Pratama *et al*. MKH (2024). 71-78
DOI: 10.20473/mkh.v35i2.2024.71-78

Detection of *Cryptosporidium* spp. in Wild Rats (*Rattus* spp.) in Surabaya, East Java

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ABSTRACT

Cryptosporidiosis is a waterborne zoonosis caused by *Cryptosporidium*, which is a parasite that causes infection of the small intestine and leads to acute diarrhea in both humans and animals. Wild rats (*Rattus* spp.) are known to carry many zoonotic pathogens including *Cryptosporidium*. Given their close proximity to humans in urban environments, the likelihood of disease transmission is high. This study aims to detect the *Cryptosporidium* spp. infection in wild rats in Surabaya City, East Java Province. Intestinal fecal samples were collected using necropsy. The flotation test and Ziehl-Neelsen stain were used to observe the presence of *Cryptosporidium* under the microscope. The results showed that 69 samples were positive for *Cryptosporidium* spp. in the oocyst stage, which appeared dark pink with a clear cavity and the size of the oocysts ranged from 2-6 µm. The high incidence of cryptosporidiosis in wild rats is a potential health threat to both animals and humans.

Keyword: *Cryptosporidium*, parasitic disease, Surabaya, wild rats

INTRODUCTION

Cryptosporidium spp. is an intestinal parasite that causes cryptosporidiosis and was discovered over a century ago. In 1976, the first case of *Cryptosporidium* infection in humans was reported, namely in an immunocompetent girl and an immunosuppressed adult with symptoms of diarrhea (Gerace et al., 2019).

Cryptosporidiosis is a waterborne zoonosis caused by microbial contamination or hazardous substances that are transmitted between living organisms through water (Mufa et al., 2020). According to the World Health Organization (WHO), waterborne diseases are responsible for 4.1% of total deaths worldwide, affecting approximately 1.8 million people annually (WHO, 2006).

More than 40 species and genotypes of *Cryptosporidium* have been identified worldwide (Zahedi et al., 2021). A total of 21 species and 21 genotypes of *Cryptosporidium* were identified in rodents (Zhao et al., 2019).

Received: 08-10-2023, Accepted: 01-12-2023, Published online: 10-05-2024
Available at https://e-journal.unair.ac.id/MKH/index

The most common *Cryptosporidium* species found in wild rats are *C. parvum* and *C. muris*. *C. parvum* has also been found in humans, cattle, sheep, deer, and swine (Koehler et al., 2018).

Cryptosporidium spp. has a monoxenous life cycle in which all stages of development, including asexual and sexual, occur in a single host (Smith et al., 2007). The infective stage of the parasite is characterized by the presence of oocysts that are excreted in feces (Edwards et al., 2009). Upon entering the digestive tract, the oocysts release infective sporozoites which attach to the intestinal epithelium and turn into trophozoites (Bouzid et al., 2018). In addition, some merozoites develop into macrogametocytes and microgametocytes for sexual reproduction (Smith et al., 2007). Sexual reproduction occurs through gametogony, which results in the formation of a zygote that undergoes further asexual development (sporogony), leading to the production of sporulated oocysts that contain four sporozoites (Ghazy et al., 2015). The entire life cycle of the parasite can be completed in two days. The period from infection to oocyst excretion ranges from one to three weeks, while the patent period ranges from a few days to months or even years. The length of infectious period can be influenced by several factors, but the most common factors are the immunocompetence of the host and the species of the parasite (Ghazy et al., 2015). Oocysts as the infective form of *Cryptosporidium* spp. are excreted in the feces of an infected host. The clinical manifestations of cryptosporidiosis depend on the immune status of the patient. In immunocompetent individuals, *Cryptosporidium* spp. Infection may be asymptomatic or result in acute diarrhea that may resolve spontaneously (Ghazy et al., 2015).

