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Health Examination of The Digestion of Proboscis Monkey (*Nasalis larvatus*) Through Bacteriological Tests on Feces

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ABSTRACT

This study aims to identify bacteria and the prevalence value of bacteria found in probosci's monkey feces as a picture of proboscis monkey digestive health. Seventeen fecal samples from each proboscis monkey, namely Mimin, Chikita, and Pedro, were taken at the Bekantan Rescue Center, Sahabat Bekantan Indonesia Foundation (SBI). Feces were identified by making macroscopic and microscopic observations. Visible observations include consistency, color, mucus, and blood in the physical feces. Microscopic observations were made by culturing feces on Mac Conkey, blood agar, EMBA, DHL agar, and NA media, followed by gram staining and biochemical tests consisting of VP (*Voges-Proskaeur*) test, methyl red test, indole test, citrate test, and lactose test. The results of identifying 17 feces samples from each probosci's monkey found the presence of *E.coli* bacteria from microscopic testing. The prevalence of *E.coli* bacteria in Mimin, Chikita, and Pedro is 41.17%; 35.29%; 35.29%, so it is concluded that the presence of *E.coli* is a typical bacterium that is not harmful to the digestion of proboscis monkeys, supported by observations on the physical macroscopic feces of good probosci's monkeys.

1. INTRODUCTION

The Proboscis monkey (*Nasalis larvatus*) is a primate from the Cercopithecidae family (Klaus et al., 2018). Proboscis monkeys are endemic primates of Borneo which are spread across three regional countries, namely Indonesia (Kalimantan), Brunei Darussalam, and Malaysia (Sabah, Sarawak) (Meijaard & Nijman, 2000). Proboscis monkeys in their habitat are starting to decrease due to the conversion of forests to residential land (Srimulyaningsih & Syaputra, 2021). The decline in the probosci's monkey population by 50-80% in nature has resulted in the species being categorized as endangered by the IUCN (*International Union for Conservation of Nature and Natural Resources*) and included in Appendix I of CITES (*Convention on International Trade in Endangered Species of Wild Fauna and Flora*) (IUCN, 2023). The causes of the decline in the probosci's monkey population include land conversion, forest fires, and poaching. These factors must be balanced with fishing efforts (Kartono et al., 2008).

In the context of and supporting government programs in the field of probosci's monkey conservation, Sahabat Bekantan Indonesia Foundation (SBI) is a non-profit foundation that has several programs such as rescue & rehabilitation activities and rehabilitation of proboscis monkeys before being released into their natural habitat (Santoso et al., 2019). Monitoring animal health

during the rehabilitation period is necessary to evaluate the readiness of animals both physically and mentally before they are released into their natural habitat. Animals that have good digestive health will also have good physical health. Digestive health is an important indicator to assess animal health in general.

Gastrointestinal disorders are a problem that is often found in primates (Rahmi et al., 2014). Digestive health examination can be performed macroscopically using a stool sample, including observing the stool's color, consistency, amount, smell, mucus, and blood (Collison et al., 2010). Gastrointestinal disorders are usually caused by enteropathogenic bacteria (Wahyuni, 1999). Microscopic examination of probosci's monkey feces to determine the presence of bacteria can be used as a supporting examination in diagnosing digestive disorders. So far, tracing of bacteria focusing on enteric bacteria in probosci's monkey feces at the Indonesian Proboscis Monkey Foundation (SBI) Rehabilitation Center has never been carried out.

Research conducted by Pramono (2021) on the feces of proboscis monkeys (*Nasalis larvatus*) by examining bacteria, namely lactic acid bacteria (BAL), and the results of this study found three types of lactic acid bacteria, the *Lactobacillus* type. In addition, several researchers have examined enteric bacteria in other primates. The results of research by Rahmi et al. (2014) reported that two Sumatran orangutans (*Pongo abelii*) at the Jantho Orangutan Reintroduction Center identified nine fecal samples contaminated with bacteria from the genera *Salmonella* and *Shigella*. Research by Virgi (2016) also reported on cases of diarrhea of two Bornean orangutans (*Pongo pymaeus*), each of which came from captive orangutans at the Yogyakarta Nature Conservation Foundation in Kulon Progo and orangutans at the Borneo Orangutan Survival Foundation in Semboja, East Kalimantan, identified two swab samples feces contained *Providencia rustigianii* and *Enterobacter agglomerans*. In addition, research from Loe et al. (2021) regarding the prevalence of bacteria in the feces of long-tailed monkeys (*Macaca fascicularis*) who experience diarrhea at the Bogor Agricultural University Captive Facility, it is known that the results of isolation and bacterial assistance show that the fecal sample is contaminated with *E.coli* (100%), *Salmonella enteritidis* (97%), and *Shigella* sp. (60%).

