by the World Wide Fund for Nature Indonesia Southern Sumatra Program team (Figure 3).

Sido Makmur III, Murah Rezeki I, Mandiri Jaya, and Block Lindung II sites are areas where a large river flows, it can be seen in (Figure 4). Elephants depend on water, so in the afternoon, elephants will usually look for water sources to drink, bath, wallow, and of course, with good quality like water pH, conductivity and total dissolved solids (Ndlovu et al. 2018). This observation is supported by Yanti et al. (2017), who suggested that elephants look for water sources to cool their bodies from the hot sun and repel insects that stick to their bodies.

An elephant needs 200-250 liters of drinking water/day (Sukoco 2012). Overall, vegetation and slope had the most significant impact on elephants (Wilson et al. 2021). The elephant's water and food supplies are influenced by physical environmental habitat factors, such as climate and soil. Changes in a habitat can disrupt the normal functioning of all existing biological systems. A suitable habitat will affect and determine the level of animal welfare, both its reproductive ability and resistance to disease (Capucchio et al. 2019). The habitat is deemed suitable if there are sufficient water and food supply for elephants. According to Kuncoro et al. (2017), elephants are susceptible to ectoparasite infections caused by flies in dirty rivers. Ectoparasites are parasites that live on the body surface of the host. The ectoparasites' disorder may manifest into various medical conditions, such as irritation, itching, inflammation, scabies, myiasis, various allergic and others. These symptoms effects an inconvenience caused and anxiety that may interfere the elephant's activity (Rizwar et al. 2017). The thing that elephants do to keep their metabolism balanced is by saltlicks (Sitienei et al. 2012). Mineral salts such as calcium, magnesium and potassium contained in the soil or cliffs taken by the

elephant's trunk (Sach et al. 2019) are deemed helpful for elephants to help strengthen their bones, teeth, and tusks.

Safety, security and convenience factors (*Elephas maximus sumatranus*)

The ecotourism sector was suggested as a form of activity that can generate income for sustainable development without sacrificing the environment and benefit the next generation. However, maintaining a tourist attraction that is always visited by tourists and is sustainable may require special attention. Tourism attractions need a security guarantee, including safe environmental conditions, opportunities to be involved in social activities, and transportation access to the destination to tolerate any disturbance to tourists, such as the risk of contracting a disease or accidents while in a tourist attraction. This is very important to note, otherwise, it will be challenging to become a tourist attraction (Kirom et al. 2018). Tourist's convenience and safety are of priority, as they want to relax without worrying during their visits to a tourist attraction for a certain amount of time. Tourists are not willing to travel to an area if the region is involved in a conflict, war, or natural disaster. For this reason, a preparation plan is needed, both in terms of the destination's image and physical assets (Rossello et al. 2020). KPH Kotaagung Utara created the treehouse to observe wild elephant's activities more closely (Figure 5).

The KPH Kotaagung Utara built tree houses to observe wild elephant's activities more closely (Figure 5). The KPH Kotaagung Utara has two treehouses of different sizes. The treehouse (A), located 1.1 km from the Main Door/Rhino Camp, is a size 2.5 m x 2 m, built at the height of 12 m. Meanwhile, the treehouse (B), located 1.8 km from the Main Door/Rhino Camp, has a size of 2.2 m x 2 m and is built at the height of 14 m. The two treehouses can fit 4-5 people. The construction of a treehouse is a unique concept, and the results were in different sizes. When building treehouses for observing elephants, essential considerations include determining the right location to face (towards the elephant's roaming area), safe geographical location, and selecting a solid tree as the building pillar. An ideal tree used as a building pillar should have solid trunks and branches, deep and strong roots, and no signs of parasites or disease that could weaken the tree. Other considerations include minimizing threats caused by other factors, such as natural factors, the environment, economic activity factors, and road access factors.

The purpose of building a treehouse in KPH Kotaagung Utara was to allow officers to observe elephants closely. However, the innovative use of treehouses can help attract tourists who want to see elephants directly. During the observation, the society and field forest ranger confirmed that they felt more secure and comfortable viewing elephants in the tree houses. This finding is in line with Maryone's (2015), which states that the purpose of building a treehouse is to avoid wildlife. Therefore, treehouses can be innovated and used for the planning and development of tourism attractions in KPH Kotaagung Utara.



Figure 3. A wild elephants (Bunga) group were looking for food in KPH Kotaagung Utara, Tanggamus Regency, Lampung Province, Indonesia (Photo: Rusmani 2020)



Figure 4. Block Lindung II, Register 31 Pematang Arah, KPH Kotaagung Utara, Tanggamus Regency, Lampung Province, Indonesia (Photo: Della Tiara Monik 2021)

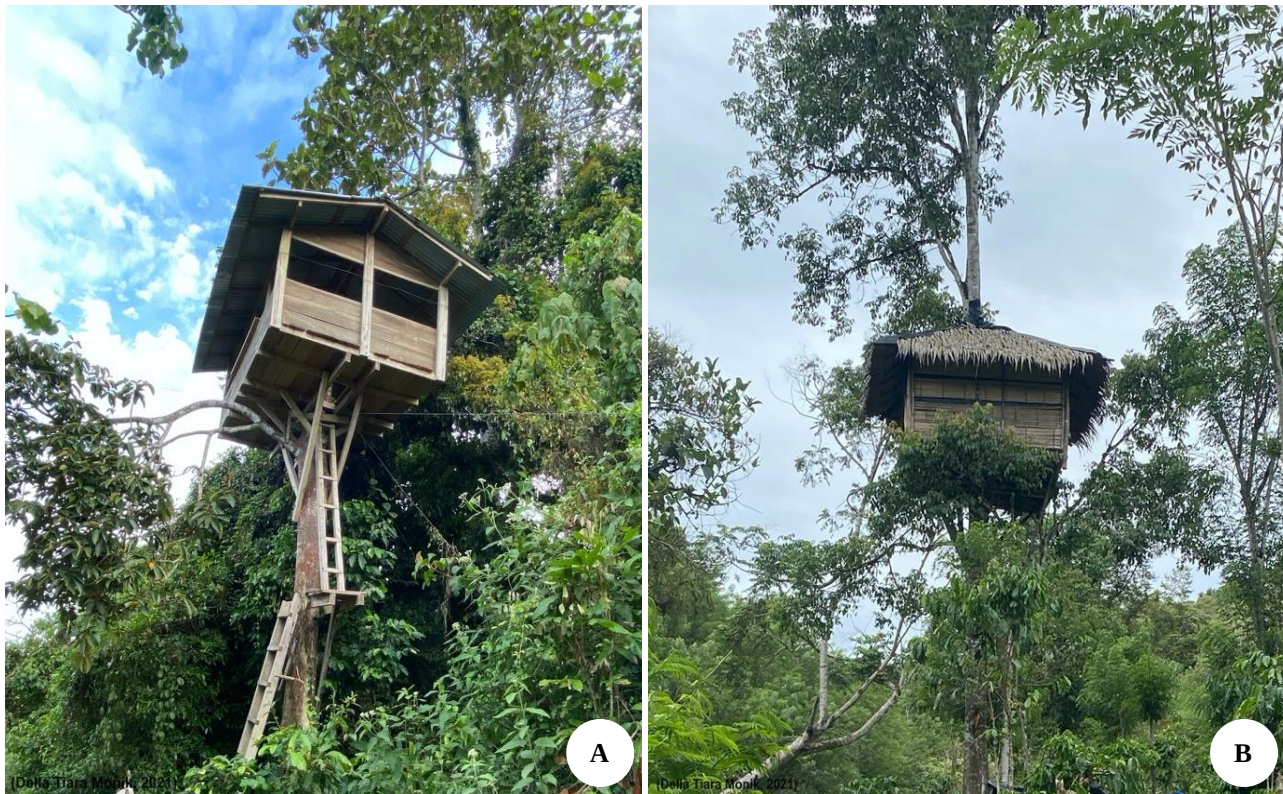


Figure 5. A. Treehouse (1) 120 m from the elephant cross trail (Photo: Della Tiara Monik 2021); B. Treehouse (2) 113 m from the elephant cross trail (Photo: Della Tiara Monik 2021)

Figure 4 shows that the elephant crossing area coincides with the river flows, especially in Mandiri Jaya and Block Lindung II. In the Block Lindung II area, there is a small waterfall and the *Tetrastigma* spp. plant is the *R. arnoldii* flower host. *Tetrastigma* spp can thrive in Mandiri Jaya and Block Lindung II compared to other areas. This is based on the availability of nutrients and supportive biotic and abiotic environmental conditions, such as temperatures of 25-29°C, ± 7 m from water sources, and surrounded by animal pollinators (Diptera) that *Tetrastigma* spp. can survive and become a healthy flower host for *R. arnoldii*. Using complex carrying capacity, Mandiri Jaya and Block Lindung II areas can be used as a tourist attraction, including the elephant crossing, *R. arnoldii* and small waterfall, and it can be seen in (Figure 6).

Rafflesia arnoldii's existence and a small waterfall in the Block Lindung II area are valuable for tourists who want to see the Sumatran Elephant at KPH Kotaagung Utara. A proper distance and access to tourism objects will make it easier for tourists to visit. The distance from the Rhino Camp location to the treehouse (A) is 1.2 km, and then to the treehouse (B) is 1.8 km. Meanwhile, the distance from the Rhino Camp location to the *R. arnoldii* location (Block A) is 1.8 km; (Block B) 1.9 km; and (Block C, F, E) is 2 km. The distance from Rhino Camp to a small waterfall is around 2.1 km. The closer the distance visitors travel to a tourist attraction, the easier it will be for visitors to come to the location.

According to Mr. Didik Purwanto as a manager of KPH Kotaagung Utara, Izin Usaha Pemanfaatan Alam (IUPA) in

the KPHL Kotaagung Utara is carried out through the Hutan Kemasyarakatan (HKM). There are 7 Gabungan Kelompok Tani Hutan (Gapoktan) in KPHL Kotaagung Utara, one of which is the 'Gapoktan Lestari Sejahtera'. The Sejahtera Lestari's Gapoktan is chaired by Mr. Rendy Hasaruddin, and this group has been operating for a long time with Government Regulation Number: SK.10098/MENLHK-PSKL/PKPS/PSL.0/12/2019. This is in accordance with the Tanggamus Regency Spatial Plan, namely the realization of an advanced, sustainable, and independent based on the potential of natural resources, without damaging the ecological aspect.

This study found that the appropriate time for tourists who want to see wild elephants in KPH Kotaagung Utara is around 7:30 to 12:00 am and 3:00 to 6:00 pm, with each session lasting 3 hours per day. During the day, the elephants will stop feeding due to the hot weather. If the weather is scorching, the Sumatran elephants will seek shade to cool down the body temperature. However, there is a better visual in the day than at night, which is why tourists want to see wild elephants in the daytime. But, if tourists want to see wild elephant's activity at night, they can use binoculars with infrared sensors. Binoculars with an infrared sensor can assist travelers in seeing in the dark and foggy. Elephants are land mammals active at night (nocturnal), from 1:00 pm to 4:00 am. According to Gravett et al. (2017), elephants can sleep in standing or lie down. One of the reasons elephants are not active during the day is because elephants have lousy eyesight. The strong daylight will cause a glare effect for the elephants.



Figure 6. Tourism Maps in Block Lindung II, Register 31, Pematang Arahan, KPH Kotaagung Utara, Tanggamus Regency, Lampung Province, Indonesia

Safety factors practiced when observing wild elephants in KPH Kotaagung Utara include an evacuation route, the use of binoculars, and Personal Protective Equipment (PPE). In an emergency, tourists can leave the treehouses using a rope ladder and follow the existing evacuation route. The evacuation route connects one area to a safe area and meets feasible building requirements to deal with the dangerous emergency response (Rahman and Sinaga 2019). The use of binoculars provides several advantages for tourists, including visual acuity, contrast sensitivity, and a clearer vision than monocular/direct vision (Kumagai et al.

2020). Thus, it facilitates for tourists to see the object. The use of PPE has a vital role in maintaining the safety and security of tourists in observing wild elephants. An accident can create a big issue (lousy reputation) for tourist destinations if an accident occurs during observation. Forestry work has significant risks and these losses can cause fatalities, so the use of PPE is necessary (Nkomo et al. 2018). Despite using PPE, technical prevention is the most important. Therefore, the main benefit of using PPE is avoiding and reducing accidents or health issues for tourists.

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Perilaku Makan Badak Sumatera di Suaka Rhino Sumatera, Taman Nasional Way Kambas, Lampung

Feeding Behaviour of Sumateran Rhino in Sumateran Rhino Sanctuary, Way Kambas National Park, Lampung

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Abstract

Research on the feeding behaviour, feeding plants and the way to access the plants has been conducted on Sumateran rhinos at and in collaboraton with Sumatran Rhino Sanctuary, Way Kambas National Park, Lampung, by scanning and rapid assessment methods. Feeding activity is done during day time, especially in the morning (05.00-10.00) and in the afternoon (14.00-18.00). Plants consumed include saplings (42%), followed by trees (30%), seedlings (20%) and liana (8%). Cutting (86%) is the most common way to access the food sources, followed by pulling (9%), pushing by its head and holding in between front legs (4%) and twisting by its horn (1%).

Key words: Sumateran Rhino, *Dicerorhinus sumatrensis*, *in-situ*, tegakan, scanning, rapid assessment.

Diterima: 29 November 2005, disetujui: 13 Maret 2006

Pendahuluan

Badak Sumatera (*Dicerorhinus sumatrensis* Fischer, 1814) terancam punah keberadaannya (Sadmoko, 1990). Penurunan populasinya disebabkan oleh perburuan liar dan perusakan habitat seperti penebangan liar, pembukaan lahan dan pemukiman, serta nilai ekonomi dari culanya (Manan dan Santiapillai, 1987; Rabinowitz, 1995).

Salah satu upaya konservasi secara *in-situ* telah dilakukan oleh Suaka Rhino Sumatera (SRS) (Anonim, 2000). Masing-masing badak ditempatkan terpisah di area seluas 6-10 ha, sesuai pola hidup alami badak Sumatera sebagai satwa soliter (Gambar 1).

Informasi tentang perilaku harian dan faktor penunjang hidup satwa ini masih sangat kurang terutama mengenai perilaku makan. Hal ini karena sulitnya perjumpaan langsung di habitat alaminya, serta pola hidupnya yang soliter dan bersifat sensitif. Tujuan penelitian ini untuk mengetahui perilaku makan,

kelompok tegakan tumbuhan pakan yang dikonsumsi dan cara memperoleh pakan badak Sumatera di habitat alaminya, di Suaka Rhino Sumatera, Taman Nasional Way Kambas, Lampung.

Metode Penelitian

Penelitian ini dilakukan sebagai bagian dari program dan bekerja sama dengan Suaka Rhino Sumatera, pada bulan Januari-Februari 2003, dilakukan di Suaka Rhino Sumatera, Taman Nasional Way Kambas, Lampung.

Pengamatan dilakukan dengan metode *scanning* (Robinowitz dalam Ma'turidi, 1998), dengan mengikuti pergerakan pada jarak tertentu, yaitu dari jarak terdekat $\pm$ 5-7 meter sampai jarak yang terjangkau untuk pengamatan dengan pertimbangan individu badak tidak terganggu. Dua ekor badak Sumatera yang terdapat di dalam suaka, badak jantan "Torgamba" dan badak betina "Bina"

diamati secara terpisah pada masing-masing paddock. Identifikasi tegakan tumbuhan pakan diamati dengan menggunakan metode *rapid assessment*, modifikasi dari *habitat assessment* (Brower *et al.*, 1990).

Pengamatan meliputi cara memperoleh pakan, kelompok tegakan (liana, seedling, sapling dan pohon) dan bagian tumbuhan pakan yang dikonsumsi, waktu memulai dan mengakhiri aktivitas makan dalam satu hari.

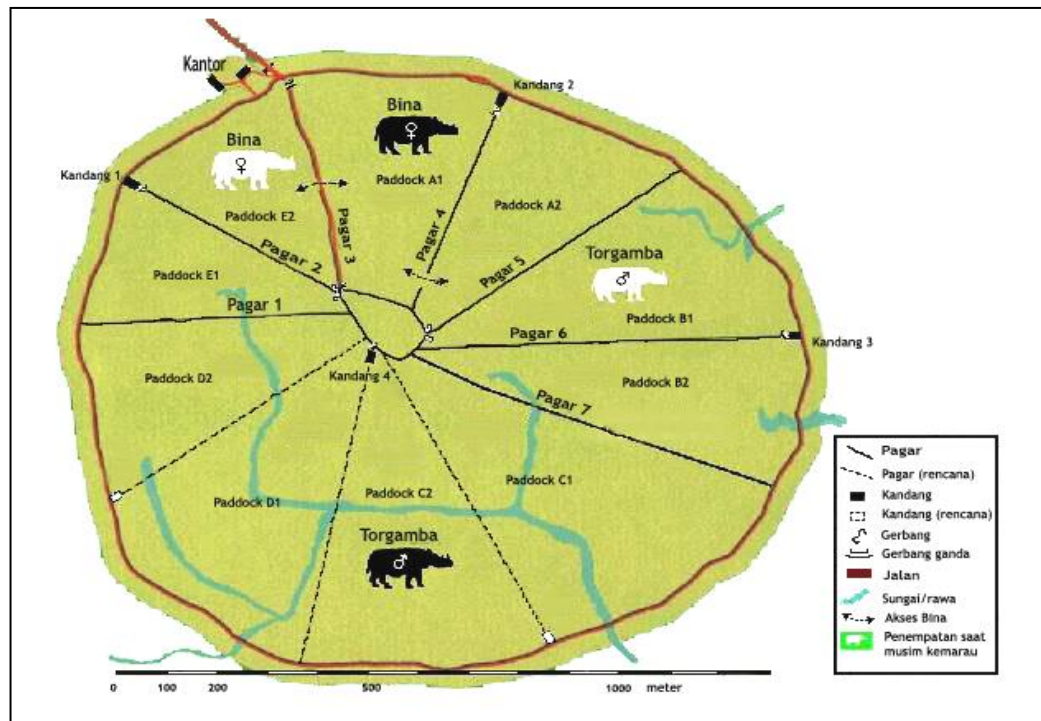
Hasil dan Pembahasan

Aktivitas makan badak Sumatera

Kedua badak Sumatera melakukan aktivitas makan hampir sepanjang hari, mulai pukul 05.00 dan berakhir pukul 19.00 (Gambar 2). Intensitas aktivitas makan tertinggi kedua badak Sumatera dilakukan pada pagi (pukul 05.00-10.00) dan sore hari pukul (14.00-18.00). Aktivitas kedua badak biasanya dimulai dengan bangun dari berkubang, kemudian berjalan sambil membersihkan tubuh dari lumpur dan memulai aktivitas makan. Pada

sore hari, aktivitas makan juga dilakukan setelah kedua badak bangun dari berkubang atau beristirahat pada siang hari (pukul 10.00-14.00) WIB atau???