In Indonesia, only a few cases of cryptosporidiosis have been reported. In addition to diarrhea, clinical manifestations of cryptosporidiosis include mild fever, dizziness, heartburn, nausea, vomiting, anorexia, and sleep disturbances, which can lead to weight loss. While *Cryptosporidium* spp. does not directly cause death, diarrhea and malnutrition can be contributing factors (Wijayanti, 2017). Kurniawan et al. (2007) reported that 34% of fecal samples from toddlers aged under three years in Jakarta were positive for *Cryptosporidium* spp.

The prevalence of *Cryptosporidium* spp. in animals varies widely, with rodents showing a rate of up to 22% in Europe (Wijayanti, 2017). A study conducted in France reported that the prevalence of *Cryptosporidium* spp. in rats ranged from 2.1% to 63.0% (Livia et al., 2021). Another study conducted in China found that 8.2% of 232 fecal samples from rats were positive for *Cryptosporidium* oocysts (Zhao et al., 2015). In Japan, 8% of 50 rats were positive for *Cryptosporidium* oocysts using the indirect fluorescent antibody (IFA) technique and 38% were positive using the nested polymerase chain reaction (PCR) technique (Kimura et al., 2007). Another study found that 27.3% of rats were positive for *Cryptosporidium* (Bahrami et al., 2012). This study aims to detect *Cryptosporidium* infection in wild rats in Surabaya City, East Java.

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MATERIALS AND METHODS

Sampling

Wild rats were captured using baited trap cages in different areas of Surabaya, including North, West, South, East, and Central Surabaya. The sampling locations included traditional market areas, high density residential areas, and residential areas. A total of 100 rat samples were collected for this study.

Microscopic Examination

Fecal samples were collected using necropsy from the digestive tract of rats induced ketamine at a dose of 50 mg/kg intramuscularly. The samples were analyzed using the centrifugal sugar flotation technique. One gram of feces was diluted in 9 mL of distilled water and centrifuged at 1500 × g for five minutes. The supernatant was discarded and 10 mL of sugar solution with a specific gravity of 1.2 was added to the sediment. The sample was centrifuged again at 1500 × g for five minutes. The floating material was transferred to a glass slide and examined under a light microscope at 400x magnification to determine the shape of the visible cyst. Subsequently, the sample was examined at 1000x magnification and documented using an OptiLab camera.

Ziehl-Neelsen Stain

For direct smears, a small amount of fecal suspension was placed on a glass slide and spread to create a thin later. The smears were stained according to the Ziehl-Neelsen method. The smear was first dried then fixed by heating it over a Bunsen burner. Subsequently, carbol fuchsin was poured onto the slide and heated until it steamed, but did not boil. After five minutes, the slide was washed with water and decolorized with 2.5% sulfuric acid for one minute, depending on the thickness of the smear. Finally, the slide was stained again with 1% methylene blue for one minute, washed, dried, and examined with immersion oil at 400x or 1000x magnification (Tahvildar and Salehi, 2020).

RESULTS AND DISCUSSION

To detect oocysts of *Cryptosporidium* spp., fecal samples from house rats (*Rattus tanezumi*) and Norway rats (*Rattus norvegicus*) in Surabaya City underwent microscopic examination using the floatation test. Out of 100 samples, oocysts of *Cryptosporidium* spp. were found in 69 samples (69%). The oocysts were round with a diameter of 3.67 µm (Figure 1). This is consistent with the findings of Mufa et al. (2020) who reported that the size of the oocysts of *Cryptosporidium* spp. ranged from two to six µm, were round, and had four sporozoites inside.

The fecal examination technique was combined with the Ziehl-Neelsen stain to confirm the diagnosis. The stain results of *Cryptosporidium* spp. showed round, dark pink oocysts with a clear internal cavity (Figure 2). This is in consistent with the findings of Hameed et al. (2021) who reported that the oocysts of *Cryptosporidium* spp. were pink as well as the findings of Rekha et al. (2016) who reported that the results of the Ziehl-Neelsen stain of *Cryptosporidium* spp. showed dark pink oocysts with a clear internal cavity