Based on the bacterial background above, it is necessary to research tracing proboscis monkey feces. This study aims to describe the digestive health of proboscis monkeys at the Bekantan Rescue Center, Sahabat Bekantan Indonesia Foundation (SBI) by tracing bacteria in feces with the hope that this research will be helpful as basic information for reference in monitoring the health of probosci's monkeys before being released back to their natural habitat.

2. MATERIALS AND METHODS

This research was conducted for five months, from February to June 2023. Sampling was conducted at the Bekantan Rescue Center, Sahabat Bekantan Indonesia (SBI). The research samples were feces from 1 adult male proboscis monkey (Pedro) and two adult female proboscis monkeys (Mimin and Chikita). Sample examination was conducted at the Banjarbaru Regional V Veterinary Investigation and Testing Laboratory, Banjarbaru Veterinary Center, South Kalimantan Province.

The tools used for this study were ziplock packs, scoops, tongs, latex sheaths, trays, ice boxes, ice gel, Petri dishes, micropipette, cover slip, slide, loop needle, bunsen, microscope, test tube, pipette, incubator, freezer, digital scales, and camera. The materials used for this study were proboscis monkey feces, DHL agar, Blood agar, Mac Conkey agar, Nutrient agar, Eosin Methylene Blue agar (EMBA), VP Media, MR Media, trypticase broth, 0.9% NaCl, alpha-naphthol solution, 40% KOH, distilled water, crystal violet, Lugol's iodine, 96% alcohol, safranin, Koser's citrate medium, tryptone broth, brilliant green lactose broth, methyl red reagent, and Kovac's reagent.

Sampling was conducted in the morning at around 07.00 - 09.00 for each adult probosci's monkey at the Bekantan Rescue Center, Sahabat Bekantan Indonesia Foundation. The macroscopic examination is done immediately after the sample is placed in the tray. Stool samples were identified through macroscopic characteristics: consistency, color, mucus, and blood. The

macroscopic characteristics obtained from the observations were then recorded. Parameters of Proboscis monkey stool consistency are known using the Fecal Consistency Score (FCS), according to Moxham (2001). Meanwhile, Microscopic observation was carried out by culturing the feces by isolating bacteria on Mac Conkey media, blood agar, EMBA, DHL agar, and NA, followed by gram staining and biochemical tests consisting of the VP test (Voges-Proskaeur), methyl red test, indole test, citrate, and lactose tests.

The data were analyzed descriptively to explain the description by correlating the macroscopic results/physical characteristics of the stool, the microscopic results (type of bacteria), and the prevalence of bacteria. Determination of the prevalence of bacterial infection is based on the results of examining the presence or absence of bacteria on stool examination.

The data obtained is presented using the formula:

$$\text{Prevalence} = \frac{\sum positive\ sample\ of\ bacteria}{\sum observed\ samples} \times 100\% \quad \text{.....(1)}$$

(Safitri et al., 2019)

3. RESULTS AND DISCUSSION

3.1.Results

The results of macroscopic observations on the feces of proboscis monkeys can be seen in (**Table 1**).