Pola aktivitas makan antara Torgamba dan Bina tidak jauh berbeda. Bina melakukan aktivitas makan yang tinggi pada pagi hari (45%) antara pukul 05.00-08.00, dan pada sore hari (46%) pada pukul 14.00-17.00. Aktivitas makan Torgamba tinggi pada pagi hari (42%) antara pukul 06.00-10.00 dan pada sore hari (51%) pukul 14.00-18.00. Antara pukul 11.00-14.00 lebih sering digunakan oleh kedua badak untuk berkubang atau beristirahat. Kegiatan berkubang merupakan kegiatan harian yang penting bagi badak dan dilakukan sekali atau dua kali sehari, selama beberapa jam (Borner, 1979). Berkubang dilakukan untuk mendinginkan tubuh, membebaskan dari gigitan serangga, serta untuk menjaga kesehatan kulitnya. Di antara aktivitas berkubang, sesekali kedua badak Sumatera masih melakukan aktivitas makan.



Gambar1. Lokasi Penelitian Suaka Rhino Sumatera, Taman Nasional Way Kambas, Lampung (Dokumentasi Suaka Rhino Sumatera) peta tdk jelas!!tidak menunjukkan wilayah lampung!!!tidak ada arah utara!!

Kelompok tegakan dan bagian tumbuhan pakan yang dikonsumsi badak Sumatera

Tumbuhan yang paling tinggi dikonsumsi kedua badak Sumatera adalah tumbuhan pada tegakan sapling (42%), diikuti pohon (30%), semak (20%), liana (8%). Sedangkan bagian tumbuhan yang dikonsumsi umumnya berupa daun, ranting atau dahan, akar dan buah. Sapling paling tinggi dikonsumsi kemungkinan karena daun dan rantingnya mudah dijangkau. Hal ini sesuai dengan pendapat McDonald (1984) yang menyatakan bahwa badak Sumatera sering mematahkan sapling untuk memakan daun-daun pucuknya.

Bina paling banyak mengonsumsi tumbuhan pakan pada tegakan sapling (68%), diikuti liana (14%), pohon (12%) dan semak (6%). Sedangkan Torgamba lebih banyak mengonsumsi pada tegakan pohon (48%), diikuti semak (31%), sapling (16%) dan liana (5%). Perbedaan kelompok tegakan tumbuhan pakan yang dikonsumsi oleh Bina dan Torgamba kemungkinan disebabkan oleh keragaman dan ketersediaannya di masing-masing kandang. Seperti dalam penelitian Muya dan Oquge (2000) pada badak hitam di Afrika bahwa keragaman, penyebaran dan

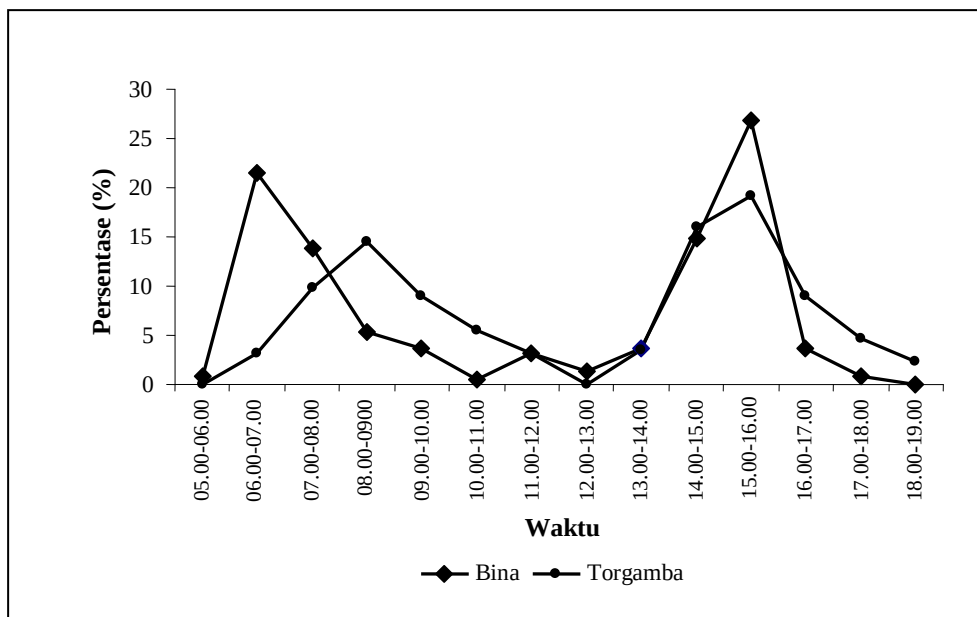
ketersediaan tumbuhan pakan mempengaruhi pemilihan pakan.

Cara memperoleh pakan badak Sumatera

Badak memperoleh pakannya dengan memangkas, menarik, merobohkan dan mematahkan (Anonim, 1982). Untuk tumbuhan yang dapat dijangkau biasanya badak langsung memangkas bagian tumbuhan yang disukai dengan giginya. Pada tumbuhan yang tergolong liana, tumbuhan ditarik terlebih dahulu dengan cara digigit atau dililit pada leher atau cula.

Cara memperoleh pakan yang paling umum dilakukan oleh Torgamba dan Bina adalah dengan memangkas (86%) terutama untuk semak. Cara ini juga dilakukan untuk menjangkau tegakan sapling dan liana. Sedangkan tumbuhan yang tidak dapat terjangkau, diperoleh dengan cara dirobuhkan terlebih dahulu yaitu dengan mendorong menggunakan kepala/dada dan kemudian memakan bagian tumbuhan yang disukainya (4%)(Gambar 4).

Liana yang dikonsumsi oleh kedua badak ini umumnya merupakan liana yang merambat pada pohon, sapling atau yang merambat diatas tanah. Liana yang merambat di pohon atau sapling diperoleh dengan cara ditarik dengan menggunakan mulutnya (9%) atau dililit dengan menggunakan cularnya (1%).

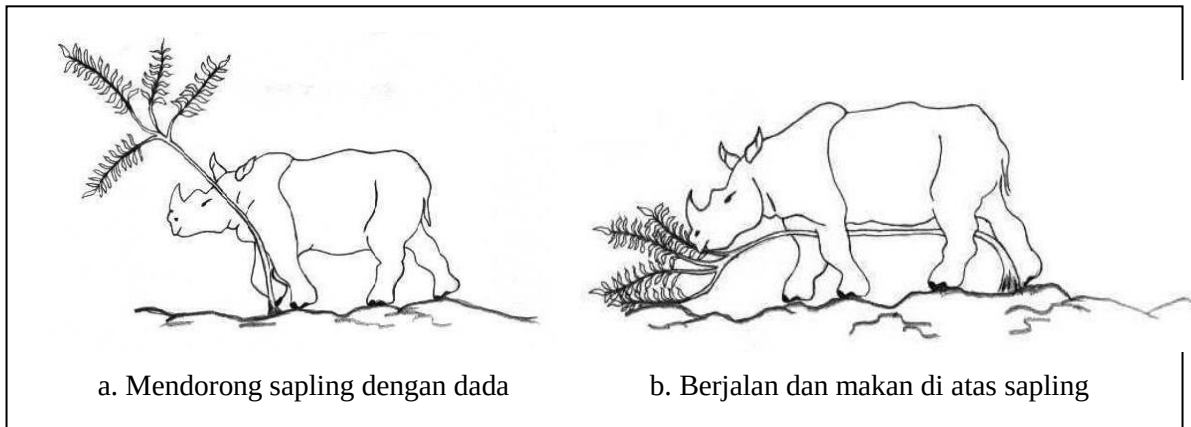


Gambar 2. Pola aktivitas makan badak Sumatera Bina dan Torgamba

Cara memperoleh pakan dengan memelintir tumbuhan pakan sangat jarang terlihat. Umumnya cara ini dilakukan untuk mengasah cula atau membersihkan cula dari kotoran atau lumpur. Perilaku ini sering terlihat dilakukan oleh Torgamba terutama pada saat defekasi dan urinasi. Untuk memakan buah yang terjatuh dari pohonnya dilakukan dengan cara menjulurkan bibir bagian atas untuk menjangkau buah tersebut.

Kesimpulan

Badak Sumatera di Suaka Rhino Sumatera melakukan aktivitas makan sepanjang hari, terutama pada pagi (pukul 05.00-10.00) dan sore hari (pukul 14.00-18.00). Kelompok tegakan tumbuhan yang dikonsumsi secara berurutan meliputi sapling (42%), pohon (30%), semak (20%) dan liana (8%) yang diperoleh dengan cara memangkas.



Gambar 4. Perilaku makan badak Sumatera (dikutip dari Borner, 1979).

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DNA Extraction of *Cynopterus brachyotis* of Labuhan Ratu VII Based on Column Method in Supporting its Molecular Species Confirmation

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Abstract

Background. Bats (Chiroptera) are true flying mammals and nocturnal. Ecologically, bats play important roles as pollinators, seed dispersers, guano producers, biocontrol, and hosts a range of viruses. *Cynopterus brachyotis* belongs to fruit-eating bats and is considered morphologically cryptic with *C. sphinx*.

Aims. To support morphological identification, molecular species confirmation needed to be conducted. DNA extraction, as an initial step, plays a crucial role in molecular analysis.

Methods. The quality and quantity of extracted DNA determine the success rate of sequencing for species confirmation in bats. In this study, oropharyngeal samples were used.

Result. The research procedures included bat capture and DNA oropharyngeal swab sample collection, sample preparation, DNA extraction using a silica column-based commercial kit, DNA concentration measurement using a Qubit assay, and electrophoresis.

Conclusion. The Qubit assay showed DNA concentrations of >1 ng/μL, while electrophoresis did not reveal bright DNA bands.

Keyword: *Cynopterus brachyotis*, DNA extraction, oropharyngeal samples, species, confirmation



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INTRODUCTION

Bats (Chiroptera) are the only flying mammals and are nocturnal. (Manek *et al.*, 2020). Based on morphological characters such as body size and diet, bats are divided into fruit-eating

and insect-eating species (Ransaleleh *et al.*, 2024). *Cynopterus brachyotis* belongs to the group of fruit-eating bats.

Cynopterus brachyotis belongs to the Pteropodidae, a frugivorous family of fruit, flower, and nectar eaters (Sapphire *et al.*, 2020), and can be found in almost all regions of Indonesia, especially Sumatra, Java, Kalimantan, and Sulawesi, except West Papua (Marlinda *et al.*, 2019). Its habitats include lowland forests, primary and secondary forests, urban areas, and areas with food resources (Ramona *et al.*, 2019). Ecologically, bats act as pollinators for flowering plants, seed dispersers in fruiting plants, and insect controls, especially insect-eating bats (Ransaleleh *et al.*, 2024). The faeces and urine produced by bats can also be used as guano fertilizer (Tangguda *et al.*, 2022). *C. brachyotis* also acts as a reservoir for pathogens such as the Nipah virus and *betacoronavirus* (bCOV) (Morcatty *et al.*, 2022).

Based on its morphological characteristics, *C. brachyotis* and *C. sphinx* are phylogenetically similar (Mubarok *et al.*, 2023). Morphological and morphometric characters are used in detail to identify *C. brachyotis*. In the identification of *C. brachyotis* the observed characteristics include the shape of the lower incisors, body colour, and the colour of the hair around the neck, the length of the forearms, the length of the tail, the ears, the tibia, the hind legs, and the weight of the body. In supporting the results of morphological identification, molecular species confirmation is carried out (Sapphire *et al.*, 2020).

Genes commonly used for species confirmation include mitochondrial genes such as Cytochrome Oxidase I (COI), the D-loop, and *Cytochrome b* (Cyt b) (Mubarok & Sandika, 2023). The *Cytochrome b* gene consists of 1,140 bp and has a high degree of variability at the species (intraspecific) and inter-species (interspecific) levels. The *Cytochrome b* gene can confirm cryptic species and their degree of kinship, making it useful for population studies and phylogenetic tree construction (Lam *et al.*, 2024).

DNA extraction aims to obtain pure DNA from organisms' tissues as the basic material for genetic identification. The results of DNA extraction greatly determine the success rate of subsequent molecular analysis stages, such as amplification, electrophoresis, and sequencing (Triasih *et al.*, 2020). Factors that affect the quality of extracted DNA include the type and condition of the initial sample, the extraction method used, the contaminant removal technique, and the storage process (Pearce *et al.*, 2024). To obtain DNA of optimal quality, this research applied *C. brachyotis* DNA sampling procedures and DNA extraction protocols.

Molecular identification has become an essential approach for accurate species confirmation in bats, particularly for taxa with cryptic morphological characteristics, such as

fruit bats within the genus *Cynopterus*. Traditional identification based on morphometric traits—such as forearm length, body coloration, and dentition—often leads to misidentification because phenotypic characters overlap among closely related species.

To overcome these limitations, mitochondrial DNA markers (e.g., COI, D-loop, and Cytochrome b genes) are widely used for species-level confirmation, phylogenetic reconstruction, and population genetic studies. The success of these molecular approaches depends heavily on the quality and quantity of extracted DNA, which in turn are influenced by sample type, extraction method, and storage conditions.

Current studies predominantly utilize tissue samples (wing membrane, blood) due to their high DNA yield; however, these methods are invasive and raise ethical concerns, especially for small-bodied bats. As a result, non-invasive or minimally invasive samples such as guano, feces, and oropharyngeal swabs have gained increasing attention. Among these, oropharyngeal swabs are considered advantageous due to reduced animal stress and suitability for pathogen surveillance.

Commercial silica column-based extraction kits are widely adopted due to their standardized protocols, high contaminant removal efficiency, and reproducibility. DNA quantification using fluorometric methods (Qubit) is currently considered more accurate than spectrophotometric methods, particularly for low-concentration DNA samples. Nevertheless, studies report inconsistent electrophoretic visualization when working with low-input DNA derived from non-invasive samples.

Overall, while molecular confirmation of bat species is well established, optimizing DNA extraction from minimally invasive samples remains an active area of research, particularly for tropical bat species.

METHODS

This research is under the Innovation and Collaboration Batch 3 scheme for the domestic year 2025 at Universitas Lampung, in collaboration with the Lampung Disease Investigation Centre. Bat life trapping by mist net and oropharyngeal swab sampling was carried out at Labuhan Ratu VII, East Lampung. At the same time, molecular analysis was performed at the Biotechnology Laboratory of the Lampung Disease Investigation Centre.

Bat sampling was carried out using mist nets, which were set up 4-6 meters high from 18.00 to 22.00. The oropharyngeal swab was collected using a sterile cotton swab, which was

inserted into the bat's oral cavity and rotated slowly. The cotton swabs are then inserted into a VTM tube containing physiological NaCl. VTM tubes were labelled and stored in a cool box.

Sample preparation for molecular analysis was performed using a VTM tube containing a homogenized sample, which was vortexed. DNA extraction was performed using silica columns with the Invitrogen Pure Link™ Viral RNA/DNA Mini Kit (Catalog #12280050) in four stages: lysis, binding, washing, and elution. The process began with breaking down the cell structure (lysis), which was performed by adding 200 µL of lysis buffer, 25 µL of proteinase K, and 200 µL of the sample to a 1.5 mL microcentrifuge tube. The solution was then homogenized using a vortex and was incubated at 56 °C for 15 minutes. It was then added to 250 µL of absolute alcohol, vortexed, and incubated at room temperature for 5 minutes. The binding stage was performed by transferring the incubated samples to a spin column and centrifuging at 10000 rpm, 4 °C for 1 minute.

The washing stage was performed twice by replacing the collection tube and adding 500 µL of wash buffer, then centrifuging at 10000 rpm at 4 °C for 1 minute. In the second wash, the collection tube containing the supernatant was discarded, and 500 µL of wash buffer was added, then centrifuged again at 10000 rpm at 4 °C for 1 minute.

In the elution stage, the collection tube was replaced with a 1.5 ml microcentrifuge tube, then 50 µL of Nuclease Free Water (NFW) was added and incubated at room temperature for 1 minute. The sample was then centrifuged at 12000 rpm and 4 °C for 1 minute. The spin column on the microtube was discarded, and then the microcentrifuge tube was closed, labelled, and the DNA was stored in the freezer at -20 °C.

