

IDENTIFICATION OF *CRYPTOSPORIDIUM* SP. IN SUMATRAN ORANGUTAN AND BORNEAN ORANGUTAN AT NORTH SUMATERA WILDLIFE PARK

(Identifikasi *Cryptosporidium* sp. pada Orangutan Sumatera dan Orangutan Kalimantan di Taman Satwa Sumatera Utara)

**Indah Serly Pohan¹, Muhammad Hanafiah^{2*}, Erdiansyah Rahmi³
Winaruddin², Teuku Zahrial Helmi⁴, Rosmaidar⁵**

¹Veterinary Student, Faculty of Veterinary Medicine, Syiah Kuala University, Jl. Teuku Nyak Arief, Banda Aceh, Aceh, Indonesia, 23114;

²Parasitology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Jl. Teuku Nyak Arief, Banda Aceh, Aceh, Indonesia, 23114;

³Histology Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Jl. Teuku Nyak Arief, Banda Aceh, Aceh, Indonesia, 23114;

⁴Biochemistry Laboratory, Faculty of Veterinary Medicine, Syiah Kuala University, Jl. Teuku Nyak Arief, Banda Aceh, Aceh, Indonesia, 23114;

⁵Pharmacology Laboratory Faculty of Veterinary Medicine, Syiah Kuala University, Jl. Teuku Nyak Arief, Banda Aceh, Aceh, Indonesia, 23114

*Corresponding author email: hanafi_2015@unsyiah.ac.id

Abstract

Cryptosporidium sp. is a zoonotic intestinal protozoa in orangutans which is transmitted through contaminated water (waterborne disease). This study aims to determine *Cryptosporidium* sp. which infects the sumatran orangutan (*Pongo abelii*) and the bornean orangutan (*Pongo pygmaeus*) in North Sumatera Wildlife Park. The samples used were 3 orangutan fecal samples from different locations, two bornean orangutans in Medan Wildlife Park and one sumatran orangutan in Pematang Siantar Animal Park. Samples were examined using the floatation method and the modified Ziehl-Neelsen method. The results showed that from 3 samples of orangutan feces (sumatran and bornean orangutan), 2 positive tails from bornean orangutans were found containing *Cryptosporidium* oocysts, namely the *C. parvum* species. In this study, it can be concluded that the orangutans in Medan Wildlife Park, the bornean orangutan were infected with cryptosporidiosis.

Keywords: *Cryptosporidium* sp.; bornean orangutan; *Pongo abelii*; *Pongo pygmaeus*; sumatran orangutan

Abstrak

Cryptosporidium sp. adalah protozoa pencernaan yang bersifat zoonosis pada orangutan yang ditularkan melalui air yang terkontaminasi (*waterborne disease*). Penelitian ini bertujuan untuk mengetahui *Cryptosporidium* sp. yang menginfeksi orangutan sumatera (*Pongo abelii*) dan orangutan kalimantan (*Pongo pygmaeus*) di Taman Satwa Sumatera Utara. Sampel yang digunakan sebanyak 3 sampel feses orangutan dari lokasi yang berbeda, yaitu 2 ekor orangutan kalimantan di Taman Margasatwa Medan dan 1 ekor orangutan sumatera di Taman Hewan Pematang Siantar. Sampel diperiksa dengan menggunakan metode floatasi dan metode *modified* Ziehl-Neelsen. Hasil penelitian menunjukkan dari 3 sampel feses orangutan didapatkan 2 ekor positif mengandung oosista *Cryptosporidium*, yaitu spesies *C. parvum*. Pada penelitian ini dapat disimpulkan bahwa orangutan yang ada di Taman Margasatwa Medan, yaitu orangutan kalimantan terinfeksi kriptosporidiosis.

Kata kunci: *Cryptosporidium* sp.; orangutan Kalimantan; orangutan Sumatera; *Pongo abelii*; *Pongo pygmaeus*.