Table 1. Results of macroscopic observations of the feces of proboscis monkey feces

No.	Nth Collection Sample	Individual Code	Macroscopic Observations			
			Color	Consistency	Presence of Mucus	Presence of Blood
1.	1	M	Greenish	Liquid, Shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
2.	2	M	Brownish yellow	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Brownish yellow	Liquid, shapeless	-	-
3.	3	M	Brownish yellow	Solid, Shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Solid, Shaped	-	-
4.	4	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Solid, Shaped	-	-
5.	5	M	Greenish	Liquid, shapeless	-	-

No.	Nth Collection Sample	Individual Code	Macroscopic Observations			
			Color	Consistency	Presence of Mucus	Presence of Blood
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Fairly liquid, Shaped	-	-
6.	6	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Solid, Shaped	-	-
7.	7	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Solid, Shaped	-	-
8.	8	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
9.	9	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Moderately dense, Shaped	-	-
10.	10	M	Greenish	Solid, Shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Fairly dense, shapeless	-	-
11.	11	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Yellowish green	Moderately dense, Shaped	-	-
12.	12	M	Greenish	Moderately dense, Adequately shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
13.	13	M	Greenish	Fairly dense, shapeless	-	-

No.	Nth Collection Sample	Individual Code	Macroscopic Observations			
			Color	Consistency	Presence of Mucus	Presence of Blood
		C	Greenish	Fairly dense, shapeless	-	-
		P	Greenish	Liquid, shapeless	-	-
14.	14	M	Greenish	Solid, Shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
15.	15	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
16.	16	M	Greenish	Liquid, shapeless	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-
17.	17	M	Greenish	Solid, Shaped	-	-
		C	Greenish	Solid, Shaped	-	-
		P	Greenish	Liquid, shapeless	-	-

Note: (-) = none

Each proboscis monkey's stool is tested microscopically, including bacterial culture, gram staining, and biochemical tests. The identified bacteria are *Escherichia coli* (*E.coli*). The results of bacterial identification from microscopic observations of proboscis monkey feces and the results of the tests are presented in (**Table 2**) and (**Table 3**).

Table 2. Results of Microscopic Observations on the Feces of Proboscis Monkeys

No.	Nth Collection Samples	Individual Code	Results of Identification of Bacteria in Proboscis Monkey Feces
1.	1	M	*E.coli*
		C	-
		P	-
2.	2	M	*E.coli*
		C	*E.coli*
		P	*E.coli*
3.	3	M	*E.coli*
		C	*E.coli*
		P	-
4.	4	M	*E.coli*
		C	-
		P	*E.coli*

No.	Nth Collection Samples	Individual Code	Results of Identification of Bacteria in Proboscis Monkey Feces
5.	5	M	-
		C	*E.coli*
		P	*E.coli*
6.	6	M	-
		C	-
		P	-
7.	7	M	-
		C	-
		P	-
8.	8	M	-
		C	-
		P	-
9.	9	M	*E.coli*
		C	*E.coli*
		P	*E.coli*
10.	10	M	-
		C	-
		P	-
11.	11	M	-
		C	-
		P	-
12.	12	M	*E.coli*
		C	*E.coli*
		P	*E.coli*
13.	13	M	-
		C	-
		P	-
14.	14	M	-
		C	-
		P	-
15.	15	M	*E.coli*
		C	*E.coli*
		P	*E.coli*
16.	16	M	-
		C	-
		P	-
17.	17	M	-
		C	-
		P	-

Note: (-) = none

Tabel 3. Microscopic Test Results

Type of Bacteria	Isolated culture					Staining Gram	Biochemical test				
	Mac Conkey Agar	Blood Agar	DHL Agar	EMBA	NA		VP	MR	Indole	Citrate	Lactose
E.coli	+	-	+	+	+	Gram Negatif	-	+	+	-	+

Note: (+) = reacts/ changes occur
(-) = no change

The prevalence of *E.coli* bacteria identified was then calculated from the total amount of feces for each proboscis monkey Mimin (M), Chikita (C), and Pedro (P). The results of calculating the percentage prevalence of bacteria found in the feces of proboscis monkeys are presented in (**Table 4**).