The DNA concentration was measured using a Qubit fluorometer to check the quantity of extracted DNA. The reagents used were 199 µL Qubit™ dsDNA BR Buffer and 1 µL Qubit™ reagent. Both reagents are put in a 0.5 ml microtube. The extracted DNA was added to a total of 10 µL to the 190 µL test reagent, resulting in a final volume of 200 µL. The sample is slowly inverted by repeatedly sucking and dispensing the liquid with a micropipette to avoid bubbles or liquid from sticking to the tube walls. The mixture was incubated for 2 minutes at room temperature, then placed in a dark place. The DNA sample is read using a Qubit fluorometer on a program that corresponds to the size expressed in ng/µL.

Electrophoresis is performed using an electrophoresis set, which provides an electrical current through the agarose gel containing DNA in the chamber. Agarose gel 1% is made by dissolving 1 g of agarose powder into 100 mL of TAE 1X buffer in an Erlenmeyer flask. The solution is homogenized by whisking, then heated in the microwave for 3 minutes. The

fluorescent dye SYBR® safe DNA gel stain was added at up to 12 µL, and the mixture was homogenized. The agarose gel mold, after being combed, poured with an agarose solution, and allowed to harden, is ready to use. The solidified agarose gel is transferred into a chamber containing 1X TAE buffer until it is submerged. For comparison, a 100 bp DNA marker was inserted as much as 6 µL. The electrophoresis process is run for 35 minutes at 100 V and 300 A.

RESULTS AND DISCUSSION

The novelty of this research lies in several key aspects:

1. Application of oropharyngeal swab samples for molecular preparation of *Cynopterus brachyotis* in Indonesia, where tissue-based sampling still dominates bat genetic studies.
2. Empirical evaluation of silica column-based DNA extraction specifically for bat oropharyngeal samples, rather than pathogen-focused applications.
3. Combined assessment of DNA quantity (Qubit fluorometry) and quality (electrophoresis) to highlight discrepancies between measurable DNA concentration and visible DNA bands.
4. Context-specific contribution by providing baseline molecular preparation data for *C. brachyotis* populations in Lampung, supporting future genetic and phylogenetic research.
5. Ethical relevance, as the study supports minimally invasive sampling strategies that reduce harm to wildlife.

This study, therefore, contributes methodologically rather than taxonomically, emphasizing sample feasibility and laboratory workflow validation for future sequencing-based confirmation. The results of the DNA concentration measurement obtained showed a value of < 1 ng/µL. (Table 1).

Table 1. DNA concentration measurement results

No	Sample	DNA concentration ng/µL
1.	1	0.698 ng/µL
2.	2	0.668 ng/µL
3.	3	0.700 ng/µL

The Qubit assay results showed the total DNA concentration per microliter of sample. Higher values indicate higher DNA concentrations in the sample (Bruijns *et al.*, 2022). The sample with the highest DNA concentration was sample 3, with a value of 0.700 ng/ μ l. The DNA concentration can be increased through amplification to at least 1 ng/ μ L or higher so that it can be used for sequencing (Socea *et al.*, 2023).

The Sanger method is commonly used in DNA sequencing to determine the nucleotide sequence of DNA. This method is based on amplifying the target DNA fragment, followed by the synthesis of a new DNA strand using the DNA polymerase enzyme—the addition of fluorescently labeled nucleotides that terminate DNA elongation results in DNA fragments of varying lengths. Sanger sequencing is capable of generating sequence reads of up to approximately ± 1000 base pairs with very high accuracy, and therefore is often regarded as the gold standard for sequence confirmation and genetic analysis (Al-Shuhaib & Hashim, 2023).

The electrophoresis test showed a thin DNA band luminescence (Figure 1). The low sample concentration may be due to its storage period.

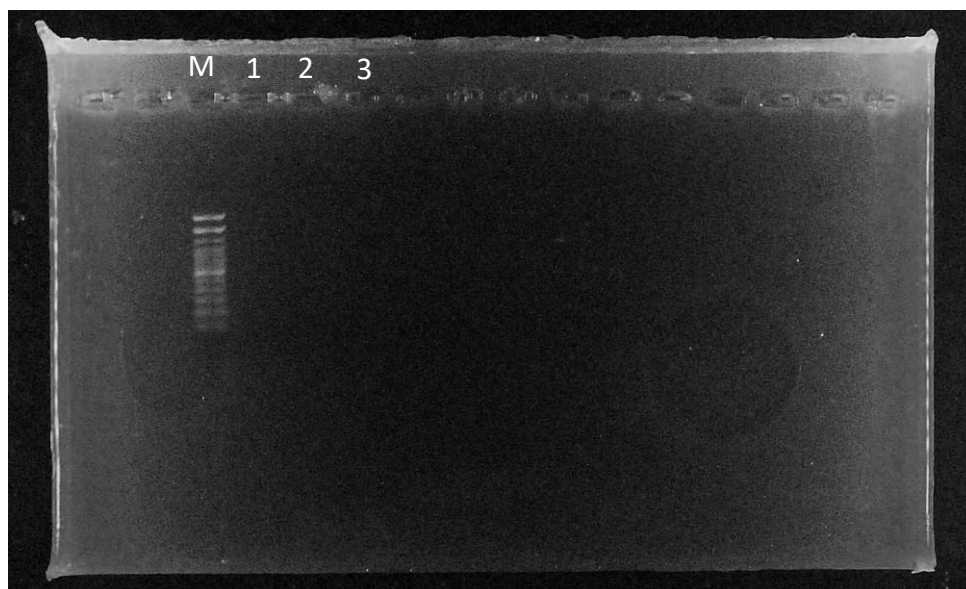


Figure 1. Electrophoresis results on bats' oropharyngeal swab samples (M: marker).

The advantage of oropharyngeal samples is their minimal invasiveness; however, the DNA concentration obtained may be relatively low. The oropharyngeal swab method has been successfully used in previous studies to sequence DNA to the species level in bats of the Vespertilionidae and Rhinolophidae in the UK (Huges *et al.*, 2024). Other types of bat samples

that can be used include tissue samples (wing membranes/uropathageum), blood samples, and guano or fecal samples (Arnaout *et al.*, 2022).

Factors that affect the quality and quantity of extracted DNA include sample type, the sample's initial condition, and storage period (Walker *et al.*, 2019). Other types of samples, such as guano/fecal samples, are more easily degraded and susceptible to contamination because they are mixed with food waste and microbes (Guan *et al.*, 2020). Tissue samples, such as wing membrane or blood, can be used to obtain higher DNA concentrations. However, the level of invasiveness is higher, given that *C. brachyotis* is a small animal; tissue sampling increases the risk of injury if not performed very carefully (Arnaout *et al.*, 2022).

Despite advances in bat molecular identification, several research gaps remain evident:

1. Limited optimization studies focusing on increasing DNA yield from oropharyngeal swabs in fruit bats, particularly under tropical field conditions.
2. Lack of comparative analysis between different non-invasive sample types (oropharyngeal swab vs. guano vs. wing membrane) using identical extraction and quantification protocols.
3. Insufficient evaluation of downstream success, such as PCR amplification efficiency and sequencing outcomes, when DNA concentrations are below 1 ng/ μ L.
4. Minimal reporting on storage duration effects on DNA degradation for swab-based bat samples prior to extraction.
5. Scarcity of region-specific molecular baseline data for Indonesian bat species to support biodiversity monitoring and zoonotic surveillance.

Addressing these gaps would improve methodological reliability and expand the applicability of non-invasive molecular techniques in chiropteran research.

CONCLUSION

The electrophoresis results showed a thin DNA band, and the Qubit assay indicated a DNA concentration of 0.6-0.7 ng/ μ L. The quality and quantity of DNA can be optimized through amplification before proceeding to further molecular analysis stages.

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**Kompilasi Publikasi Kajian DNA Gajah Sumatera dan Badak Sumatera
Dosen dan Mahasiswa Biologi FMIPA Universitas Lampung**

**Oleh
Tim Wildlife ID
di bawah
Dra. Elly Lestari Rustiati, M.Sc.**



**Jurusan Biologi
Fakultas Matematika dan Ilmu Pengetahuan Alam
Universitas Lampung
2021**

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SHORT COMMUNICATION: IDENTIFICATION AND CHARACTERIZATION OF COI GENE IN FEMALE SUMATRAN ELEPHANT (*Elephas maximus sumatranus*) IN ELEPHANT TRAINING CENTRE, WAY KAMBAS NATIONAL PARK

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Abstract :

Sumatran Elephant is a subspecies of endemic Asian elephants on the island of Sumatra and is included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. The building of the Elephant Training Centre (ETC) in Way Kambas National Park (WKNP) is one of the conservation efforts of Sumatran elephants. Small and closed population size lead to an increased risk of inbreeding that triggers reduction in genetic variation and viability and increases the risk of extinction. The phylogenetic pattern of Sumatran elephants in Indonesia has shown a low population genetic diversity. Genetic diversity information is indispensable to support the direction of decision making in Sumatran elephant conservation policy. The DNA isolation of Sumatran elephants in ETC, WKNP has performed as a first step to trace its genetic variation. The advanced step of DNA isolation is the use of Cytochrome Oxidase subunit I (COI) gene for identification of genetic characteristics in Sumatran elephants. The COI gene is one of the genes on the mitochondrial genome and in molecular studies it is used as a genetic marker to study genetic characteristics between species and individuals. Identification and characterisation are done by sequencing process and data analysis in the form of electroforegram using Molecular Evolution Genetics Analysis (MEGA) software version 6.0. to see the genetic diversity of the female Sumatran elephant population in ETC, WKNP. Based on the results of the analysis it is indicated that the genetic distance of 24 individual female Sumatran elephant from PLG, TNWK is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded all individual female Sumatran elephants in the PLG, TNWK is derived from one population group.

Keywords: Sumatran elephant, COI gene, genetic distance, homology, phylogenetic

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1. Introduction

Sumatran elephant is a subspecies of Asian elephants endemic to the island of Sumatra. It is also included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. Sumatran elephants characterized as a land mammal with a grouping pattern led by adult females (matrilineal) [1] [2]. One of the conservation efforts site for Sumatran elephants is in ETC, WKNP which is part of the area of the natural habitat of Sumatran elephants. The presence of ETC plays an important role in conservation efforts, such as handling Sumatran elephants that are affected by habitat fragmentation, wild hunting, and human-wildlife conflict with national park buffer village communities. The population size of Sumatran elephants in ETC, WKNP in 2016 shows a total of 66 individuals with 36 male elephants, and 30 female elephants [2]. Currently with the birth of two elephants, the total number of Sumatran elephants increases to 68 individuals [3].

Sumatran elephants as critically endangered animals depend on their ability to maintain their survival rate, while in closed populations such as ETC increases the chances of inbreeding, which is caused by the absence of supporting data describing the genetic diversity of the elephant population within it. The genetic diversity of Sumatran elephants in the population affects its ability to adapt to changing environmental conditions. Reduced genetic diversity increases the potential for extinction. Sumatran elephant as an umbrella species with various challenges for its survival; including low genetic diversity should be saved from such threats. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity at the population level. COI gene as one of genetic markers is used to study genetic characteristics of female Sumatran elephants in ETC, WKNP.

2. Materials and Methods

2.1 Materials

The tools used in this research are 0.2 µl micro tubes, Laminar Air Flow (LAF), vortex, Micropipette, Microtip, Veriti Thermal Cycler, agarose gel

electrophoresis, Digital Documents (Digi doc), Canon cameras, QI Aquick column, Spin column, centrifuge, Microtube, System sequencer type 3130 Applied Biosystem. The material used is the blood DNA extracts of female Sumatran elephant, and a master mix consisting of MyTaq™ HS Red Mix, nuclease-free water as well as primary forward and reverse cytochrome oxidase subunit I (COI) gene, Tris-Acetate-EDTA (TAE) buffer solution, 100 bp SYBR safe marker.

2.2 Methods

Process of PCR started with the making of Master mix by homogenizing MyTaq™ HS Red Mix and primary reverse and forward cytochrome oxidase subunit I (COI) gene Cat No. 10336022 Invitrogen (table 1) and nuclease free water (NFW). A total of 30 µl of the master mix is homogenized with 5 µl of DNA extraction results.

Table 1. COI primary sequence for Sumatran elephants

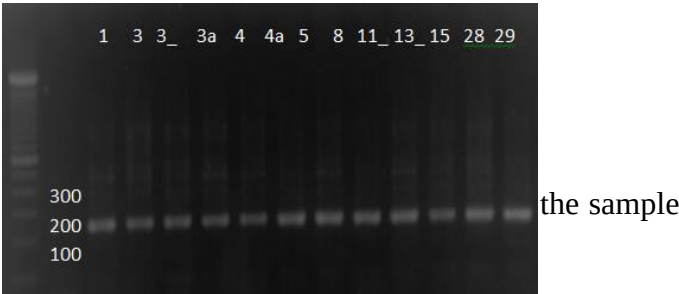
Primary	Sequence
Forward	5'GTGTCATTGTCACAGCACAC '3
Reverse	5'CTGCCAGAGGAGGATATCG '3

The results of the PCR further processed with agarose gel electrophoresis and visualized by Digi Doc. The result then purified to obtain DNA fragments. The process with sequencer takes up as many as 25 cycles with predenaturation, denaturation, forging and extension stages. Data analysis of the sequencing results were analysed using Molecular Evolution Genetics Analysis (MEGA) software version 6.0 [4]. To obtain phylogenetic tree from the COI gene sequencing result of 24 female Sumatran elephants in ETC, WKNP.

3. Results and Discussion

The DNA quality test of the amplification results of the blood samples carried out on 24 individual with a dissolved agarose gel electrophoresis. The DNA samples used are the extraction results of female Sumatran elephant blood sample that has been amplified using PCR. Visualisation of the results of agarose gel electrophoresis is done by using digital document (Digi doc). The results obtained from the visualization of agarose gel using a Digi doc in the form of DNA ribbon luminescence are visible in 13 samples.

Agarose gel electrophoresis test result for 13 DNA samples for individuals Pepi with isolation number (IN) 1, Dita (IN 3), Sulli (IN 3_), Rahmi (IN 3a), Wulan (IN 4), Poniym (IN 4a), Gunturia (IN 5), Mega (IN 8), Lingling (IN 11_), Karmila (IN 13_), Kartijah (IN 15), Arni (IN 28), and Dona (IN 29) after PCR, showed good DNA ribbon except in sample named Pepi (IN 1) (Figure 1).



Agarose gel electrophoresis test result for 12 DNA samples for individuals Bunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34), Yulia (IN 8a), Amalia (IN 14_), Yeti (IN 24), Mela (IN 28_), Heli (IN 36), Pleno (IN 12) Pepi (IN 1) dan Queen (IN 1_) after PCR showed good DNA ribbon except in samples identified as Bunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34) (Figure 2).

Figure 2. Qualitative DNA test results of the sample extracts (12 samples)

The next step was to re-extract the 4 DNA samples; Bunga (IN 27) Riska (IN 30), Meli (IN 32) dan Alma (IN 34) which then showed good results (Figure 3).

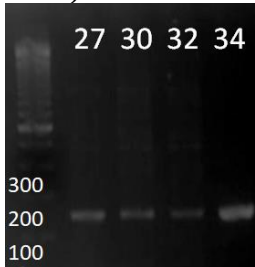


Figure 3. Qualitative DNA test results of the re-extracted sample extracts (4 samples)

After testing the quality of DNA extraction results, the samples were proceeded to sequencing and data analysis process of the electroforegram using the Molecular Evolution Genetics Analysis (MEGA)

software version 6.0 [5]. to read the genetic variations of Sumatran elephants in ETC, WKNP based on its nucleic acid to determine its genetic distance and homology [6]. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity in the population level. The COI gene as one of the genetic markers is used to study genetic characteristics of female Sumatran in ETC, WKNP. Based on the results of the sequential data analysis, it is known that the genetic distance from the 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree.

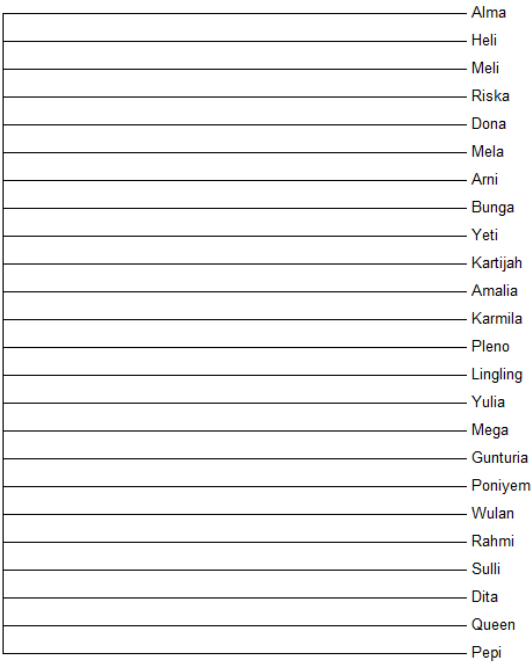


Figure 4. Constructed phylogenetic tree of female Sumatran elephants in ETC, WKNP

The absence of genetic distance as shown in Figure 4 indicates a close kinship relationship between the female individuals in the population in in ETC, WKNP, so that the entire population was derived from one population group. The future of Sumatran elephant conservation effort will need genetic information as a way to identify each of the Sumatran elephant individual. The data results of this research of the can be

used as a supporting reference to the decision making in policy direction of Sumatran elephant conservation effort in captivity, for example to determine whether or not translocation of elephants from and to ETC as an attempt to reduce the probability of inbreeding is needed.