INTRODUCTION

Indonesia is one of the countries with high primate diversity. One of the primates that are threatened with extinction is the orangutan. There are three extant orangutan species, two of which are on the island of Sumatera (*Pongo abelii* and *Pongo tapanuliensis*) and one on the island of Kalimantan (*Pongo pygmaeus*) (Condro *et al.*, 2021). Orangutans are classified by the International Union for Conservation of Nature (IUCN) as critically endangered or critically endangered because they have experienced rapid population decline (Chua *et al.*, 2020).

One of the efforts of ex-situ conservation is to carry out captive breeding with the aim of education and promotion of conservatives through the management of animal parks or zoos. In Medan, there is Medan Wildlife Park which has long been known to have tourism potential, has attractions such as typical animals from North Sumatera (Tamba and Prana, 2020). In addition, in Siantar, there is also a breeding place, namely Pematang Siantar Animal Park (THPS). In 2018, there were 2 orangutans (1 male and 1 female) in Pematang Siantar Animal Park. However, in 2021 there will be only one male orangutan because another orangutan has died. The habitat environment between natural and enclosures in zoos is very different. The parameters for the success of ex-situ conservation are seen from the number of orangutans that are able to reproduce and survive with the treatment and treatment given at the time of conservation (Tarigan *et al.*, 2021).

Primate populations in animal parks act as potential reservoirs for pathogens that infect humans, such as viruses, bacteria and parasites that can cause zoonoses. Several studies have reported that the enteric protozoan pathogen, namely *Giardia intestinalis*, *Entamoeba histolytica* and *Cryptosporidium* sp. can cause gastrointestinal enteritis in primates (gibbons, orangutans, marmosets, gorillas

and chimpanzees) (Menu *et al.*, 2021). *Cryptosporidium* sp. is a zoonotic protozoan. *Cryptosporidium* sp. can cause diarrhea that is acute to chronic diarrhea (Maryanti, 2017). Transmission of *Cryptosporidium* sp. can occur in animals and humans when consuming contaminated water (waterborne disease) (Xiao and Feng, 2008).

Until now there has been no report regarding the identification of *Cryptosporidium* sp. in sumatran and bornean orangutans, so it is necessary to identify *Cryptosporidium* sp. which infests sumatran and bornean orangutans in North Sumatra Wildlife Park as an effort to improve health and estimate potential zoonotic risks as well as to maintain the life of protected orangutans.

RESEARCH METHODS

Place and Time of Research

This research was conducted in January 2022 in three locations, the Medan Wildlife Park, Pematang Siantar Animal Park, North Sumatra and Parasitology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh.

Research Tools and Materials

The tools used in this study were ice boxes, smartphone cameras, refrigerators, masks, gloves, sterile bottles/plastics, digital scales, mortar, stirring rods, spatulas, centrifuges, centrifuge tubes, centrifuge tube racks, tea filters, dropper drops glasses, measuring instrument, object glass, cover glass, lens tissue, CX-3 microscope, label paper, markers, and pen.

The materials used in this study were 3 fecal samples, consisting of 2 samples of the bornean orangutans feces taken at Medan Wildlife Park and 1 sumatran orangutan feces samples from Pematang Siantar Animal Park, North Sumatra, methylene blue 0,1%, NaCl saturated, aquades, acid alcohol 3%, formalin 10%, methanol, carbol fuchsin 1% , immersion oil, and water.

Research Sample

The samples used in this study were fresh fecal samples taken from 3 orangutans, including 2 bornean orangutans, male and female in Medan Wildlife Park and 1 male sumatran orangutan at Pematang Siantar Animal Park, North Sumatra.

Research Procedure

Sampling method.

Stool samples of sumatran orangutan and bornean orangutans were taken immediately after the orangutans defecated using a spatula by taking the middle or inner part of the pile of feces as much as ± 3 g and put into bottles/plastic samples filled with 10% formalin solution in a ratio of 1:1, then homogenized and stored in an ice box. Furthermore, the samples in the ice box were brought aseptically to Parasitology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University for examination.

Sample Inspection Method

Floatation method.