Tabel 4. Prevalence of *E.coli* in Proboscis Monkey Feces

Type of Bacteria	Percentage of Bacteria in Mimin (M)	Percentage of Bacteria in Chikta (C)	Percentage of Bacteria in Pedro (P)
E.coli	41.17%	35.29%	35.29%

3.2. Discussion

Macroscopic observations include the characteristics of consistency, color, presence of blood, and mucus. The existence of this observation is intended as preliminary research to support microscopic observations, which are then carried out. According to Kasırga (2019), finding out the presence of diseases that occur in the digestive tract can be obtained from stool examination with several options, namely macroscopic and microscopic. Stool consistency was observed based on the provisions of the Fecal Consistency Score (FCS), with a score range of 1 (dry and hard stools) to 5 (watery diarrhea) (Ridwan & Batan, 2021). From all observations, Chikita's (C) feces showed normal stool consistency at a score of 2-2.5 with an oval and dense shape but not too dry and hard or soft so that it only left a few marks when lifted.

The consistency of Mimin's (M) and Pedro's (P) stools had a consistency score of 2.5-3.5, with the observation that there were generally oval stools but not completely oval, and the surface was quite moist. In addition, Mimin and Pedro's feces were excreted at level 3, which had a slightly denser, almost liquid form with a very moist surface. According to research by Mirsageri et al. (2015), macroscopic observations of feces show that the consistency of orangutan feces is soft to hard. Hard stool consistency is often found in orangutan puppies with a blackish-brown to yellowish-green color, while relatively soft stool consistency is found in adults. This research is directly proportional to the results of stool macroscopic observations conducted in this study. Judging from the age of the proboscis monkey Chikita is included in the category of juvenile proboscis monkeys or still in the process of reaching adulthood, similar to Mirsageri's research, which found hard feces in baby orangutans, while Pedro and Mimin's proboscis monkeys are in the category of mature probosci's monkeys with a relatively soft faecal texture. (soft) corresponds to macroscopic observations of relatively soft faeces in orangutans.

In addition, differences in the texture of the feces excreted by individual proboscis monkeys cannot be separated from the feed consumption, digestive metabolism, and eating habits of each observed proboscis monkey. The main feed is fresh green vegetables, namely kelakai, cassava leaves, kale, raw corn, and cassava. The proboscis monkeys are also given fruits such as unripe papayas, bananas, and milk in the afternoon. Giving food in the form of fresh vegetables and fruit is an effort to fulfill the nutrition in the form of protein, fat, and vitamins needed by adjusting their natural needs (Alkatiri, 2020). Giving corn to proboscis monkeys is in line with the statement of Sharafina (2017) in her research, which stated that giving corn can be a source of energy and fat for primate feed.

Feeding green vegetables to each proboscis monkey is given at least three bunches of vegetables of each type at three meals interspersed with tubers and fruits. The provision of these green vegetables contributes fiber to the process of food metabolism in the proboscis monkey's body at the Proboscis Monkey Rehabilitation Center. The presence of dietary fiber in the stool causes the stool to absorb more water so that the stool volume becomes large and has a soft texture. A large

volume of feces will accelerate intestinal contractions to pass water faster, so food transit time will be quick (Faidruz & Nisa, 2015).

Research by Kusumorini et al. (2014) found a difference in the texture of the feces of slow lorises, where the texture of the feces tends to be denser because slow lorises eat bananas more. In contrast, those who prefer papaya tend to be softer. From the results of observing the texture of the proboscis monkey stools studied, Mimin and Pedro's stools were more liquid and slightly denser because Mimin and Pedro ate fewer green vegetables and preferred fruit to eat. Meanwhile, Chikita eats anything with gusto.

From the texture of the excreted feces, all observations confirmed that the three proboscis monkeys did not experience constipation or difficulty defecating. The overall condition or texture of the stool is in the range of 2-3.5, where the water and moisture content on the stool's surface is still visible. *Constipation* is a reduction, loss, or difficulty defecating (Garcia-Pertierra et al., 2017). Constipation can be caused by dehydration due to a lack of water intake that enters the body (Watrous, 1983).

The color of the feces of all three proboscis monkeys is greenish, in line with the research by Mirsageri et al. (2015), which states that orangutan feces are yellowish green to black. The ingested feed can cause the presence of a different color in the physical proboscis feces. According to Lisana et al. (2015), feed digestibility is influenced by the content of nutrients in feed, especially crude fiber and lignin. Various green vegetables are given to the proboscis monkeys. Kalakai has a yellowish-green leaf color, while cassava leaves have a darker green color. Kale gives the same green as cassava leaves. In addition, pieces of corn, unripe bananas, and papaya give it a yellow color.