4. Conclusion

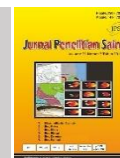
Genetic distance from 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with the homology value of 100%, supported by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded that all individual female Sumatran elephants in ETC, WKNP is derived from one population group.

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Research Articles

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

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Keyword:

Molecular method;
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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 μ l proteinase K and 200 μ l 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 (MgCl₂) 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'-ACAGCCACGCCNGTICCGATGC-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'-GCAAGATCATNTTYRTTTCITC-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).

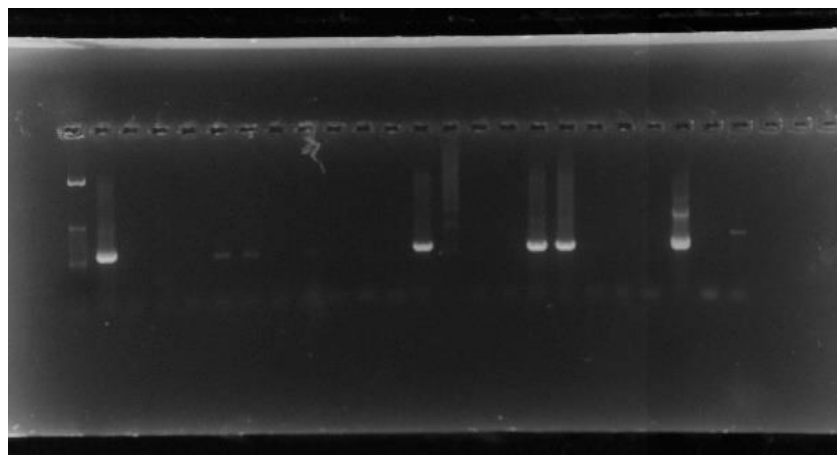


Figure 3. Electrophoresis result of Herpesviridae.

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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The Essential Contribution of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park for Wildlife Genetics Conservation

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Abstract:

Way Kambas National Park (WKNP) is home of five protected big mammals including sumatran elephants. It shares its border with 22 of 40 villages surrounding the national park. Understanding their existence in the wild is a priority, and wildlife genetics is a crucially needed. Besides poaching and habitat fragmentation, wildlife-human conflict is one big issue. Elephant Training Center (ETC) in WKNP is built for semi in-situ conservation effort on captive sumatran elephants that mainly have conflict histories with local people. Participative observation and bio-molecular analysis were conducted to learn the importance of captive Sumatran elephant for conservation effort. Through captive sumatran elephants, database and applicable methods are expected to be developed supporting the conservation of their population in the wild. Participative observation and molecular identification was carried on captive sumatran elephants in ETC, WKNP under multiple year Terapan grant of Ministry of Research and Technology Higher Education, Indonesia. Gene sequence and cytological analyses showed that the captive sumatran elephants are closely related and tend to be domesticated. Translocation among ETC to avoid inbreeding, and maintaining the captive sumatran elephant as natural as possible are highly recommended. Developing genetic database can be a reference for both captive and wild sumatran elephants.

Keywords: captive Sumatran elephant, Elephant Training Center, Way Kambas National Park, genetic conservation, wildlife

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1. Introduction

Way Kambas National Park (WKNP) is the natural habitat of the Sumatran elephant (*Elephas maximus sumatranus*). Since 2011 the presence of Sumatran elephants has been recorded in critical status in the International Union for Conservation of Nature and Natural Resources (IUCN) [1]. This is caused by the pressure of habitat fragmentation, illegal hunting and conflicts with humans [2].

Efforts to maintain the existence and continued preservation of Sumatran elephants, such as monitoring, mapping Sumatran elephant range areas and capacity analysis Sumatran elephant habitat in order to survive in

the area adjacent to the human activity. The Elephant Training Center (ETC), WKNP was established with the aim of rescuing Sumatran elephants and supporting the efforts of semi-situ conservation of Sumatran elephants that are in conflict with humans. Currently there are 69 Sumatran elephants built with 39 individual males, 26 females and 4 tillers individual. The existence of the Sumatran elephant built in ETC, WKNP raises the question of being limited population movements, their role in conservation efforts and the possibility of release as well as wildlife attractions.

The presence of elephant populations in ETC, closed WKNP is very vulnerable to the threat of deep cross-breeding. In addition to information from the location, bio-

logical information of Sumatran elephants fostered in ETC, WKNP is not widely known including information on molecular biology. Kinship analysis is needed to prevent the decline of genetic diversity in the population and prevent the occurrence of deep intermarriage. This paper was compiled to see the important role of the built Sumatran elephants in ETC, WKNP in supporting the efforts of Sumatran elephant conservation in natural habitats.

2. Materials and Methods

DNA isolation has been carried out as the beginning of the process of phylogenetic map construction of Sumatran elephants from Sumatran elephants fostered in ETC, WKNP [3,4]. The process of taking blood samples in the Sumatran elephants fostered was carried out in collaboration with the medical team of the elephant hospital Rubini Atmawidjaja, ETC, WKNP. Method optimization has been done both through simple and molecular methods, and comparing the two [5]. Molecular analysis was continued to the level of DNA sequencing carried out to see genetic variation and phylogenetic map construction of elephants [6, 7, 8]. Phylogenetic maps of Sumatran elephants were analyzed from the results of DNA sequencing (702 base pairs) [9] 65 individual Sumatran elephants fostered. Phylogenetic map of individual Sumatran elephants fostered from 65 sequenced DNA profiles and cytological profiles of 21 Sumatran elephants under the guidance of ETC WKNP were built [9, 10].

3. Results And Discussion

Conservation efforts require molecular information as a basis for determining the appropriate policies and management, including the Sumatran elephant rescue. For this reason, it is necessary to sample the source of genetic material such as blood and feces as basic data material. In compiling biomolecular information on Sumatran elephants, Sumatran elephants fostered in ETC, WKNP can be the best source of genetic material. This is because the Sumatran elephant fostered is already familiar with human existence, located in a closed location so that it is easily accessible. Blood samples are possible to be taken because of the routine examination of Sumatran elephants fostered in ETC, WKNP.

Every Sumatran elephant is guided in ETC, WKNP is accompanied by at least one mahout who is very understanding of the characteristics and behavior of each of the Sumatran elephants fostered [9]. This is very helpful and provides convenience in handling blood sampling for molecular analysis without going through anesthesia and arrest (Figure 1).



Figure 1. Handling and blood sampling in Sumatran elephants fostered in ETC, WKNP by a medical team accompanied by mahout

With the assistance of mahout and the medical team that are well known it can be carried out invasive and participatory blood sampling. Further sampling can be done by ensuring the individual, sex and age both invasive (blood samples) and non-invasive (fecal samples) [9]. From the results of molecular analysis genetic information can be obtained from fostered Sumatran elephants in the form of phylogenetic maps of fostered Sumatran elephants from 65 sequenced DNA profiles (Figure 2-3) [9] and cytological profiles of 21 individual Sumatran elephants guided by ETC WKNP (Figure 4) [10] has been awakened.

Sumatran elephants fostered in ETC, WKNP come from the same large group, and are feared to have a close kinship. Further analysis of kinship is needed. If an individual exchange from another ETC is not carried out in the long term, it is feared that deep cross marriages will occur. Cytologically shows that Sumatran elephants fostered in ETC WKNP tend to be domesticated. This is indicated by 3 forms of nonsymmetric chromosomes, namely at number 12, 24 and 27.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG
PEPI_01		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
WULAN_04	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
GUNTURIA_05	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
SALMON_06	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
RENDI_07	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
SANDI_09	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
SOGOL_10	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
TONY_11	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
INDRA_14	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CUNI_16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
MILO_22	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
YANDO_23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
YETI_24	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
KARNANGUN25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
BAYU_26	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
BUNGA_27	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
ALMA_34	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
RENOLO_43	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100	100
QUEEN_44	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100	100
SULLI_47	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100	100
RAHMI_48	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100	100
GADAR_52	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100	100
SUGENG_53	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100	100
VERDI_54	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100	100
DAENG_56	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100	100
AGAM_57	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100	100
TOMI_60	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100	100
MELA_62	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100	100
HARYONO_46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		100	100	100

Figure 2. Homology values and genetic distance of the Sumatran elephants fostered in ETC, TNWK

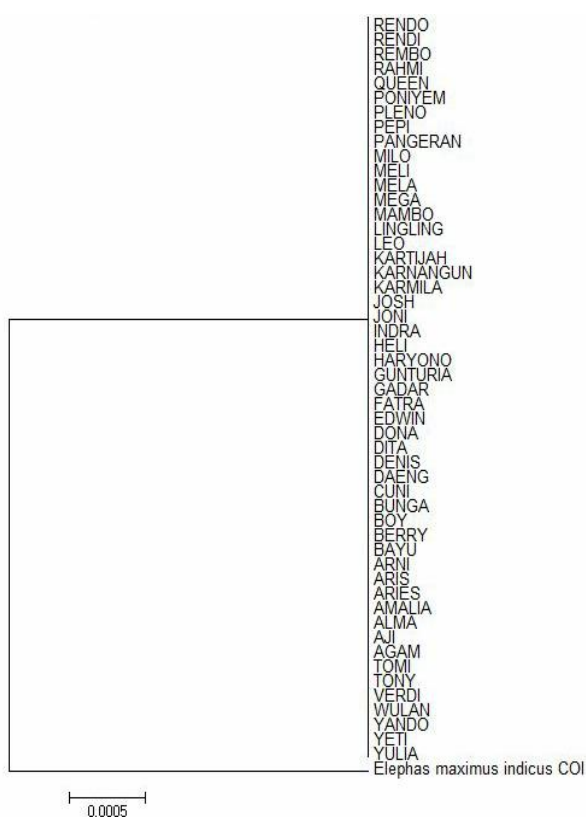


Figure 3. Phylogenetic map of fostered Sumatran elephants in ETC, TNWK

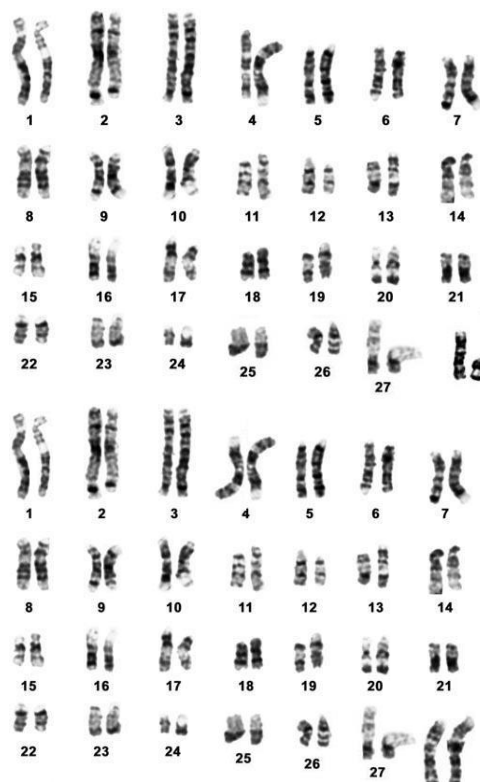


Figure 4. Cytological profiles of Sumatran elephants under the guidance of ETC, TNWK: (a) male individuals (Agam) and (b) female individuals (Lingling)

Sumatran elephants fostered in ETC, WKNP in general are a group of relatives of Sumatran elephants from a large population and have a tendency to have experienced domestication, which is already accustomed to human presence and intervention. Therefore, the release of fostered Sumatran elephants is not recommended. In connection with these results, the identification and marking of individual molecular Sumatran elephants fostered in ETC WKNP and other locations is needed, so that exchange of fostered Sumatran elephants can be carried out between captive breeding groups that have different target Sumatran elephant populations, and adjusting the management of the built Sumatran elephant, and ultimately building a data center for Sumatran elephants to be guided by wild Sumatran elephants in their natural habitat. Although already in coaching, Sumatran elephants in ETC, WKNP have an important contribution as a source of genetic material in formulating policy on the conservation of Sumatran elephants in Indonesia.

4. Conclusions

Sumatran elephants that have been fostered can make a significant contribution as a source of genetic material to the world of conservation both to determine the form of efforts and management policies

5. Acknowledgement

Thanks and appreciation are given to the Ministry of Research and Technology of Higher Education for Applied research grants, Way Kambas National Park, and Lampung Veterinary Center for their support and cooperation.

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DNA Isolation on Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park: A First Step towards Its ID Card

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Abstract

Elephant Training Center (ETC) Way Kambas National Park (WKNP) was built to support human-elephant mitigation conflict. The small population of captive sumatran elephant in ETC WKNP need a comprehensive strategy in order to maintain the genetic variation of each individual and avoid inbreeding drive. Currently, genetic studies have opened new field studies in ecology, included conservation ecology. Patterns in variation of population has been investigated by molecular method supporting species conservation effort. The captive sumatran elephant's ID Card is a necessary in database building, which included morphology, health status, and genetic profile. Genetic profile in each ID Card was filled by cytogenetic and molecular profile for RADP result, that initiated with DNA isolation. The DNA sources collected by blood sampling protocol described by Asiyah *et al.* (2016) from captive sumatran elephant in ETC, WKNP, and be carried to laboratory in cold condition. The DNA sources stored at 4°C and isolated following commercial protocol. The result of DNA isolation stored at -20°C until amplification analysis. DNA isolation was successfully done, for further individual genetic ID building.

Keywords: Conservation, DNA isolation, sumatran elephant's ID Card, WKNP

Background

Elephants belongs to the order of Proboscidea with only two living genera, *Loxodonta* (African elephant) and *Elephas* (asiatic elephant). Asian elephants are widely distributed, from South Asia to a larger part of Southeast Asia, in Malaysia and Indonesia, including Sumatra and Borneo (Fernando *et al.*, 2003; Vidya *et al.*, 2005). However, the asian elephant population has shown constant sign of decreased and in 1986 was listed as endangered species by the IUCN. One of the reason for such decrease can be found in the intensive illegal hunting of wild elephants (Stiles, 2004; Sukumar, 2006). However, the most serious threat faced by the population of elephants is habitat loss and fragmentation resulting from deforestation activities (Sukumar, 2006). These lost habitat combines with high human-

population density, like in Asia, has resulted in Human-Elephant Conflict (HEC), where elephants can damage millions of dollars' worth of agricultural crops and numerous people and elephants are killed as a result. Based on those tragic consequences, HEC is considered to be one of the biggest conservation issues in Asia and presents an urgent challenge for governments and policy makers.

To mitigate such conflict, the Elephant Training Centre (ETC) in Way Kambas National Park, East Lampung, Sumatra, Indonesia was established in the 1985 by the Indonesian government to host wild sumatran elephants (*Elephas maximus sumatranus*) in conflict. As the wild elephant population decreased, inbreeding is one of population pressure that might occurs among captive elephants within the EEC and no lineage history were ever undertaken.

As a result, the risk of inbreeding in ETC is relatively high, thus presenting a serious threat for a successful and sustainable an elephant conservation program. To mitigate such risk, DNA profile for each animal was carried out. The small population of captive sumatran elephant in ETC WKNP need a comprehensive strategy in order to maintain the genetic variation of each individual and avoid inbreeding drive. Therefore; DNA bank data needs to be built (Priyambodo *et al.*, 2017) and blood sampling were carried out in collaboration with WKNP and medical teams (Rustiati *et al.*, 2017).

Nowadays, genetic studies have become one of new field studies in ecology, included conservation ecology. Patterns in variation of population has been investigated by molecular method supporting species conservation effort. The captive sumatran elephant's ID Card is a necessary in database building, which included morphology, health status, and genetic profile. Genetic profile in each ID Card was filled by cytogenetic and molecular profile for RADP result, that initiated with DNA isolation.

Materials and Methods

This project is conducted under research grant from Directorate of Research and Community Services, Higher Education Indonesia, and in collaboration with Way Kambas National Park. The DNA sources collected by whole blood sampling protocol described by Asiyah *et al.* (2016) from captive sumatran elephant in ETC, WKNP, and be carried to laboratory in cold condition. Consideration on each individual elephant was taken (Rustiati *et al.*, 2017). Twentyeight individual elephants have been whole blood sampled. DNA isolation was conducted successfully by silica gel column methods with commercial QIAGEN Dneasy and tissue kit for blood and tissue.

Three main steps included cell preparation/lysis, DNA isolation from contaminants and DNA precipitation. Detergent contained buffer was applied for lysis. It is done to break cell and nucleus membrane so that the chromosome can be isolated from the cell. DNA isolation using

gradual wash buffer to separate DNA from RNA, protein and other contaminants with proteinase K and buffer AL and buffer AW. DNA was bound on silica gel and eluted with buffer AE. In the last step, DNA isolation is to precipitate DNA using elution buffer. DNA isolation success was qualitatively analyses by 1% agarose gel electrophoresis technique and observed under UV transiluminator. Loading dye was added to DNA to ease its movement during the electrophoresis. The DNA sources stored at 4°C and isolated following commercial protocol. The result of DNA isolation stored at -20°C until amplification analysis.

Results and Discussion

DNA was successfully separated from protein and others component (Figure 1) and kept in -40°C. Qualitative test using agarose gel electrophoresis technique.



Figure 1. Isolated DNA genome in buffer solution

In electrophoresis process 1% agarose gel was applied as stationer phase, and Tris-acetate EDTA (TAE) as movement phase. DNA genome was separated in agarose gel based on electro mobility of its negative charge of DNA molecule in certain distance. Loading dye addition on DNA may clarify migration distance. The results were visualized under UV transiluminator (Figure 2). The yield of DNA was shown by electrophoretic migration of the undigested DNA samples. The success of DNA isolation process was shown on the existence of DNA band. Sumatran eleph-

ant's DNA genome was predicted more than 10kb. It is shown by its short migration distance from the well. Isolated DNA will be used for further molecular analysis, PCR-RAPD and DNA sequencing.