The principle of the floatation method is that the specific gravity of the protozoa is lighter than the specific gravity of the solution used, so that the protozoa will float on the surface. Fecal samples taken as much as ± 2 g were put into a mortar, added with distilled water and stirred until homogeneous. The preparation was filtered, then put into a centrifuge tube up to tube and centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, then saturated NaCl was added and stirred until homogeneous. Spin again at 1500 rpm for 5 minutes. The preparation was placed on a tube rack perpendicularly and dripped with saturated NaCl using a pipette until the surface of the liquid in the tube became convex and left for 3 minutes. Paste the object glass on the convex surface carefully and then flip it quickly. The surface of the object glass was covered with a cover glass and examined for gastrointestinal protozoa under a microscope.

Ziehl-neelsen modified method.

Fecal samples taken ± 1 g were put into the mortar, added with distilled water and crushed until homogeneous, then filtered through a tea filter and dripped 2-3 drops on the object glass. Then, the preparation is made like a thin blood smear and waits for it to dry. After drying, the preparation was fixed in methanol for 3 minutes, and was flooded with carbol fuchsin for 15-20 minutes, then washed with running water. The preparation is rinsed again with acid 3% alcohol and allowed to stand for 15-20 seconds, then washed with running water and waited for it to dry. Then dripped with methylene blue into the preparation for 1 minute and observed under a microscope. The surface of the object glass was closed using a cover glass and examined for *Cryptosporidium* sp. under a microscope with a magnification of 400x or 1000x.

Species Identification

Protozoa identification is done by looking at the morphological characteristics as well as the size and shape of the protozoa. Furthermore, to determine the species of *Cryptosporidium* sp. measurements were made by connecting a sigma camera to the microscope and then displayed on the monitor screen and measurements were made using the Topup view application .

Data Analysis

The research data obtained were tabulated for further descriptive analysis.

RESULTS AND DISCUSSION

Examination of the fecal from sumatran orangutan and bornean orangutans using the floatation method and the modified Ziehl-Neelsen method to detect the oocysts of *Cryptosporidium* sp. which is presented in Table 1.

Based on Table 1, it can be seen that from 3 samples of orangutans, 2 orangutans from Kalimantan (*Pongo pygmaeus*) was positive *Cryptosporidium parvum* using the floatation method and the modified Ziehl-Neelsen method.

Infection *C. parvum* in bornean orangutans has been reported by Mynářová *et al.* (2016), there were 2 orangutans positive *C. parvum* from 298 samples. Species identification in this study was carried out by observing the characteristics, morphology, and shape of the protozoan then measurements were made to determine the species of *Cryptosporidium* sp.

Based on Figure 1, the oocyst of *Cryptosporidium* sp. observed with Ziehl-Neelsen method are round, pink in color and have a size of $2.21 \times 2.25 \mu\text{m}$. In Figure 2, the *Cryptosporidium* sp. oocyst observed by the floatation method are round with a size of $2.43 \times 2.65 \mu\text{m}$. According to Mufa *et al.* (2020), oocyst *Cryptosporidium* spp. has a round, oval shape and 2-6 μm in diameter, while the size of *Cryptosporidium parvum* oocysts is 2-3.2 μm in diameter and spherical (Mehlhorn, 2012; Taylor *et al.*, 2016). Based on this, the species *Cryptosporidium* sp. observed was *C. parvum*.

Cryptosporidium parvum is a species that has been found in orangutans (Cibot *et al.*, 2021) and the most pathogenic species in humans with more than 90% of cryptosporidiosis cases in humans worldwide and is responsible for almost all causes of cryptosporidiosis (Khan *et al.*, 2018; Zhao *et al.*, 2019).

Cryptosporidium is a zoonotic protozoan commonly found in primates. As a result, there is a potential for transmission of this pathogen to humans and vice versa (Zhao *et al.*, 2019). Close and frequent contact between orangutans and cage staff can increase the risk of transmitting parasitic diseases from orangutans to cage staff or vice versa. Primates with high levels of human contact are more often infected with *Cryptosporidium*, for example semi-wild and captive animals (Mynářová *et al.*, 2016).