Observations in the field provide clues about where the feces produced will be brighter in color, such as light green or brownish yellow, when proboscis monkeys prefer to eat light green vegetables and fruit such as kalakai, bananas, papaya, and corn. Meanwhile, the feces are greenish when the food consumed mainly contains dark green leaves such as cassava and kalakai. Green or yellow-green food contains lots of spinach and other green vegetables. The laxative comes from vegetables. Food passes through the intestines quickly so that bile pigments have no time to oxidize (Faidruz & Nisa, 2015).

Apart from the type of leaves or fruit that is given, the behavior that is carried out because of each proboscis monkey's appetite also affects the excreted physical feces. Proboscis monkey eating behavior is caused by feed stimulation (environmental stimulation) and a need or hunger (internal stimulation) because the energy has been used for metabolism during rest or sleep. This stimulation from inside and outside the proboscis monkey's body will respond to the body's action of digesting food (Alikodra, 1993). Chikita has a stable appetite, where he will eat the food given when the time comes until the food runs out (Picture 3), while Mimin does not immediately eat the food given.

Mimin will look for the position where she usually eats and carefully sorts out the parts of the vegetables to be eaten (Picture 4). Besides this picky nature, Mimin often ignores food to watch over her child, Hanny. Pedro has a great appetite when it is time to eat but will occasionally drop his food (Plate 5). The feed given is usually eaten immediately on the spot or near the place where the feed is placed (Alikodra, 1993). With its multi-chambered stomach, the proboscis monkey digests natural food from various proportions of leaves and mostly unripe fruits/seeds (Matsuda et al., 2010).

In macroscopic observation of the feces of the three proboscis monkeys, no blood or mucus was found during the observation. These observations' results stated that the excreted feces were normal feces without mucus and blood. According to Widman (1995), mucus indicates irritation or inflammation of the intestinal wall. Meanwhile, the presence of blood in the stool may indicate acute inflammatory diarrhea (Zein et al., 2004)

Furthermore, the results of microscopic observations on seventeen stool samples from each proboscis monkey identified the presence of *Escherichia coli* (*E.coli*) bacteria. In general, these

bacteria are in the feces of proboscis monkeys Mimin (M), Chikita (C), and Pedro (P). The feces of the three proboscis monkeys indicated *E. coli* in the 2nd, 9th, 12th, and 15th collection samples. In contrast, other collection samples found it in one or two individuals until they were indicated in the entire collection samples. Samples of the 6th, 7th, 8th, 10th, 11th, 13th, 14th, 16th, and 17th collections did not find *E. coli* in the three proboscis monkeys.

E.coli bacteria are sequentially examined through microscopic stages, from bacterial culture and gram staining to being tested through biochemical testing. Bacterial culture or purification as an isolation step by growing colonies on certain media. Purification occurs until a colony grows separately or singly from the bacteria (Putri & Kusdiyantini, 2018). Positive results on MCA media (Mac Conkey agar) are indicated by the growth of round colonies accompanied by changes in the color of the media. These results are consistent with the observations of Bakri et al. (2015) stated that *E.coli* colonies grew on Mac Conkey media to appear round in shape, with flat edges and smooth surfaces with colony color. Mac Conkey agar media is a selective medium for isolating gram-negative bacteria with a media composition containing bile salts which can inhibit the growth of gram-positive bacteria (Amri et al., 2017) and lactose as a source of carbohydrates (Jawetz et al., 2013).

According to Champoux et al. (2004) production of acid formed in MCA media as a form of discoloration of bacterial colonies, which were initially pink due to neutral red absorbance due to a decrease in pH. This product proves lactose fermenting bacteria's success on MCA media. In addition, these bacteria can also form gas from fermented lactose (Jawetz et al., 2013). Loey et al. (2021) used Mac Conkey media in the feces of long-tailed monkeys with a positive result for *E.coli* bacteria.