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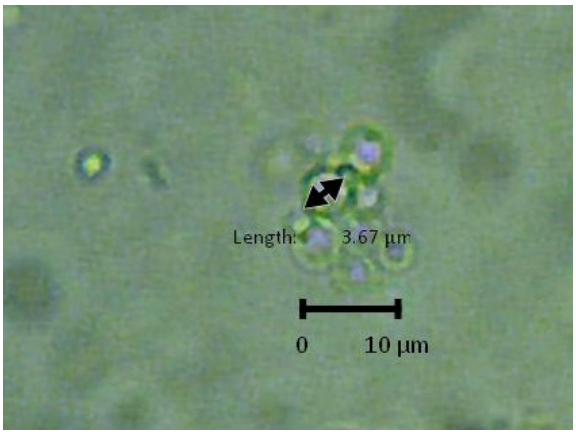

Figure 1. Oocysts of *Cryptosporidium* spp. at 1000x magnification

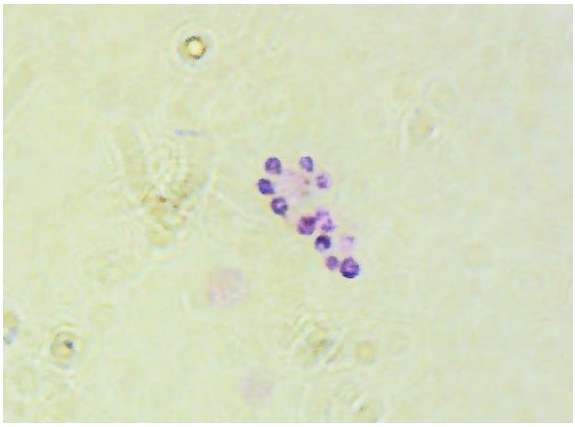

Figure 2. Oocysts of *Cryptosporidium* spp. using the Ziehl-Neelsen stain at 400x magnification

Wild rats (*Rattus* spp.) are carriers of numerous zoonotic pathogens, including *Cryptosporidium*. Given their close proximity to humans in urban environments, the likelihood of disease transmission is high (Zhao et al., 2019). Rodents play a significant role in the transmission of emerging pathogens, such as viruses, bacteria, rickettsiae, and protozoa (Meerburg et al., 2009). Wild rats are the most common rodents and are usually found in populated areas, especially in rural areas with poor sanitation or in densely populated slums. Rodent migration facilitates disease transmission because they are numerous, nomadic, and resistant to pathogens (Koehler et al., 2018). In Tehran, Iran, 13% and 27.3% of the 77 mice were found to be positive for *Cryptosporidium* oocysts microscopically and molecularly, respectively (Bahrami et al., 2012).

Rodents have the potential to transmit infection. Mechanical transmission by birds, insects, and humans may also occur (Martin & Aitken, 2000). Other potential risk factors include the size of the herd and the season of the year. In addition, the large number of oocysts released during infection ensures a high level of environmental contamination (Dixon et al., 2011). A single oocyst is enough

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to cause infection and disease in a susceptible host. Environmentally resistant oocysts are transmitted by the fecal-oral route. Disease transmission can occur in a variety of ways, including animal to animal, animal to human, human to animal, or human to human, through ingestion of contaminated water or food or contact with contaminated surfaces (Smith et al., 2007).

Host infection occurs by ingesting infective oocysts. Once ingested, excystation occurs in the upper gastrointestinal tract. The oocysts release infective sporozoites, which attach to the intestinal epithelial cells and become trophozoites (Bouzid et al., 2018). Excystation can be triggered by several factors, including reduced host immunity, carbon dioxide, temperature, pancreatic enzymes, and bile salts (Ghazy et al., 2015). *Cryptosporidium* infection occurs between the cytoplasm and the cell membrane and is distinguished from other enteric protozoa, especially *C. parvum*, by its unique ability to autoinfect, inherent antimicrobial resistance, and general lack of host specificity (Gerace et al., 2019).

The dynamics of zoonotic pathogens in rat populations and the risk of pathogen transmission from rats to humans are significantly influenced by ecology. However, many studies of rat-associated zoonoses (RAZ) fail to take into account the characteristics of rat populations during sampling or data analysis. This is problematic because it may hinder the development of a comprehensive understanding of the ecology of pathogens in rat populations. Without comparison and synthesis of different research findings, incorrect conclusions about the dynamics of RAZ may be drawn (Himsworth et al., 2014).