Infection *Cryptosporidium parvum* in the bornean orangutan (*Pongo pygmaeus*)

at Medan Wildlife Park was caused by several factors, like water supply, environment, and cage maintenance management. A drinking holder with an open container made of cement is placed in the cage, allowing contamination of the drinking water which is a factor causing cryptosporidiosis (waterborne disease). This is in accordance with the statement of Wahdini *et al.* (2021), who stated that poor water quality contributed to the high prevalence of digestive protozoan infections. Meanwhile, Osman *et al.* (2016) reported that consumption of untreated water tripled the risk of protozoal infection compared to water treated with chlorite. However, chlorination of drinking water could not kill oocysts *Cryptosporidium* sp. (Maryanti, 2017). An effective disinfectant to kill *Cryptosporidium* sp. are ammonia, formol saline 10%, hydrogen peroxide 3%, and commercial bleach 50%. In addition, freezing below freezing point and heating at a temperature $>65 \text{ }^\circ\text{C}$ for 30 minutes can also kill it (Wijayanti, 2017).

The condition of the orangutan cage in the Medan Wildlife Park is cleaned once a day in the morning. The cage staff did not wear personal protective equipment such as masks and gloves to prevent contamination of *Cryptosporidium* sp. oocysts. According to Rachmawati (2019), the habit of washing hands, cutting nails, using personal protective equipment such as masks and gloves, and cleaning the cage regularly are steps to prevent oocyst contamination.

Apart from poor maintenance management, cryptosporidiosis can also be infected through contamination of food given to orangutans. At Medan Wildlife Park, orangutans are given fresh food in the form of fruits and vegetables, such as papaya, banana, corn, kale, and mustard greens once a day. Orangutans are also given additional feed such as boiled eggs, honey and milk when stocks become available. The feed is given directly to the orangutans without being cleaned first.

Contamination of the feed given to orangutans may occur because the fruits and vegetables given are grown in areas that have been contaminated with the oocyst *Cryptosporidium* sp. This is in accordance with the statement of Zhao *et al.* (2019) that cryptosporidiosis can be transmitted through water and food, such as fruits, vegetables, and milk contaminated with the oocyst *Cryptosporidium* sp.

According to information received, the bornean orangutan in Medan Wildlife Park came from the Ragunan Zoo in 2019 and was never given antiprotozoal drugs and antibiotics. The orangutans were only given vitamins. Athaillah *et al.* (2021) stated that the high prevalence of protozoal infestations could occur because primates were never given antiprotozoal drugs.

Cryptosporidium sp. is one of the opportunistic intestinal protozoa which is generally asymptomatic or causes mild symptoms and is self-limited in immunocompetent individuals (Wijayanti, 2017). This is similar to this study where the bornean orangutan infected with cryptosporidiosis showed no symptoms (asymptomatic) and had a soft stool consistency, like solid but not hard.

The clinical symptoms and severity of cryptosporidiosis can vary widely, largely depending on the patient's immune status. Patients with immune system disorders, especially patients with Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS) have a high mortality rate (Zhao *et al.*, 2019). The infection can become chronic and life-threatening with frequent defecation leading to dehydration. In severe cases, the oocyst of *Cryptosporidium* sp. can infected the digestive tract (epithelial cells of the small intestine villi), it can also reach the respiratory tract, liver, bladder, and pancreas (Fayer, 2004). Antiretroviral therapy can be given to increase the immune system and reduce symptoms of cryptosporidiosis (Mohammed *et al.*, 2017).

Sumatran orangutan (*Pongo abelii*) in Pematang Siantar Animal Park was not found oocysts *Cryptosporidium* sp. with the floatation method and the modified Ziehl-Neelsen method. This is probably due to good management of cage maintenance, provision of drinking water and feed at THPS. Orangutan cages are cleaned twice a day, in the morning and evening. Orangutans are given regular deworming medicine every 3 months and herbal deworming medicine once a month such as papaya leaves and chinese petai which are mixed into their daily diet. Feed is given in the morning (once a day) in the form of bananas, papaya, sapodilla, cucumber, corn, salak, oranges, and long beans which are washed first. On Wednesdays and Sundays, they are given additional feed, namely one boiled egg and coconut.