The isolation medium for the subsequent bacterial culture is eosin methylene blue agar (EMBA). The positive results of *E.coli* in the study were seen from bacterial growth on the EMBA media with a change in color to shiny green. According to Rashid et al. (2020), *E.coli* bacteria on EMBA media have a characteristic that is metallic green in color with a flat surface, and there is a black spot in the middle of the colony. *E.coli* were also found in EMBA media from a study conducted by Loey et al. (2021) as a culture medium from long-tailed monkey feces. The metallic green color change in the EMBA media is because *E.coli* can ferment lactose, which increases the media's acid levels (Lal & Cheeptam, 2007).

The EMBA media used, according to Antony et al. (2013), is a selective and differential medium for growing *E.coli* bacteria. EMBA effectively grows gram-negative enteric bacteria and inhibits the growth of gram-positive bacteria, including yeast growth. This media contains methylene blue dye, which is toxic to microorganisms except for enteric bacteria. Research conducted by Rasyid et al. (2020) showed that the *E.coli* on this media were metallic green. This feature distinguishes *E.coli* from other enteric bacteria that grow on EMBA media.

Negative results were found in Blood agar media in the absence of *E.coli* colony growth capable of lysing erythrocytes in the media. The same result was also found in a study by Tivani et al. (2019) that *E. coli* cannot carry out hemolysis, characterized by no color change in the area near the bacterial colony. Blood Agar Plate (BAP) is a culture medium enriched with 5-10% sheep blood for cultivating selective organisms such as *Streptococcus* spp. (Cappucino & Sherman, 2014; Krihariyani et al., 2016).

Addition of blood to BAP media to determine the ability of particular microorganisms, especially *Streptococcus* spp., in lysing erythrocytes (hemolysis) and classified into three hemolytic activities, namely gamma, alpha, and beta hemolysis (Collins et al., 1989; Cappucino & Sherman, 2013). McKane & Kandel (1998) stated that bacteria that can completely lyse erythrocytes by forming a clear zone are classified into β-hemolysis. In contrast, if the hemolysis area is not clear, accompanied by a change in the color of the media from greenish to brownish, it is grouped as α-hemolysis, and the gamma hemolysis group indicates no hemolysis.

The presence of *E.coli* bacteria on DHL agar was indicated by the presence of red colonies, which was consistent with the results of a study by Sato et al. (2020) that the isolation of bacteria

suspected of being *E. coli* was found to be characterized by pink colonies after being incubated for 24 hours at 37°C. Sakazaki et al. (1960) described DHL agar, also known as Deoxycholate Sulfide Lactose Agar, which contains a rich nitrogenous base to allow the growth of enteric bacteria, especially *Salmonella* and *Shigella* strains. The result of the differentiation of enteric bacterial colonies depends on lactose fermentation. Lactose fermenting bacteria produce acid and form pink to red-colored colonies, whereas non-lactose fermenting bacteria form colorless colonies. Most normal gut bacteria ferment lactose with red colonies, whereas *Salmonella* spp. and *Shigella* spp. can not ferment lactose (colorless colonies) (Sakazaki et al., 1971).

Furthermore, positive results in NA media were indicated by the presence of colonies on the surface of the media by Putri & Kusdiyantini's opinion (2018) that NA media is a non-selective medium so that all bacteria can grow. Pelczar et al. (2008) stated that NA media contains nutrients that support bacterial growth. These nutrients come from a nitrogen source, so this medium is commonly used as a standard medium for the growth of microorganisms in laboratory bacteriological procedures (Amri et al., 2017; Putri & Kusdiyantini, 2018). NA media in this study is a comparison medium for the growth of bacteria growing on Mac Conkey, EMBA, Blood agar, and DHL agar media which refers to Putri & Kusdiyantini's research (2018) using NA media as a comparison medium for the growth of bacteria growing on MRSA media.

The gram staining results at the microscopic stage showed that the observed bacteria were gram-negative with a red color and short rod shape. The same is in line with Darmawan (2017) that the pink color at the time of staining shows the characteristics of gram-negative and rod-shaped bacteria in general, which shows the characteristics of enteric bacteria. Gram staining was performed to confirm the shape of the bacteria and classify bacteria based on differences in response to the gram reaction on the physical and chemical properties of the cell wall (Bisen, 2014; Wibowo et al., 2016; Katon et al., 2020).