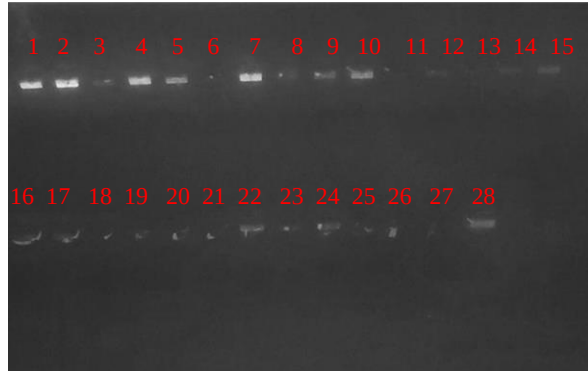


Figure 2. Visualization of qualitative test for isolation of DNA genome under UV transiluminator

Conclusion

Twentyeight whole blood samples have been successfully prepared for isolation of DNA. DNA samples were available for further individual genetic ID building and kept in -20°C.

Acknowledgement

Our high gratitude to Directorate of Research and Community Services, Higher Education Indonesia for research grant, Way Kambas National Park for the scientific collaboration and Veterinary Bureau Lampung Province for Biomolecular analysis works. Our thanks to Dr. Mikael Jazdzyk for literature supports

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Comparative Cytogenetic Study on Male and Female Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park

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Abstract

Sumatran elephant (*Elephas maximus sumatranus*) was one of five big mammals in Way Kambas National Park (WKNP). Cytogenetic study was a part of conservation effort for sumatran elephant, especially in conservation genetic field. Captive sumatran elephant's blood in Elephant Training Center (ETC) WKNP was collected to karyotype analysis. Captive sumatran elephant's chromosome was analyzed by squashing technique with pretreating cells in a hypotonic solution. The study of karyotypes were made by giemsa staining applied to captive elephant's blood cell. The diploid number of captive sumatran elephant was 56 both in male and female. The karyotype analysis performed different fundamental number in male and female, both of total fundamental number (FN) and autosomal fundamental number (FNa). Chromosome of female sumatran elephant showed the FN = 8, and FNa = 6, but in male sumatran elephant FN = 7, and FNa = . The karyotypes of X chromosome was large submetacentric and Y chromosome was small acrocentric.. The karyotype presented here will compiled with DNA fingerprinting analysis of all captive sumatran elephant in ETC WKNP as references in future breeding policy.

Keywords: captive sumatran elephant, karyotype analysis

Background

Sumatran elephant (*Elephas maximus sumatranus*) was one of five big mammals in Way Kambas National Park (WKNP), besides sumatran rhino, sumatran tiger, tapir, and bear. International Union for Conservation of Nature and Natural Resources (IUCN) classified the sumatran elephant as critically endangered species since 2011 (Gopala et al., 2011). In WKNP, sumatran elephant was categorized as wild elephant and captive elephant. The captive sumatran elephant can be found in Elephant Training Center (ETC) WKNP.

Rustiati & Priyambodo (2016) were collected the DNA samples of sumatran elephant for preliminary study of phylogenetic analysis in order to decreased the negative effect of gene flow in small population of ETC WKNP. The cytogenetic study of captive sumatran elephant in ETC WKNP has not been done. Vaughn (1986) has suggested that proboscideans may have entered the final stages of their most interesting history. Despite this, these giants

among terrestrial mammals are relatively poorly studied cytogenetically. Previous cytogenetic studies have been reported for asiatic elephant (Hungerford et al., 1966; Norberg, 1969). To our knowledge, however, no banded karyotypes have been published for either species, although Cand R-banding and NOR staining were used in a cursory cytogenetic examination of the asiatic elephant (Hartl et al., 1995).

Materials and Methods

Chromosomes from two sumatran elephants were harvested from cultured blood with fixated by formaldehyd. All the samples were acquired form captive population in Elephant Training Center, Way Kambas National Park, Lampung, Indonesia. Pro-metaphase chromosomes of all samples were examined by Giemsa staining method. Autosomes were arranged in order of size with acrocentric/telocentric autosomal pairs preceding metacentric/submetacentric pair). All karyotypes presen-

ted here represent the complete chromosome complement of single pro-metaphase cell.

The fundamental number (FN) of each karyotype were calculated from visible major chromosomal arms per set of chromosomes, and the autosomal fundamental number (FNa) of a karyotype each karyotype were calculated from visible major chromosomal arms per set of autosomes (non-sex-linked chromosomes) (Kim et al, 2005).

Results and Discussion

The normal diploid number of the sumatran elephant was 56 (Fig. 1). Hartl (1995) reported 10 individuals with diploid chromosome numbers of 56, as well as one individual with a diploid number of 55 derived by centric fusion. The normal ($2n = 56$) karyotype consisted of 24 acrocentric/telocentric autosomal pairs and three metacentric/submetacentric autosomal pairs. Chromosome complements were $2n = 56$ although it was distinguished by having one less small acrocentric pair and one additional small submetacentric pair.

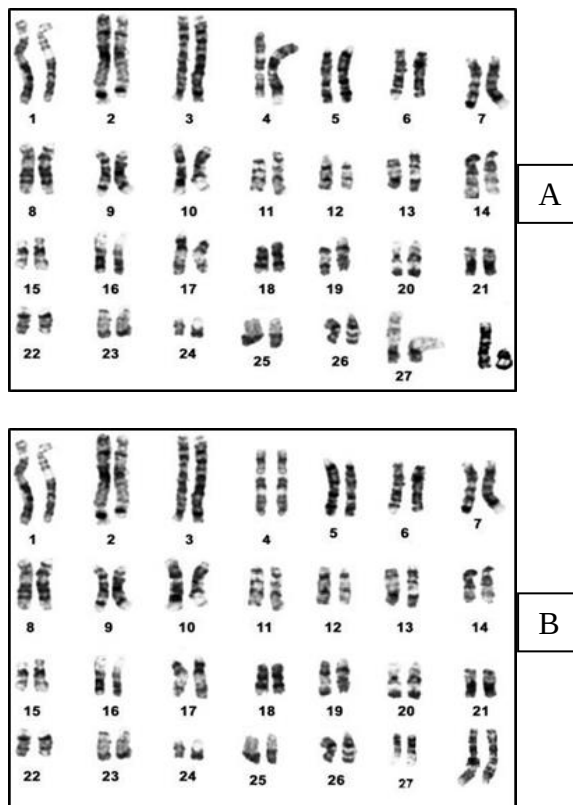


Figure 1. Cytogenetic analysis for Agam (A) and Bunga (B)

Chromosome complements were $2n = 56$ in *E. maximus* was distinguished by having one less small acrocentric pair and one additional small submetacentric pair. The presence of a distinct heterochromatic short arm for metacentric pair 27 of *E. Maximus*. Fundamental number (FN) is the number of visible mayor chromosomal arm per set chromosomes. Fundamental autosomal number (FNa) is the number of visible mayor autosomal arm per set chromosomes. Agam has FN = 7, and FNa = 6; Bunga has FN = 8, FNa = 6.

Conclusion

Both of male and female sumatran elephant have 27 autosomes, included 23 acrocentric/telocentric, and three submetacentric/metacentric. Both of male and female sumatran elephant has large submetacentric X, and small acrocentric Y found in male sumatran elephant

Acknowledgement

We thank to Directorate of Research and Community Services, Higher Education Indonesia for research grant, Way Kambas National Park for the scientific collaboration and Veterinary Bureau Lampung Province for Biomolecular analysis works. The authors appreciate greatly the help of Berry Fachry Hanifa, M.Sc. for all supports.

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Comparative Study on DNA Extraction's Qualitative Analysis of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park

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Comparative Study on DNA Extraction's Qualitative Analysis of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park

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Abstract. DNA extraction procedures may exhibit different levels of sensitivity. A preliminary effort to obtain DNA from captive Sumatran elephants (*Elephas maximus sumatranus*) in Elephant Training Center Way Kambas National Park, Lampung for building their molecular individual identification was done. DNA of 30 male and 23 female captive elephants' blood samples was extracted by QIAGEN. Qualitative analysis of DNA extraction of captive elephant was conducted using two methods, simple electrophoresis and electrophoresis based on PCR product. The aim of this project is to compare those two methods of qualitative analysis of DNA extraction. The first method used 1% agarose gel electrophoresis in the TAE buffer (Tris-Acetate-EDTA), while the second method used Polymerase Chain Reaction (PCR) technique with Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) primer. The simple electrophoresis showed 41.5 % positive samples, while the second method showed 86.7% positive samples. The electrophoresis based on PCR product exhibited more sensitivity to detect the DNA from blood of each captive elephant.

1. Introduction

Way Kambas National Park (WKNP) with 125,621.3 ha area, is the natural habitats for five big mammals including Sumatran elephant (*Elephas maximus sumatranus*) [1]. In the last 25 years, Sumatran elephant lost its habitat and its population decreased rapidly [2]. Elephant Training Center in WKNP is one of the conservation efforts for captive Sumatran elephant due to human-elephant conflict. Genetic information is needed in elephant conservation, especially in captivity which has high inbreeding pressure. Low genetic diversity in a population will affect its survival [3]. Molecular genetic analysis approach in conservation can be applied to determine genetic diversity in Sumatran elephant. Sequencing can be done using base material, good DNA extract. Technique for DNA quality test include spectrophotometer and electrophoresis to detect gene *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH).

2. Materials and Methods

This research was carried out between January and February 2018, in collaboration with Way Kambas National Park for sample collection and conducted in Biotechnology Veterinary Bureau Lampung.



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There were 53 Sumatran elephants' blood samples, consisting of 23 female and 30 male samples. The samples were taken from Prof. Dr. Ir. H. Rubini Atmawidjaja Elephant Hospital, Elephant Training Center, WKNP [4]. The DNA extraction process were referencing to the extraction protocol of DNeasyR Blood & Tissue Kit from QIAGEN.

DNA extract quality was tested with a simple technique using agarose gel electrophoresis 1% with the addition of SYBR safe as stains and TAE buffer as mobile phase. The electrophoresis step used 100V for 17 minutes and the DNA molecules with good quality will show band luminescence that can be monitored by digi doc under UV light. DNA extract quality test in molecular technique was done with *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH) primary that consist a pair of primers, *forward primer* and *reverse primer* (table 1).

Table 1. GAPDH primary sequence.

PriPrimary	SeSequence
FoForward	5' 5'ATCACTGCCACCCAGAAGAC3'
RReverse	5' 3'CATGCCAGTGAGCTTCCCGTT5'

DNA extract quality test with PCR GAPDH was started by mix mastering, homogenizing Platinum Blue PCR SuperMix with GAPDH primary (Reverse primer and Forward primer) and DNA. In pre denaturation phase, 95°C temperature was applied for 5 minutes. Pre denaturation phase was applied to make sure the DNA genome double chains can be separated into single ones. The second step was denaturation step with 94°C temperature for 20 seconds. Denaturation was the early process to separate DNA double chains into two separate chains. The third step, annealing, 57°C temperature were applied for 45 seconds, as primary identifier for DNA target with unique base pairs in an organism. The fourth step is extension phase in 72°C for 1 minute. Extension phase was the DNA new chain elongation phase. The fifth step was post-extension phase in 72°C temperature for 5 minutes and 4°C to complete the last step. The first and fifth steps were repeated once and the second, third and fourth were repeated for 35 times.

The PCR results then observed and separated with 1.5% agarose gel electrophoresis. The first well contained 100bp marker as amplicon length indicator. Electrode then connected with the power supply to induce movement from the DNA for 30 minutes with 100V. The gel moved from electrophoresis device into digi doc, visualization result was used to compare the quality test result of simple and molecular techniques.

3. Results and Discussion

Based on electrophoresis analysis on DNA quality, 13 of 23 female blood samples (figure 1) and 9 of 30 male blood samples (figure 2) showed positive results.



Figure 1. Electrophoresis results of female Sumatran elephant blood samples (n=23)

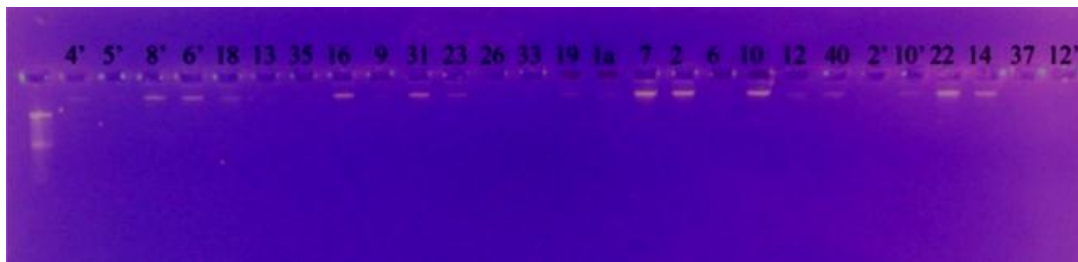


Figure 2. Electrophoresis results of male Sumatran elephant blood samples (n=30)

Positive results in electrophoresis analysis were showed by DNA band illuminescence, from SYBR *safe* bonded with the DNA. There are five factors causing the positive results, 1) high number DNA extracted, 2) suitable protocol selection, 3) good quality protocol and have been used for the same samples for better success during extraction process, 3) steril condition, 4) suitable operational standard in treating samples, 5) samples and materials condition supporting the extraction. Negative results in electrophoresis process may be affected by samples' condition, external contaminant (storage procedure, handling and shipping) and internally (changing in chemical structure in blood samples due to additive solution for preservation, the small number of DNA extracts that caused low quality of DNA band.

DNA molecular quality test using Primer of *Glyceraldehyde-3-phosphate Dehydrogenase* (GAPDH) via Polymerase Chain Reaction on certain temperature and time so that the amplification process can be proceeded and targeted DNA can be detected. Electrophoresis on its DNA molecules showed positive results on all female Sumatran elephant blood samples (figure 3), and 23 of 30 males samples (figure 4).

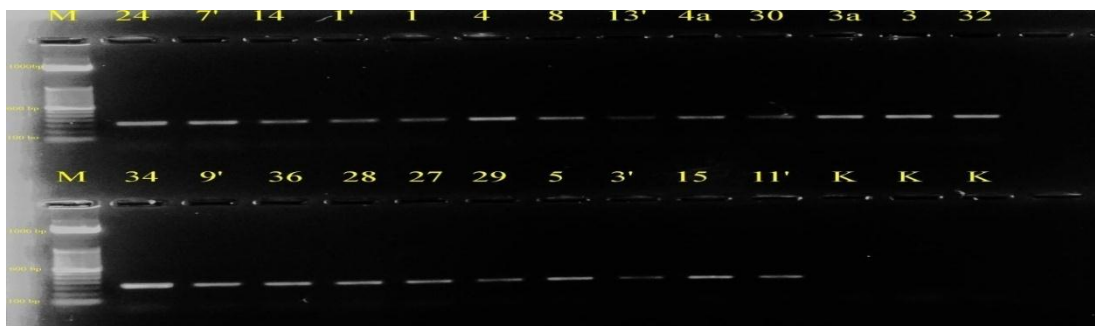


Figure 3. DNA qualitative test of female Sumatran elephants' blood samples using molecular technique

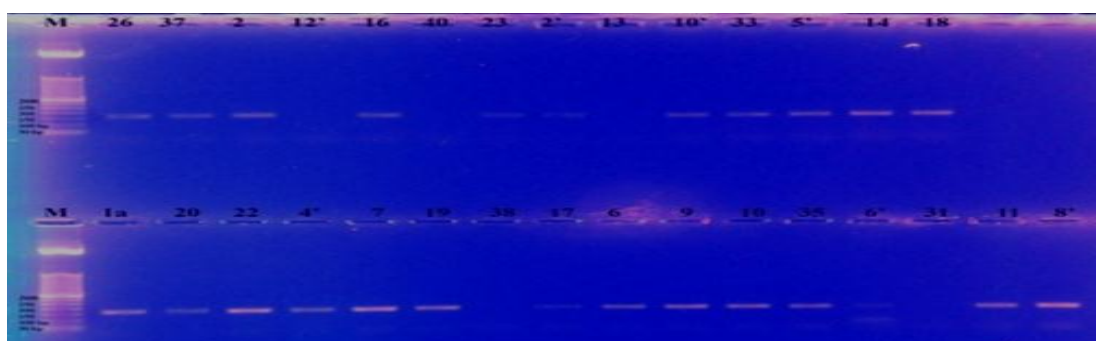


Figure 4. DNA qualitative test of male Sumatran elephants' blood samples using molecular technique

Molecular technique using GAPDH had better results than the simple technique, as primer GAPDH is gene acted coding GAPDH enzyme in organisms. Therefore, it helped to see the DNA amplicon band from the extraction that had small number DNA. The DNA molecules solved in the buffer has been multiplied through Xn PCR. Seven samples gave negative results in both simple and molecular

techniques. It may be because of the degraded samples during preservation. DNA degradation may be happened when the samples kept for a long period of time. Stable temperature during preservation (-20 °C) is necessary to maintain the DNA quality of the samples [5]. Freezing/thawing process may affect the DNA quality, especially on DNA nitrogen base [6]. Extraction maybe also affected by the materials for homogenization and the solvent to eliminate the residue [7].

4. Conclusions

Different results were observed on DNA quality extraction when two different techniques were applied. Of 53 samples, technique using only electrophoresis showed 41.5% positive results while the second technique using PCR and GAPDH primer showed 86.7% positive results. The latter technique offer significant increase of efficiency for DNA extraction, thus reducing the time and cost associated with repeated DNA extraction.