Oocyst of *Cryptosporidium* sp. is the infective stage that is commonly found in the feces of infected humans and animals. Diagnosis can be made by finding oocysts from feces through floatation or stool concentration methods. Fresh stool specimens can be concentrated with sheater sugar solution and preparations with cover glass examined under a microscope. In this study, the floatation method and the modified Ziehl-Neelsen method were used. In the floatation method, positive results were obtained or the discovery of *Cryptosporidium* sp. oocysts. This method must have a high eye sensitivity to look for *Cryptosporidium* sp. considering how small they are under a microscope.

The modified Ziehl-Neelsen method is a simple and specific method to detect *Cryptosporidium* sp. (Maryanti *et al.*, 2014) which will be colored reddish-pink and easily distinguished from the background (Kusumasari and Syairaji, 2020). This method has an accuracy of about 37-90% (Mahardianti *et al.*, 2020). The same thing in this study, in the modified Ziehl-Neelsen method the discovery of oocysts *Cryptosporidium* sp.

was pink under a microscope at 1000x magnification.

CONCLUSION AND SUGGESTIONS

Conclusion

From the results of the examination of 3 samples of orangutan feces at different locations, 1 sumatran orangutan (*Pongo abelii*) at Pematang Siantar Animal Park and 2 bornean orangutans (*Pongo pygmaeus*) at Medan Wildlife Park, it was found that 2 bornean orangutans were infected by the protozoan *Cryptosporidium parvum*.

Suggestion

It is necessary to carry out good and correct management of cage maintenance to prevent cryptosporidiosis and further serological research is needed, such as ELISA (Enzyme-linked Immunosorbent Assay) to confirm *Cryptosporidium* oocyst species from samples obtained.

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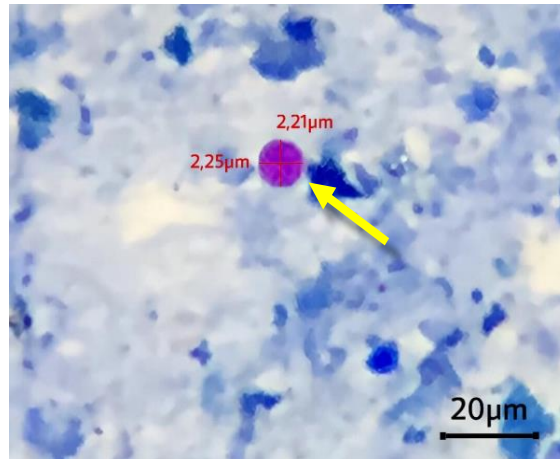


Figure 1. Results inspection oocyst of *Cryptosporidium parvum* in orangutan's feces with a diameter $2.21 \times 2.25 \mu\text{m}$ using the modified Ziehl-Neelsen method

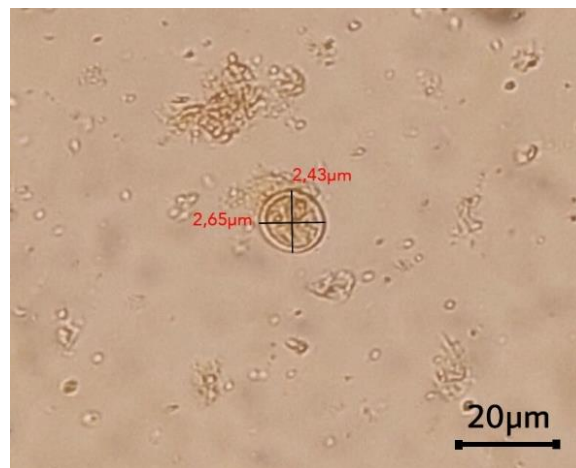


Figure 2. Results inspection oocyst of *Cryptosporidium parvum* in orangutan's feces with a diameter $2.43 \times 2.65 \mu\text{m}$ using the floatation method

Table 1. *Cryptosporidium* sp. found in orangutans in North Sumatra Wildlife Park

Type of orangutan	Gender	Method	
		Floatation	Modified ziehl-neelsen
Sumatran orangutan (<i>Pongo abelii</i>)	Male	-	-
Bornean orangutan (<i>Pongo pygmaeus</i>)	Male	+	+
Bornean orangutan (<i>Pongo pygmaeus</i>)	Female	+	+