Putri & Kusdiyantini (2018) explained that the principle of gram staining is that gram-positive bacteria will absorb the color from the crystal violet solution so that it turns purple. Meanwhile, gram-negative bacteria will release a crystal violet solution after washing with alcohol and then absorb safranin so that it is red. This explanation refers to the results of observations of gram staining showing red-colored bacteria because bacteria have a thin cell wall, so they cannot hold crystal violet solutions. The observation results are strengthened by the opinion of Amri et al. (2017) gram-negative bacteria have a thin peptidoglycan layer and high enough permeability to release crystal violet substances quickly, and bacteria only absorb safranin. Gram-negative bacteria cannot retain crystal violet because of their cell walls' high lipid and protein content (Marzuki et al., 2014).

The final stage is to carry out biochemical testing. Biochemical tests were carried out to confirm previously obtained examination results (Rasyid et al., 2020). The test results were declared hostile in the VP (Voges-Proskauer) test because there was no change or formation of a red color on the surface of the test tube. These results suggest that *E. coli* cannot decompose glucose into acetoin but produces glucose which is in line with the research of Gunawan et al. (2022) that the negative results of *E.coli* in the VP test were because *E.coli* is a fecal group that cannot produce acetoin so that when KOH and alpha-naphthol are dropped, there will be no color change.

Escherichia coli in the MR test tested positive with a change in the surface of the media becoming red that same with is consistent with the research by Gunawan et al. (2022) that the positive test for *E. coli* on MR media is with the formation of red color in the media. Methyl red, as a reagent (indicator) that is reacted with MR media, can detect acid products from the fermentation of glucose so that it forms a red color and lowers the pH of the media in an acidic environment (Hemraj et al., 2013).

Positive test results for *E.coli* on indole media were indicated by the presence of a red ring on the surface of the media, with the explanation from Darmayanti et al. (2015) that *Escherichia coli* produces tryptophanase enzymes to decompose tryptophan amino acids into indole compounds and utilize tryptophan as a carbon source. According to Anggraini et al. (2016), some bacteria can

produce tryptophane enzymes to catalyze the macromolecules of the amino acid tryptophan into pyruvic acid, ammonia, and indole. The red ring formed on indole media indicates the ability of the bacteria to degrade tryptophan. The indole product formed will react with Kovac's reagent to form a red ring (Hemraj et al., 2013).

E.coli was declared hostile on the citrate test results with no color change in the citrate medium. According to Pastra et al. (2012), *Escherichia coli* does not utilize citrate as a carbon source. Citrate media is used to differentiate enteric bacteria as a carbon source, with the media composition containing bromothymol blue as an indicator of a green-to-blue color change in the media (Hemraj et al., 2013). In addition, the positive test results for *E. coli* on the glucose test are known by the presence of bubbles that appear in the test tube, which follows the statement of Hemraj et al. (2013) that the presence of air bubbles in the Durham tube is the result of carbohydrate fermentation. The lactose test was carried out in the same way as the glucose test where according to Nurhidayati et al. (2015), a glucose test was carried out to determine which bacteria are capable of fermenting glucose with a positive test result that will cause bubbles in the Durham tube which indicates the formation of gas while no bubbles indicate a negative result.

The characteristics of *E.coli* in the reference book stated positive results on Mac Conkey agar media, indol test, MR test, and lactose test, while negative results on the VP test and citrate test. The presence of these characteristics is consistent with existing microscopic tests. *Escherichia coli* is a bacterial species of the genus *Escherichia* and the family of Enterobacteriaceae (Cowan & Steel, 1993). Jawetz et al. (2008) stated that the Enterobacteriaceae family is a gram-negative rod that is short and has a distinctive morphology, but the morphology varies significantly in each species. The classification of E.coli is as follows.