Acknowledgment

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PROSIDING SEMINAR NASIONAL HASIL PENELITIAN

Bandar Lampung, 13 Desember 2018

Tema:

**Diseminasi Hasil Penelitian Dalam Mendukung
Pembangunan Berkelanjutan**



**LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
UNIVERSITAS LAMPUNG**

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**TEKNIK PENGENALAN TANDA TIDAK LANGSUNG KEBERADAAN BADAK
SUMATERA (*Dicerorhinus sumatrensis*) di SUAKA RHINO SUMATERA, TAMAN
NASIONAL WAY KAMBAS**

**TECHNIQUES THE DISCOVERY OF INDIRECT MARKS FROM THE SUMATRAN
RHINO (*Dicerorhinus sumatrensis*) IN SUMATRAN RHINO SANCTUARY WAY
KAMBAS NATIONAL PARK**

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ABSTRAK

Badak sumatera (Dicerorhinus sumatrensis) merupakan spesies badak terkecil di dunia merupakan satu dari 5 spesies badak yang masih mampu bertahan dari kepunahan selain badak Jawa. Badak sumatera terdaftar dalam Convention on International Trade in Endangered Species of Wild Fauna and Flora (*CITES*) Apendiks I sejak tahun 1975. Penelitian ini bertujuan untuk mengetahui karakteristik tanda tidak langsung badak sumatera, pada bulan Januari – Februari 2018 di Suaka Rhino Sumatera, Taman Nasional Way Kambas. Karakteristik tanda tidak langsung termasuk gesekan badan, bekas urinasi, tapak kaki. Tanda tidak langsung badak sumatera berperan dalam penandaan teritori, menarik pasangan, serta membedakan karakteristik tiap individu badak sumatera.

Kata kunci—Badak sumatera, tanda tidak langsung, Suaka Rhino Sumatera, Taman Nasional Way Kambas

Abstract—The Sumatran rhino (*Dicerorhinus sumatrensis*) is the smallest rhino species in the world, which is one of 5 rhino species that are still able to survive extinction in addition to the Javan rhino. The Sumatran rhino is registered in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (*CITES*) Appendix I since 1975. Research was conducted to learn the indirect signs of the sumatran rhino including their characteristics. Research was done in January - February 2018 at the Sumatra Rhino Sanctuary, Way Kambas National Park. Observations were carried out by measuring indirect signs found, including body friction, urine spray, footprints. The indirect signs of sumatran rhino is used to mark territories, attract mates, and distinguish the characteristics of each individual Sumatran rhino

Keyword—Sumatran rhino, indirect sign, Sumatra Rhino Sanctuary, Way Kambas National Park

PENDAHULUAN

Suaka Rhino Sumatera (SRS) adalah suatu lembaga konservasi semi in-situ yang dinaungi oleh Taman Nasional Way Kambas (TNWK) bekerja sama dengan Yayasan Badak Indonesia (YABI) untuk menyediakan kawasan yang sangat luas dan lebih alami, yang diharapkan akan membuat badak sumatera (*Dicerorhinus sumatrensis*) yang sudah terancam punah ini dapat berkembang biak, mempertahankan hidupnya, kebutuhan pakan terpenuhi, mendapatkan areal jelajah yang luas, serta habitat yang lebih alami. Pemeliharaan sealami mungkin

adalah upaya untuk meningkatkan kesejahteraan dan reproduksi badak, sehingga mendekati kondisi normal seperti di habitat alamnya. Suaka Rhino Sumatera mencoba untuk mengungkap semua fakta informasi tentang badak sumatera secara ilmiah sehingga diharapkan menjadi pusat riset dan pengembangbiakan badak. Suaka Rhino Sumatera berupa kandang berhutan seluas 100 ha, yang terbagi menjadi 10 petak, dan dikelilingi oleh pagar beraliran listrik, guna mengamankan badak di dalam kawasan tersebut, serta mencegah gangguan satwa liar.

Badak sumatera (*Dicerorhinus sumatrensis*) merupakan salah satu spesies badak yang dimiliki Indonesia selain badak jawa (*Rhinoceros sondaicus*). Badak sumatera juga merupakan spesies badak terkecil di dunia merupakan satu dari 5 spesies badak yang masih mampu bertahan dari kepunahan selain badak jawa (Strien, 1974; Groves *et al*, 2010; Goossens *et al*, 2013), badak india, badak hitam afrika, dan badak putih afrika. Diperkirakan populasi badak bercula dua ini hanya berkisar antara 220-275 ekor. *International Union for Conservation of Nature and Natural Resources* (IUCN) Redlist memasukkan badak sumatera (*Dicerorhinus sumatrensis*) dalam daftar status konservasi *critically endangered* (kritis; CE). Selain itu, badak sumatera juga terdaftar dalam *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) Apendiks I sejak tahun 1975. CITES Apendiks I berarti badak sumatera dilindungi secara internasional dari segala bentuk perdagangan (CITES, 2011).

Adapun tujuan dari pengamatan ini adalah sebagai berikut :

1. Untuk mempelajari ciri-ciri dan bentuk keberadaan badak sumatera
2. Untuk mengetahui daerah teritori badak sumatera
3. Untuk membedakan karakteristik tiap individu badak sumatera

METODE

Pengamatan dilakukan pada tanggal 18 Januari 2018 – 21 Februari 2018 di Suaka Rhino Sumatera, Taman Nasional Way Kambas. Dimulai setiap hari pada pukul 07:00 - 11:00 WIB dan 13:00 -16:00 WIB. Terdapat 2 ekor badak jantan yaitu Harapan, Andatu dan 3 ekor betina yaitu Bina, Ratu, dan Delilah..

Pengamatan dilakukan dengan cara berjalan di sepanjang jalan setapak yang ada di hutan. Tahapan kerja yang dilakukan dalam pengamatan yaitu :

1. Pengukuran gesekan tubuh badak sumatera yang terdapat di pohon (lumpur), *Urine spray* yang masih segar, tapak kaki
2. Pengambilan gambar dari setiap temuan tanda tidak langsung di hutan
3. Analisis data secara deskriptif.

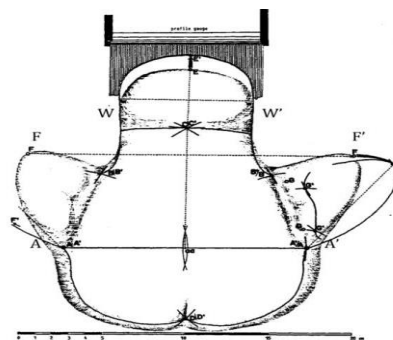
HASIL DAN PEMBAHASAN

Suaka Rhino Sumatera dikatakan sebagai kontrol positif tanda tidak langsung keberadaan badak sumatera, karena data sekunder badak sumatera seperti tapak kaki, gesekan badan, gesekan cula, *urine spray*, kubangan, feses, pelintiran, patahan, dan gigitan sudah pasti ditemukan dengan luas jelajah yang hanya mencapai 10 ha setiap badak. Berbeda dengan di hutan yang merupakan kontrol negatif, dimana tanda sekunder badak sulit ditemukan karena merupakan badak liar yang biasanya hanya ditemukan pada *Camera trap* dan hampir tidak dijumpai secara langsung oleh petugas Taman Nasional atau Lembaga Swadaya Masyarakat. Pengamatan dilakukan dengan didampingi serta adanya informasi dari *keeper* dan dokter. Tiap badak memiliki karakteristik yang berbeda setiap individu antara jantan, betina, remaja, dan anakan.

A. Hasil Pengukuran

1. Tapak Kaki Badak Sumatera

Pada pengukuran jejak badak sumatera dilakukan dengan mengukur kuku-kuku badak selama pengamatan. Masing-masing jejak yang ditemui diambil beberapa jejak terbaik yang diukur berdasarkan cara pengukuran yang diperkenalkan oleh Strien pada penelitiannya.



Gambar 1. Teknik pengukuran jejak yang diperkenalkan oleh Strien (1985)

Keterangan : W^W = panjang kaki tengah

F^F = panjang kuku samping

A^A = jarak antar kedua sisi kuku

Tabel 1. Hasil pengukuran tapak kaki badak sumatera

Hari / tanggal	Nama badak	Jenis kelamin	Umur (tahun)	Jejak kaki (cm)		
				W^W	F^F	A^A
Minggu, 21-1-2018	Delilah	Betina	2	8	19	16
Minggu, 21-1-2018	Ratu	Betina	15-18	8	21,5	18
Selasa, 23-1-2018	Bina	Betina	32	10	25	18
Jumat, 26-1-2018	Harapan	Jantan	10	11	24	18
Senin, 29-1-2018	Andatu	Jantan	6	11	24	16

2. Luasan *Urine Spray* Badak Sumatera

Urine spray adalah *urine* yang disemprotkan pada daun atau semak. *Urine spray* dikeluarkan pada saat badak stres, birahi, merasa terganggu, menarik perhatian pasangan dan menandai daerah teritori. Menurut beberapa pengamatan diketahui bahwa *urine spray* badak jantan lebih tinggi daripada badak betina.

Tabel 2. Hasil pengukuran *urine spray* badak sumatera

Hari / tanggal	Nama badak	Panjang (cm)	Lebar (cm)	Tinggi (cm)
Minggu/21-1-2018	Delilah	300	159	106
Senin/22-1-2018	Ratu	365	145	130
Selasa/23-1-2018	Bina	320	120	116
Jumat/26-1-2018	Harapan	300	200	150
Sabtu/17-2-2018	Harapan	668	228	175
Senin/29-1-2018	Andatu	500	270	180
Minggu/18-2-2018	Andatu	500	300	160

3. Tinggi Badan Badak Sumatera

Pengukuran tinggi badan badak sumatera bertujuan untuk membedakan tiap individu. Diduga bahwa apabila luasan *urine spray* lebih tinggi daripada tinggi badan, maka badak tersebut sedang menarik perhatian lawan jenis (merasa dirinya gagah).

Tabel 3. Hasil pengukuran tinggi badan badak sumatera

Hari/tanggal	Nama badak	Tinggi badan (cm)
Kamis, 15-2-1018	Harapan	130.5
Kamis, 15-2-1018	Andatu	120
Jum'at, 16-2-2018	Bina	128
Jum'at, 16-2-2018	Ratu	110
Jum'at, 16-2-2018	Delilah	116

B. Hasil Temuan

Hasil temuan yang didapatkan di hutan berupa kubangan, defekasi, pelintiran, gesekan cula, gigitan, serta patahan sapling.



Gambar 2. Kubangan Andatu.



Gambar 3. Pelintiran



Gambar 4. Kopian (*Antidesma montanum*)



Gambar 5. Defekasi



Gambar 6. Gesekan cula



Gambar 7. Tapak kaki Andatu

KESIMPULAN

Dari hasil pengamatan yang telah dilakukan, dapat disimpulkan bahwa semua tanda tidak langsung dan hasil temuan digunakan badak untuk menandai teritori dan menarik pasangan. Suaka Rhino Sumatera sebagai kontrol positif badak sumatera sehingga hasil pengamatan tanda tidak langsung ini dapat digunakan sebagai informasi tim rhino dan masyarakat.

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Puji syukur kehadiran Allah SWT yang telah memberi kesempatan ini. Penulis mengucapkan terimakasih kepada :

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GENOMIC DNA ISOLATION OF GAJAH SUMATERA (*Elephas maximus sumatrensis*) IN ELEPHANT TRAINING CENTER, WAY KAMBAS NATIONAL PARK, EAST LAMPUNG

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ABSTRACT

Elephant Training Center, Way Kambas National Park holds 44 captive sumatran elephants (*Elephas maximus sumatrensis*). This *critically endangered species* small population is in high risk of *inbreeding*. Its genomic DNA isolation was done to provide DNA amplification material for phylogenetic analysis. Individual blood samples were collected from 8 different individuals based on age and sex characteristics and stored at EDTA-anticoagulated blood tubes. Qualitative test by 1% agarose gel electrophoresis, visualized by UV transilluminator. Isolated genomic DNA was kept in -20°C elution buffer solution.

Keywords: sumatran elephant, blood, DNA isolation, Way Kambas National Park.

1. INTRODUCTION

Sumatran elephant (*Elephas maximus sumatrensis*) is endemic asian elephant that lives at seven provinces in Sumatera, included Lampung (Soeharsono, 2007). Since 2011, International Union for Conservation of Nature and Natural Resources (IUCN) classified the sumatran elephant as critically endangered species (Gopala *et al.*, 2011). Elephant Training Center in Way Kambas National Park was established to be one of solution of this problem.

Elephant Training Center in Way Kambas National Park holds 44 captive sumatran elephants. The small size of the population of the sumatran elephant allows to increased the inbreeding probability, then will made negative effect on gene flow in the sumatran elephant population. The bad gene flow of the population can adversely affect the viability of individual members of the population.

Research kinship patterns sumatran elephant has begun to do, especially regarding the relationship filogenik Asian elephants in Indonesia, Nepal, India and the elephants of the African continent. Fernando *et al* (2003) stated that the Sumatran elephants are related to elephants in Asia with diverse levels of phylogenetic closeness, while Sulandari and Zein (2012) states based on mitochondrial DNA genetic variation among populations of Sumatran elephants in Lampung, South Sumatra and Bengkulu is low.

Kinship patterns of captive sumatran elephants in Elephant Training Center in Way Kambas National Park has not been done. Potential high inbreeding and low genetic variation push for an immediate kinship between individual data collection of sumatran elephants in Elephant Training Center in Way Kambas National Park. Kinship patterns can be analyzed with DNA finger printing methods, for example methods Random Amplified Polymorphism DNA (RAPD) (Kumar and Gurusubramain, 2011). RAPD molecular markers are widely used in the analysis of genetic variation because it can be done without the need for data on the nucleotide sequence of the DNA template to be amplified (Yadav *et al.*, 2012). Therefore, research on the molecular genetics of the Sumatran elephant population is an important step that must be waged. Through genetics approach, expected to conservation efforts more focused and able to rescue survival of the Sumatran elephant. RAPD molecular markers require genomic DNA as a base material for the amplification and analysis of genetic kinship.

2. MATERIALS AND METHODS

Blood sampling

Venous blood samples were obtained from 8 individual sumatran elephants in Elephant Training

Center, Way Kambas National Park, based on age and sex characteristics (Table 1). Whole bloods were collected by syringe and stored at 3 mL EDTA-anticoagulated blood tube (Pic. 1). All samples were kept at 4°C before DNA isolation was performed.

Table 1. Sumatran elephants in Elephant Training Center, Way Kambas National Park

Number of Sample	Name of elephant	Sex	Age(years)
1	Agam	male	37
2	Daeng	male	29
3	Pangeran	male	2
4	Sugeng	male	6
5	Lingling	female	38
6	Suci	female	27
7	Yulia	female	3
8	Queen	female	5



Pic 1. Whole bloods were collected at 3 mL EDTA-anticoagulated blood tube

DNA Isolation

DNA was isolated from sumatran elephant's whole blood using Dneasy Blood & Tissue Protocol. Well mixed of 200 μ L EDTA-anticoagulant-treated blood, 20 μ L proteinase K and 200 μ L buffer AL in 1,5 mL microtube was incubate in 56°C for 10 minutes. Then 200 μ L ethanol 96% was added followed by centrifugation at 8000 rpm for 1 minute. Washing step was done by added AW1 and AW2 with high rise centrifugation. AE buffer was added to center of the spin column for ellution step than centrifuge at 8000 rpm for 1 minute.

DNA Qualitative Test

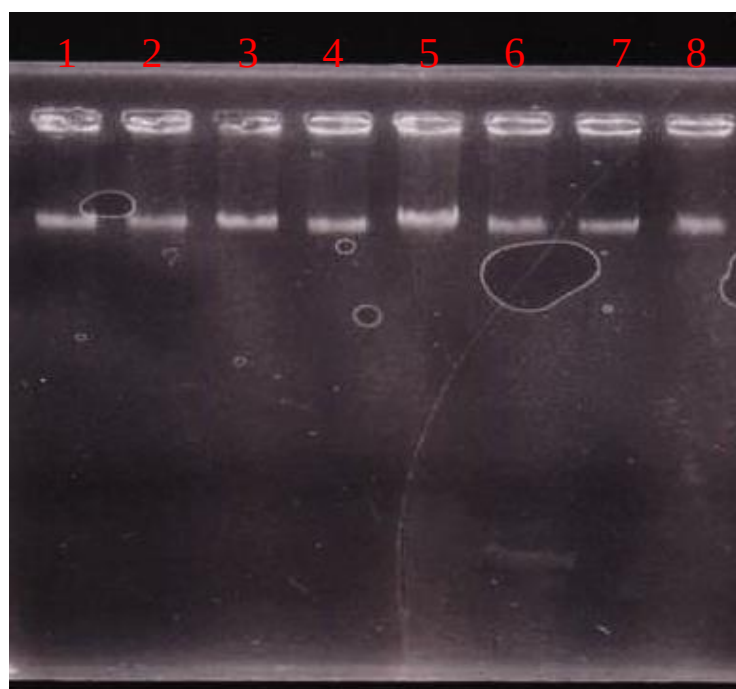
Isolated DNA was test by qualitative method, loaded in 1% agarose gell electrophoresis. DNA was visualized by good view staining and photographed under ultraviolet light. Isolated DNA kept at -20°C before DNA amplification and another reaction performed.

RESULTS

Genomic DNA from 8 individual sumatran elephant was eluted in AE buffer sollution than kept in -20°C (Pic 2). DNA was visualized by good view staining and photographed under ultraviolet light as shown at Pic 3.



Pic 2. Genomic DNA from elephant eluted in buffer solution



Pic 3. Photograph of qualitative test from genomic DNA under UV light
(1: Agam, 2: Daeng, 3: Pangeran, 4: Sugeng, 5: Lingling, 6: Suci, 7: Yulia, 8: Queen)

3. DISCUSSION

The type and condition of specimen and tissues, according to its origin, are key factors in selecting a DNA isolation method. The total genomic DNA from whole blood of sumatran elephant was isolated with Dneasy Blood & Tissue Protocol from QIAGEN®. The protocol consists of three steps, lysis, washing, and elution, respectively. Lysis step using AL buffer, proteinase-K under incubating condition in 56°C. This step will destruct the cell wall and nucleus membrane. Second step was done by twice washing step, with AW1 and AW2 buffer with centrifugation condition. This step will removed the other materials besides DNA. Third step will eluted the DNA from the silica gel in buffer solution with AE buffer.

Using the silica-coated gell in DNA isolation step allows to reversibly bind and purify DNA away from cell debris, proteins, and another materials released upon directed cell lysis. The binding of DNA to silica gell seems to be driven by dehydration and hydrogen bond formation, which competes against weak electro-static repulsion. Hence, a high concentration of slat will help drive DNA adsorption onto silica, and low concentration will help to release the DNA from the silica gell. The total genomic DNA will kept in -20°C condition before PCR analysis to construct the phylogenetic tree between sumatran elephant in Elephant Training Center, Way Kambas National Park.

4. CONCLUSION

The resulted quantity of genomic DNA is enough to conduct further PCR reactions. Using the above methode, good quality DNA samples from a sumatran elephant whole blood were isolated to study the phylogenetic analysis in sumatran elephant population.

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SHORT COMMUNICATION: IDENTIFICATION AND CHARACTERIZATION OF COI GENE IN FEMALE SUMATRAN ELEPHANT (*Elephas maximus sumatranus*) IN ELEPHANT TRAINING CENTRE, WAY KAMBAS NATIONAL PARK

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Abstract :

Sumatran Elephant is a subspecies of endemic Asian elephants on the island of Sumatra and is included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. The building of the Elephant Training Centre (ETC) in Way Kambas National Park (WKNP) is one of the conservation efforts of Sumatran elephants. Small and closed population size lead to an increased risk of inbreeding that triggers reduction in genetic variation and viability and increases the risk of extinction. The phylogenetic pattern of Sumatran elephants in Indonesia has shown a low population genetic diversity. Genetic diversity information is indispensable to support the direction of decision making in Sumatran elephant conservation policy. The DNA isolation of Sumatran elephants in ETC, WKNP has performed as a first step to trace its genetic variation. The advanced step of DNA isolation is the use of Cytochrome Oxidase subunit I (COI) gene for identification of genetic characteristics in Sumatran elephants. The COI gene is one of the genes on the mitochondrial genome and in molecular studies it is used as a genetic marker to study genetic characteristics between species and individuals. Identification and characterisation are done by sequencing process and data analysis in the form of electroforegram using Molecular Evolution Genetics Analysis (MEGA) software version 6.0. to see the genetic diversity of the female Sumatran elephant population in ETC, WKNP. Based on the results of the analysis it is indicated that the genetic distance of 24 individual female Sumatran elephant from PLG, TNWK is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded all individual female Sumatran elephants in the PLG, TNWK is derived from one population group.

Keywords: Sumatran elephant, COI gene, genetic distance, homology, phylogenetic

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1. Introduction

Sumatran elephant is a subspecies of Asian elephants endemic to the island of Sumatra. It is also included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. Sumatran elephants characterized as a land mammal with a grouping pattern led by adult females (matrilineal) [1] [2]. One of the conservation efforts site for Sumatran elephants is in ETC, WKNP which is part of the area of the natural habitat of Sumatran elephants. The presence of ETC plays an important role in conservation efforts, such as handling Sumatran elephants that are affected by habitat fragmentation, wild hunting, and human-wildlife conflict with national park buffer village communities. The population size of Sumatran elephants in ETC, WKNP in 2016 shows a total of 66 individuals with 36 male elephants, and 30 female elephants [2]. Currently with the birth of two elephants, the total number of Sumatran elephants increases to 68 individuals [3].

Sumatran elephants as critically endangered animals depend on their ability to maintain their survival rate, while in closed populations such as ETC increases the chances of inbreeding, which is caused by the absence of supporting data describing the genetic diversity of the elephant population within it. The genetic diversity of Sumatran elephants in the population affects its ability to adapt to changing environmental conditions. Reduced genetic diversity increases the potential for extinction. Sumatran elephant as an umbrella species with various challenges for its survival; including low genetic diversity should be saved from such threats. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity at the population level. COI gene as one of genetic markers is used to study genetic characteristics of female Sumatran elephants in ETC, WKNP.

2. Materials and Methods

2.1 Materials

The tools used in this research are 0.2 µl micro tubes, Laminar Air Flow (LAF), vortex, Micropipette, Microtip, Veriti Thermal Cycler, agarose gel

electrophoresis, Digital Documents (Digi doc), Canon cameras, QI Aquick column, Spin column, centrifuge, Microtube, System sequencer type 3130 Applied Biosystem. The material used is the blood DNA extracts of female Sumatran elephant, and a master mix consisting of MyTaq™ HS Red Mix, nuclease-free water as well as primary forward and reverse cytochrome oxidase subunit I (COI) gene, Tris-Acetate-EDTA (TAE) buffer solution, 100 bp SYBR safe marker.

2.2 Methods

Process of PCR started with the making of Master mix by homogenizing MyTaq™ HS Red Mix and primary reverse and forward cytochrome oxidase subunit I (COI) gene Cat No. 10336022 Invitrogen (table 1) and nuclease free water (NFW). A total of 30 µl of the master mix is homogenized with 5 µl of DNA extraction results.

Table 1. COI primary sequence for Sumatran elephants

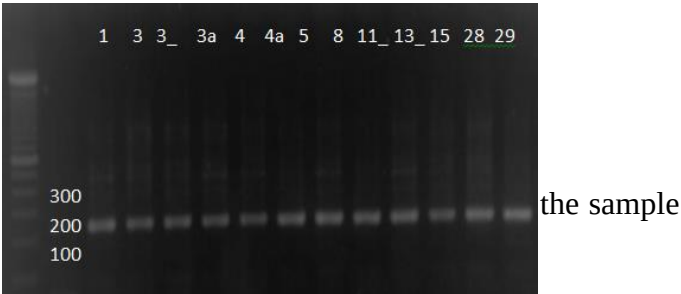
Primary	Sequence
Forward	5'GTGTCATTGTCACAGCACAC '3
Reverse	5'CTGCCAGAGGAGGATATCG '3

The results of the PCR further processed with agarose gel electrophoresis and visualized by Digi Doc. The result then purified to obtain DNA fragments. The process with sequencer takes up as many as 25 cycles with predenaturation, denaturation, forging and extension stages. Data analysis of the sequencing results were analysed using Molecular Evolution Genetics Analysis (MEGA) software version 6.0 [4]. To obtain phylogenetic tree from the COI gene sequencing result of 24 female Sumatran elephants in ETC, WKNP.

3. Results and Discussion

The DNA quality test of the amplification results of the blood samples carried out on 24 individual with a dissolved agarose gel electrophoresis. The DNA samples used are the extraction results of female Sumatran elephant blood sample that has been amplified using PCR. Visualisation of the results of agarose gel electrophoresis is done by using digital document (Digi doc). The results obtained from the visualization of agarose gel using a Digi doc in the form of DNA ribbon luminescence are visible in 13 samples.

Agarose gel electrophoresis test result for 13 DNA samples for individuals Pepi with isolation number (IN) 1, Dita (IN 3), Sulli (IN 3_), Rahmi (IN 3a), Wulan (IN 4), Poniym (IN 4a), Gunturia (IN 5), Mega (IN 8), Lingling (IN 11_), Karmila (IN 13_), Kartijah (IN 15), Arni (IN 28), and Dona (IN 29) after PCR, showed good DNA ribbon except in sample named Pepi (IN 1) (Figure 1).



Agarose gel electrophoresis test result for 12 DNA samples for individuals Bunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34), Yulia (IN 8a), Amalia (IN 14_), Yeti (IN 24), Mela (IN 28_), Heli (IN 36), Pleno (IN 12) Pepi (IN 1) dan Queen (IN 1_) after PCR showed good DNA ribbon except in samples identified as Bunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34) (Figure 2).

Figure 2. Qualitative DNA test results of the sample extracts (12 samples)

The next step was to re-extract the 4 DNA samples; Bunga (IN 27) Riska (IN 30), Meli (IN 32) dan Alma (IN 34) which then showed good results (Figure 3).

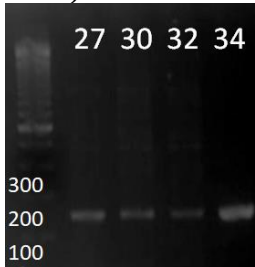


Figure 3. Qualitative DNA test results of the re-extracted sample extracts (4 samples)

After testing the quality of DNA extraction results, the samples were proceeded to sequencing and data analysis process of the electroforegram using the Molecular Evolution Genetics Analysis (MEGA)

software version 6.0 [5]. to read the genetic variations of Sumatran elephants in ETC, WKNP based on its nucleic acid to determine its genetic distance and homology [6]. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity in the population level. The COI gene as one of the genetic markers is used to study genetic characteristics of female Sumatran in ETC, WKNP. Based on the results of the sequential data analysis, it is known that the genetic distance from the 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree.

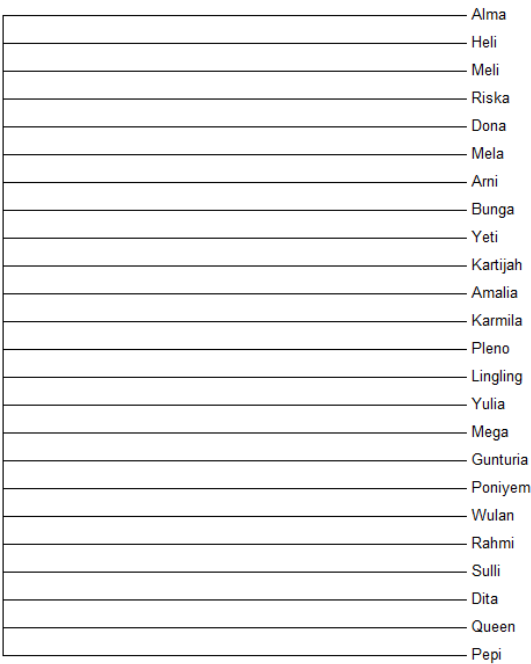


Figure 4. Constructed phylogenetic tree of female Sumatran elephants in ETC, WKNP

The absence of genetic distance as shown in Figure 4 indicates a close kinship relationship between the female individuals in the population in in ETC, WKNP, so that the entire population was derived from one population group. The future of Sumatran elephant conservation effort will need genetic information as a way to identify each of the Sumatran elephant individual. The data results of this research of the can be

used as a supporting reference to the decision making in policy direction of Sumatran elephant conservation effort in captivity, for example to determine whether or not translocation of elephants from and to ETC as an attempt to reduce the probability of inbreeding is needed.

4. Conclusion

Genetic distance from 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with the homology value of 100%, supported by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded that all individual female Sumatran elephants in ETC, WKNP is derived from one population group.

5. Acknowledgement

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DNA Detection Sensitivity Test from Blood Sample Extraction of Sumatran Elephant (*Elephas Maximus Sumatranus*) Male at Elephant Training Center, Way Kambas National Park Using Simple and Molecular Methods

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Abstract. DNA detection of male sumatran elephants' (*Elephas maximus sumatranus*) blood samples using simple and molecular methods are ways to find out the DNA quality from extracted samples. Molecular biology analysis was done in Biotechnology Laboratory, Lampung Disease Investigation Center, under the Institution Nation Strategy Grant, Directorate General of Research Empowerment and Development, Ministri of Research, Technology and Higher Education 2018 Number 393/UN26.21/PN/2018. Sensitivity test on 12 blood samples was conducted using simple and molecular methods. Simple method is based on 1% gel agarose electrophoresis in TAE (Tris-acetate EDTA) buffer while molecular method involves Polymerase Chain Reaction (PCR) based Glyseraldehyde-3-Phosphate Dehydrogenase (GAPDH) primer. Of 12 samples extracted and tested, 10 samples using molecular method showed DNA bands. Sensitivity value (83.33%) of diagnostic calculation showed that molecular method is more sensitive in detecting DNA.

Keywords: DNA, Molecular Analysis, Sensitivity, Sumatran Elephant, Way Kambas National Park

INTRODUCTION

Elephants are one of the large mammals whose existence is almost extinct and declared *critically endangered* status (IUCN, 2011). One of the subspecies of large mammals is the Sumatran elephant which lives in Lampung. The natural habitat of Sumatran elephants in Lampung is in Bukit Barisan Selatan National Park (TNBBS) and Way Kambas National Park (TNWK). The Sumatran elephant population in TNWK was 247 individuals in 2010 with an estimated 220-278 individuals (WWF, 2010). Sumatran elephant populations are declining due to the threat of habitat fragmentation, conflict with humans and poaching (Frankham et al., 2002). One of the rescue efforts carried out by TNWK is the construction of an Elephant Training Center (PLG) for the management of Sumatran elephants involved

in conflicts with the community. The population size of Sumatran elephants in PLG TNWK in 2016 was recorded at 66 heads with 36 male elephants and 30 female elephants (Rustiati et al., 2017). The number of male elephant individuals that are more than the number of female elephant individuals can also increase the chances of *inbreeding* which causes a decrease in genetic diversity and *viability* of Sumatran elephants (Frankham et al., 2002).

Information on the genetic diversity of Sumatran elephants is needed in determining policies, management and strategies for Sumatran elephant conservation efforts. Molecular genetic analysis approaches can be performed by sequencing tests to determine genetic diversity in Sumatran elephants. Sequencing tests are carried out using extracted DNA that has good quality. One of the methods used in testing the quality of DNA extraction results is using agarose gel electrophoresis (Asiah, 2016). Another DNA extraction quality test method is the molecular method with PCR (*Polymerase Chain Reaction*) using GAPDH (*Glyceraldehyde 3 phosphate dehydrogenase*) primers (Rustiati et al., 2018). The PCR method is a very precise technique and is often used for molecular biology because it is easier and faster (Chen et al., 2009). DNA amplification techniques from specific DNA sources from a small number of different genes (Yusuf, 2010). GAPDH primers are sequences of nitrogenous bases that encode the enzyme GAPDH. GAPDH primers are used to determine the efficiency of mRNA synthesis (cDNA sequence) by *reverse transcription* and mRNA expression quality test.

Diagnostic testing is one way to determine the sensitivity of a method. Diagnostic testing is a test performed to determine the probabilistic of a case. Sensitivity, specificity and agreement value (Kappa test) are the results obtained in diagnostic testing. Sensitivity is the ability of a test to detect a material until the material is no longer detected (gives a result of 0) (Dohoo et al., 2003). Research on the sensitivity of DNA detection from the extraction of male Sumatran elephant (*Elephas maximus sumatranus*) blood samples at the Way Kambas National Park Elephant Training Center using simple and molecular methods with GAPDH was carried out with the aim of determining the sensitivity value of simple and molecular methods in detecting the presence of extracted DNA in Sumatran elephant blood samples so as to provide information on good Sumatran elephant DNA analysis methods for support efforts to trace the genetic diversity of Sumatran elephants at PLG TNWK.

METHOD

A. Simple test

A total of 12 DNA samples from the extraction of blood of male Sumatran elephants (*Elephas maximus sumatranus*) were used as material in this study. DNA samples from the extraction of male Sumatran elephant blood used came from elephant blood sample collections at PLG TNWK (Rustiati et al., 2017). A simple method is a DNA quality test extracted from male Sumatran elephant blood samples (N = 12) with 1% agarose gel electrophoresis in TAE buffer (TriacetateEDTA). Electrophoresis is performed at a voltage of 100 V for 17 minutes in the *chamber*. The results of electrophoresis are visualized using *Digital document (Digidoc)*.

B. Molecular Test

A total of 12 DNA samples extracted from the blood extraction of male Sumatran elephants (*Elephas maximus sumatranus*) were used as samples in this study. DNA samples from the extraction of male Sumatran elephant blood used came from elephant blood sample collections at PLG TNWK (Rustiati et al., 2017). The molecular method used in this study was to test the quality of DNA extracted from male Sumatran elephant blood samples using the *Polymerase chain reaction* (PCR) technique with *Glyseraldehyde-3-Phosphate Dehydrogenase* (GAPDH) primers .

Test the quality of extracted DNA by molecular methods through three stages, namely the *mix master* and *template* stages, the amplification stage, and the agarose gel electrophoresis stage. The amplification stage with PCR goes through five stages, namely predenaturation with a temperature of 95 0 C in 5 minutes, denaturation with a temperature of 94 0 C in 20 seconds, *annealling* with a temperature of 57 0 C in 45 seconds, extension with a temperature of 72 0 C in 1 minute, and post-extension with a temperature of 72⁰ C in 5 minutes. The denaturation, *annealling*, and *extension* stages undergo 35 cycles of repetition. PCR products (amplicon) are electrophoresis with 1.5% agarose gel in TAE buffer. Electrophoresis is performed at a voltage of 100 V for 30 minutes in the *chamber*. The results of electrophoresis are visualized using *Digital document (Digidoc)*.