Classification of *E.coli*, according to Songer & Post (2006), is as follows:

Kingdom : Bacteria
Phylum : Proteobacteria
Class : Gamma Proteobacteria
Order : Enterobacteriales
Family : Enterobacteriaceae
Genus : *Escherichia*
Species : *Escherichia coli* (*E.coli*)

The prevalence or level of presence of *E.coli* in each sample of proboscis monkey stools Mimin (M), Chikita (C), and Pedro (P) were sequentially 41.17%, 35.29%, and 35.29%. According Isnawati (2012), the high percentage of presence is due to *Escherichia* bacteria, usually present in the digestive organs of humans and animals. The high *E. coli* bacteria can protect the proboscis monkey's digestion from pathogenic bacteria because pathogenic bacteria are unable to compete with normal bacteria in the digestive tract, such as the genus *Escherichia* and lactic acid bacteria (LAB) (Supardi & Sukanto, 1999).

Escherichia coli is a commensal bacteria or normal microflora in warm-blooded animals and humans' peritoneum or lower intestine (Elliot et al., 2013). The intestinal microflora is a term for microorganisms commonly found living in the digestive tract and beneficial to the host (Salminen & Wright, 1993). *E. coli* becomes a pathogen if the number increases beyond normal or is outside the intestine. These bacteria can be spread through water contaminated with feces or urine from animals suffering from digestive infections so that they can be transmitted to other animals (Amri et al., 2017).

Infections that arise in the digestive tract due to the attack of *E.coli* bacteria on the intestinal wall cause movement of intestinal fluids in the large intestine and can damage the electrolyte balance in the mucous membrane and then can cause reduced water absorption in the intestinal wall and diarrhea (Soeliongan et al., 2013). Some strains produce enterotoxins and virulence factors to invade tissues and can cause diarrhea (Bergey's Manual, 1994). Pathogenic *E.coli* pathogens in animals that can cause diarrhea include enterotoxigenic *E.coli* (ETEC),

enteropathogenic *E.coli* (EPEC), Shiga toxin (stx) producing *E.coli* (STEC), and extraintestinal pathogenic *E.coli* (EIEC). (Gyles & Fairbrother, 2010).

Several researchers have previously researched the presence of bacteria in primate feces. Wibowo et al. (2016) tested orangutan feces from the BOS Foundation and successfully identified a type of bacteria, *Escherichia coli*. In addition, Rahmi et al. (2014) also reported the results of monitoring the feces of releasing orangutans at the Pinus Jantho Nature Reserve, Aceh, and it was known from the monitoring results that *Salmonella* sp., *Shigella* sp., and *E.coli* bacteria were identified. Research on feces of long-tailed monkeys by Loe et al. (2021) at the Bogor Agricultural Institute Captive Facility in Dramaga succeeded in isolating and identifying 100% *E.coli* bacteria (30/30), *Salmonella* sp. 97% (29/30), and *Shigella* sp. 60% (18/30).

The feces examined macroscopically and microscopically are expected to illustrate the digestion of proboscis monkeys in rehabilitation centers that are in good condition. The existence of proboscis monkeys in rescue center is continuously maintained to avoid digestive diseases by paying attention to the cleanliness of the cage as a place to live and the quality of food provided as well as routine checks by veterinarians (Wibowo et al., 2016; Amri et al., 2017).

Rahmi et al. (2014) stated that orangutans in rehabilitation have difficulty finding food because, at the training level, it causes orangutans to pick up leftover food that has fallen and is contaminated with feces. Individual proboscis monkeys also carry out the activity of picking up leftover food at the Bekantan Rescue Center Sahabat Bekantan Indonesia Foundation. Hence, the keepers need to pay attention to the cage's cleanliness daily to minimize any leftover food the proboscis monkeys can take. Wibowo et al. (2016) suggested that washing the cage floor with disinfection is vital to reduce disease-causing pathogens. The analysis in this study is expected to be used as material for further evaluation in supporting the sustainability of proboscis monkey conservation.

4. CONCLUSIONS

The bacteria identified from 17 stool samples from each proboscis monkey Mimin, Chikita, and Pedro were *Escherichia coli*. The prevalence or level of presence of *E. coli* in the proboscis monkey stool samples Mimin, Chikita, and Pedro were sequentially 41.17%, 35.29%, and 35.29%.

5. ACKNOWLEDGMENTS

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