C. Diagnostic Tests

Measurement of sensitivity values of simple and molecular methods is carried out using diagnostic testing with the following formula:

$$\text{Sensitivitas} = \frac{a}{a+c} \times 100\%$$

DISCUSSION

The electrophoresis results of DNA samples extracted from Sumatran elephant blood extraction using simple and molecular methods were visualized using Digidoc. The results obtained from visualization of agarose gel using *Digidoc* are DNA band luminescence (Figure 1). In the simple method test, DNA luminescence was seen in 5 DNA samples, while as many as 7 other samples did not show DNA band luminescence. In the molecular test of 12 DNA samples extracted that have gone through the PCR stage with GAPDH primers and electrophoresis, DNA samples that showed the luminescence of DNA bands were seen as many as 10 samples. Many of the other 2 samples showed no visible luminescence of DNA bands. The number of samples that showed visible luminescence of DNA bands was more than 80% of the total sample.

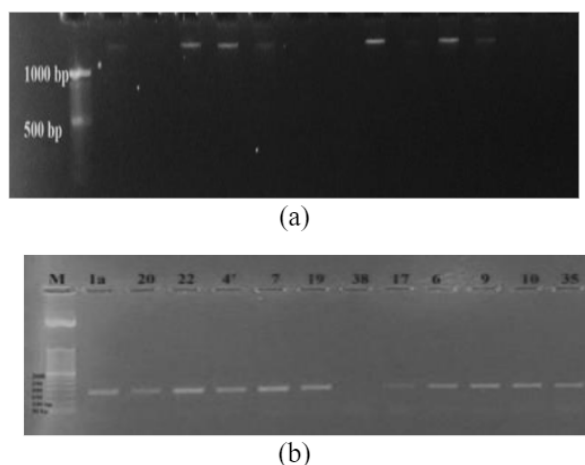


Figure 1. DNA visualization of male Sumatran elephant extraction at Way Kambas National Park Elephant Training Center using (a) simple and (b) molecular methods.

In a simple method, the absence of DNA band luminescence in 7 extracted DNA samples can be caused by DNA degradation in the sample so that the quality of the DNA produced is low and the small amount of DNA extracted also results in invisible DNA band luminescence during electrophoresis. Fachtiyah (2011) stated that the quality of DNA and the amount of DNA extracted affect the thickness of DNA band luminescence during electrophoresis. The number of samples that showed invisible luminescence of DNA bands was more than 50% of the total samples.

The molecular method using the *Polymerase chain reaction* (PCR) technique with *Glyseraldehyde-3-Phosphate Dehydrogenase* (GAPDH) primer was chosen because it has

more sensitivity and specificity to DNA molecules. Test the quality of extracted DNA by molecular methods through three stages, namely the *mix master* and *template* stages, the amplification stage, and the agarose gel electrophoresis stage. The amplification stage with PCR is regulated with a certain temperature and time so that the amplification process can run and a target DNA sample is obtained. PCR products (amplicon) are electrophoresis on 1.5% agarose gel. A higher concentration of agarose gel is expected to result in more DNA molecules being captured. Higher gel concentrations increase the agarose gel density value so that it can detect amplicon DNA that has a smaller size than genomic DNA.

The results obtained from agarose gel visualization using *Digidoc* showed that of the 12 DNA samples extracted from electrophoresis, 10 DNA samples from the extraction showed DNA band luminescence while the other 2 samples did not show visible DNA band luminescence. PCR electronics play a role in doubling the amount of DNA contained in the sample. In the amplification stage, samples containing a small amount of DNA are multiplied through five stages, namely *predenaturation*, *denaturation*, *annealing*, *extension*, and *post extension*. At the amplification stage, temperature optimization is carried out to obtain an optimal temperature so that DNA with good quality is obtained. The denaturation, *annealing* and *extension* stages are repeated 35 times so that samples with a greater amount of DNA than before are produced. As Mullis and Fallona (1989) in Yuwono (2006) said that this *polymerase chain reaction* (PCR) is used to multiply DNA molecules. This shows the advantages of the PCR method, where this method can be used on samples with a small amount of DNA.

The invisibility of DNA band luminescence can be caused by two things. First is the damage to the sample due to the storage process. DNA degradation can occur in samples that are stored for too long so that the quality of the DNA produced is relatively low, and the sample storage temperature must be stable (-20°C) to maintain the quality of DNA in the sample so that DNA degradation does not occur (Manela C., 2015). The second is the *freezing/thawing* process during the research process can also cause DNA damage which causes low quality DNA samples. The *freezing/thawing* process has an effect on the strength of DNA nitrogenous base strands (Davis *et al.*, 2000). The presence of DNA in the sample is influenced by the stage of sample extraction. Samples that have been damaged cause DNA extraction with low quality or damaged. Factors that can affect the extraction yield are the homogenization of the material used and the type of solvent used to remove the

extracted residues (Syafaruddin et al., 2011). The extraction stage is very important because it affects the damage to the DNA used as a sample.

Comparison of test results with simple and molecular methods, namely simple tests showed positive results as many as 5 samples, while molecular tests showed positive results as many as 10 samples (Table 1). The molecular test results show samples with more positive results than simple test results. This is because the number of DNA molecules in the sample has been doubled through the PCR process, so the possibility of the sample showing positive results on visualization will be higher.

Table 1. DNA detection results from the extraction of male Sumatran elephant blood samples at PLG TNWK using simple and molecular methods

No	Elephant Name	Simple Test Results	Molecular Test Results
1	Boy	+	+
2	Cuni	+	+
3	Rendi	+	+
4	Rendo	+	+
5	Renggo	+	+
6	Daeng	-	+
7	Edwin	-	+
8	Fatra	-	+
9	Senses	-	+
10	Joni	-	+
11	Josh	-	-
12	Karnangun	-	-

Diagnostic calculations in tests with simple methods produce a sensitivity value of 41.67% and a sensitivity value obtained in molecular tests of 83.33% (Table 2). This shows that a simple test carried out can detect not only the presence of the GAPDH gene in the DNA sample but can detect the presence of other genes in the DNA so that it is not specific (specific). Simple tests performed are not very good at detecting the presence or absence of a gene in the sample. Themolecular test only detects the presence of the GAPDH gene in the DNA sample and does not detect other genes. Molecular tests carried out can detect the presence or absence of DNA well. Molecular tests have a higher sensitivity value when compared to simple so they are considered better to use.

Table 2. Diagnostic test results of Sumatran elephant DNA samples

Test Method	Simple test	Molecular assay
Visible DNA samples	5 samples	10 samples
Sensitivity	41,67%	83,33%

A sensitivity value of a test close to 0% indicates that the test method is not sensitive. The insensitivity of the method indicates that the method used is insensitive in detecting the presence of a material. A sensitivity value of a test close to 100% indicates that the test method is very sensitive. The high sensitivity value of the method shows that the method used is very sensitive in detecting the presence of a material. The sensitivity and specificity values will show the accuracy and precision of the tests that have been carried out.

CONCLUSION

DNA detection from the extraction of blood samples of male Sumatran elephants (*Elephas maximus sumatranus*) using simple and molecular methods showed that more DNA band luminescence was found in molecular tests compared to simple tests. Molecular tests are more sensitive to the presence of DNA compared to simple tests as evidenced by the sensitivity value of molecular tests that are higher than the sensitivity value of simple tests.

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Exploring environmental DNA for barcoding analysis of Sumatran rhino in Way Kambas National Park

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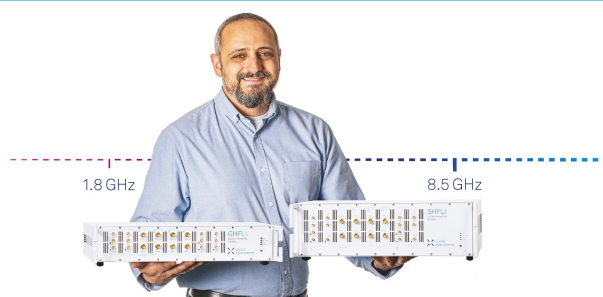
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 New

Exploring Environmental DNA for Barcoding Analysis of Sumatran Rhino in Way Kambas National Park

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Abstract. The Sumatran rhino (*Dicerorhinus sumatrensis*) is one of five species of rhino in the world. The existence of the Sumatran rhino is also considered very vulnerable to habitat degradation, internal cross-breeding, disease, and hunting. The Sumatran rhino population will experience extinction if there are no mature management measures for the long term. It is assumed that the dynamics of the natural ecosystem in the Sumatran rhino's natural habitat will harm the existence of its population. Sumatran rhino wallow water is one of the remaining sources of environmental genetic material. Extraction of eDNA in the wallow water of individual Sumatran rhinos in Sumatra Rhino Sanctuary, Way Kambas National Park aims to determine the results of testing the quality of genetic material from Sumatran rhino wallow water in the Sumatran Rhino Reserve, Way Kambas National Park using simple and molecular methods. DNA extraction using a simple method detects 0/12 samples, whereas using molecular methods can detect 11/12 samples. These results should be followed by detection using specific primers to ensure that the eDNA extracted is Sumatran rhino eDNA.

INTRODUCTION

In the world there are five species of rhinoceros, according to their distribution, two species are found on the African continent and three species are found on the Asian continent. Indonesia has two rhino species, namely the Javan rhino (*Rhinoceros sondaicus*) and the Sumatran rhino (*Dicerorhinus sumatrensis*) [1]. The Sumatran rhinoceros was once widespread in Southeast Asia, currently, it is estimated that only about 30 individuals in Sumatra and Kalimantan [2] are critically endangered and are included in the CITES Appendix I list [3]. Threats facing the Sumatran rhino include poaching, habitat destruction, and extremely low reproductive rates. Many efforts have been made to conserve the Sumatran rhinoceros to avoid extinction. The Sumatran rhino population will experience extinction if there is no long-term rescue action. The dynamics of natural ecosystems in the natural habitat of the Sumatran rhino are expected to have an impact on the existence of its population [4].

Sampling for DNA analysis can be done using invasive methods, namely sampling that is in direct contact with individuals, through taking blood or body parts from animals. This method requires capturing or coming into direct contact with the animal, which can result in a risk of physiological stress or dangerous side effects from the overuse

of the drug. Currently, DNA sampling is starting to develop, one of which is using non-invasive methods [5]. This method utilizes feces, urine, scrapes, and wallows of water on these animals [3].

METHOD

The tools and materials needed for collecting wallow samples are 10 ml vacutainer tubes, large syringes, labels, plastic, dippers, and iceboxes. A sampling of wallow water in the area at 08.00-10.00 WIB. The Sumatran rhinoceros wallow was found in an area ± 100 m from the captive location, the active Sumatran rhino wallow sample was a wallow that was still routinely used to wallow the Sumatran rhino, while the inactive sample was a wallow that was no longer used for the Sumatran rhino wallow.

A sampling of wallow water was carried out at two points, namely the edge point and the middle point. At the edge point, sufficient water samples were taken using a large syringe, then put into a 10 ml vacutainer tube. Taking at the midpoint, a sufficient sample of wallow water was taken using a dipper that had been tied with wood, then the sample was taken using a large syringe and put into a 10 ml vacutainer tube. Each sample taken was taken by taking pictures and recording important data related to individual names, conditions, sampling points, and information about the time of the Sumatran rhino wallow into the survey datasheet. All of the wallow samples that had been put into a 10 ml vacutainer tube were stored in the refrigerator.

Extraction of eDNA from Sumatran rhinoceros wallows samples was carried out concerning the QIAmp® Fast DNA Stool extraction protocol Mini Kit (50) catalog number: 51504 from Genecraft. Testing the quality of DNA extracted from Sumatran rhino wallows with a simple technique using 1% agarose gel electrophoresis. 1 g of agarose powder was added with 100 ml of Tris Acetate-EDTA (TAE) buffer which was used as a buffer solution, then heated in the microwave for 3 minutes, then 1.5 l of safe SYBR was added until homogeneous which served as a dye to see the extracted DNA after it's done electrophoresis.

The molecular method used in this test is to see the accuracy of the DNA quality test results extracted on the method simple. The technique used is Polymerase Chain Reaction (PCR) on Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) primers which have three stages, namely the master mix and template stages, amplification, and electrophoresis stages. The master mix and template stage aims to mix all the reaction components, including DNA templates, reagents, and other materials. The amplification stage aims to double the amount of sample DNA, and the electrophoresis step agarose gel aims to check the results of the PCR reaction.

RESULT AND DISCUSSION

Water samples were collected from six of the seven Sumatran rhinos at SRS, TNWK (Table 1). Sumatran rhino Rosa could not be sampled as a source of genetic material, this is because the Sumatran rhino Rosa is more sensitive than the other six Sumatran rhinos to human presence in the area, which will cause stress to the Sumatran rhino. Water samples were taken when the Sumatran rhino went to the captivity, which was led by the keeper with the condition of the Sumatran rhino being muddy, indicating that the Sumatran rhino had just finished wallowing in the morning and dry muddy conditions indicating that the Sumatran rhino was wallowing at night.

TABLE 1. Identity of Samples

Name of Rhino	Age	Source of Genetic Material	
		Active wallow	Nonactive wallow
Andatu	8	1	1
Andalas	19	1	1
Harapan	13	1	1
Ratu	15	1	1
Bina	37	1	1
Delilah	4	1	1

The characteristics of active wallows are that the wallows that are often used by the Sumatran rhino for wallowing have more soft mud and less water, while inactive wallows are wallows that have not been used for wallowing by the Sumatran rhino for about 1-3 months, in inactive wallows more water is collected. pooled because the wallow had never been used for wallowing and around the wallow it looked neat, there were no footprints or traces of the Sumatran

rhino's feet (Figure 1). The sampling point of the wallow water is at the center point which is the wallowing axis of the Sumatran rhinoceros, and the edge point is the place to scavenge the Sumatran rhino. The wallow water sample at the midpoint was taken using a dipper and the edge point was taken using a large syringe and then put into a non-EDTA vacutainer tube which was made in airtight conditions.



FIGURE 1. (a) active wallow, (b) inactive wallow

The vacutainer tube is labeled with the individual name of the Sumatran rhino, type of wallow, collection point, date of sampling, then the vacutainer tube is put into an icebox for transportation to Bandar Lampung to the Biotechnology Laboratory, Lampung Veterinary Center, the wallow samples are stored in the freezer (-20°C) until the next process.

Qualitative test results using a simple method of water samples Sumatran rhinoceros wallow showing no visible luminescence DNA bands, so that treatment is carried out using the method Another, a more sensitive method is the molecular method. This is done to ascertain whether the results of the electrophoresis of water samples Sumatran rhinoceros wallow that does not show DNA band luminescence caused by the absence of a remaining source of genetic material in the Sumatran rhino wallow or caused by the amount of DNA which is too little so that it is not detected in qualitative tests using a simple method. Visualization of agarose gel electrophoresis results was carried out using digital documents (digidoc). The results were obtained from the gel visualization agarose using Digidoc visible as DNA band luminescence (Figure 2).



FIGURE. 2 Qualitative test results using a simple method of water samples Sumatran rhinoceros wallow

The amplification process is carried out by setting a certain temperature and time which will produce 12 DNA amplicons. Electrophoresis of DNA results from the amplification process is visualized using digidoc. Visualization results of 12 pool water samples of individual Sumatran rhinoceros showed 11 positive samples and 1 negative sample (Figure 3).



FIGURE. 3. Visualization results of 12 amplicons of GAPDH fragment

Delilah Sumatran rhino wallow water sample at point collection center that gives a negative result or does not show luminescence DNA bands on the molecular method. The sample is also not showing the presence of luminescence of DNA bands in a simple method. Thing This can be caused by the absence of genetic material left behind in an inactive pond individual Sumatran rhino Delilah or genetic material damage. The results of the quality test of the simple method and the molecular method shows there is a difference, that the PCR reaction can increase the number of Extracted DNA so that during electrophoresis it can capture the DNA and produces DNA bands glow [6]. The use of GAPDH primers can detect enzymes found in Sumatran rhinoceros wallows so that the method.

Molecular GAPDH is more sensitive in detecting the presence or absence of genetic material in the Sumatran rhinoceros wallow. The quality of DNA and the amount of DNA extracted affect the thickness thin luminescence of DNA bands during electrophoresis. Luminescence thickness DNA bands on the visualization results are directly proportional to the quality of DNA. The luminescence of the DNA bands that are getting thicker indicates a higher quality of DNA getting better [7]. The age of use of the wallow affects the thickness of the luminescence of the DNA band, the thicker the luminescence DNA bands obtained, the wallow is actively used for a wallow. The thinner the luminescence of the DNA bands obtained, the age the use of wallows is not being actively used by rhinos Sumatra.

This initial study was conducted for the manufacture of DNA banks to support the Sumatran rhinoceros phylogenetic tracing source in SRS, TNWK [8]. Sumatran rhino feces samples that were isolated DNA [3]. eDNA extraction was carried out to determine the presence of genetic material left in rhino wallows Sumatra in SRS, TNWK using a simple method and method molecular. Of the 12 DNA extracted from the wallows of the Sumatran rhinoceros in SRS, TNWK showed 11 samples were suitable for testing further steps such as the DNA sequencing stage so that this research can support molecular-based conservation efforts. While 1 sample cannot be used for further tests due to the possibility of the presence of defects in the sample or the absence of a source of genetic material what is left is like in an inactive wallow of the Sumatran rhino Delilah.

CONCLUSION

This study concludes that the results of the DNA quality test shows there are differences in the results of quality tests using a simple method and molecular method. Quality test results using molecular methods are more accurate in detecting the presence or absence of a source of genetic material that left in the wallows of the Sumatran rhino, while the results of the quality test using a simple method is less specific if it is used for determining the presence of environmental genetic material sources left behind in the Sumatran rhino wallow.

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