Due to the high potential for transfer of *Cryptosporidium* oocysts from soil to water sources, populations residing near rivers should be considered as potential causes of waterborne diseases (Ghazy et al., 2015). *Cryptosporidium* oocysts have been found in untreated surface waters, such as rivers, lakes and reservoirs, as well as in untreated and treated wastes, swimming pool, and even water treated for human consumption (Gerace et al., 2019). Nitazoxanide has been shown to be an effective antiparasitic drug and should be taken twice a day orally for three days at a dose of 500 mg for individuals aged over 12 years, 200 mg for those aged between 4 and 11 years, or 100 mg for children aged between one and three years (Pantenburg et al., 2009).

CONCLUSION

The results of microscopic examination of 100 fecal samples of wild rats in Surabaya City showed that 69% of the samples contained oocysts of *Cryptosporidium* spp., and the Ziehl-Neelsen stain showed that the oocysts were dark pink with a clear internal cavity.

Further molecular identification, such as sequencing, is necessary to determine the genetic characteristics of *Cryptosporidium* spp. and to establish a diagnosis. In addition, educational efforts are needed to increase public awareness of potential transmission of *Cryptosporidium* spp. through oocyst contamination in the environment and food.

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APPROVAL OF ETHICAL COMISSION

The experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number 2.KEH.080.07.2022.

ACKNOWLEDGEMENT

The authors would like to thank the Directorate General of Higher Education for the research grant for doctorate students with a decree number 759/UN3.15/PT/2022.

REFERENCES

Bahrami, F, Sadraei J, and Frozandeh M. 2012. Molecular Characterization of *Cryptosporidium spp*. in Wild Rats of Tehran, Iran Using 18s rRNA Gene and PCR_RFLP Method. Jundishapur J Microbiol.5(3):486–490

Bouzid, M, Kintz E, and Hunter P. 2018. Risk factors for *Cryptosporidium* infection in low and middle income countries: A systematic review and meta-analysis. PLoS Neglected Trop Dis.12(6):1-13

Dixon, B, Parrington L, Cook A, Pintar K, Pollari F, Kelton D, and Farber J. 2011. The potential for zoonotic transmission of *Giardia duodenalis* and *Cryptosporidium spp*. from beef and dairy cattle in Ontario, Canada. VetParasitol. 175(1–2):6-20.

Edwards, Hanna, Thompson R, Armstrong T, and Clode P. 2009. Morphological characterization of Cryptosporidium parvum life-cycle stages in an in vitro model system. Parasitology. 137:13-26.

Gerace, Elisabetta, Marco V, and Biondo C. 2019. Cryptosporidium Infection: Epidemiology, Pathogenesis, and Differential Diagnosis. European Journal of Microbiology and Immunology. 9:1-5.

Ghazy, A, Abdel S, and Shaapan R. 2015. Cryptosporidiosis in Animals and Man: 1. Taxonomic Classification, Life Cycle, Epidemiology and Zoonotic Importance. Asian Journal of Epidemiology. 8:48-63.

Hamed, W, Yousef N, Mazrou Y, Elkholy W, El-Refaiy A, Elfeky F, Albadrani M, El-Tokhy A, and Abdelaal K. 2021. Anticryptosporidium Efficacy of Olea europaea and Ficus carica Leaves Extract in Immunocompromised Rat Associated with Biochemical Characters and Antioxidative System. Cells. 10.

Himsworth C, Jardine C, Parsons K, Feng A, and Patrick D. 2014. The Characteristics of Wild Rat (*Rattus* spp.) Populations from an Inner-City Neighborhood with a Focus on Factors Critical to the Understanding of Rat-Associated Zoonoses. PLoS ONE. 9(3).

Kimura, A, Edagawa A, Okada K, Takimoto A, Yonesho S, and Karanis P. 2007. Detection and genotyping of Cryptosporidium from brown rats (*Rattus norvegicus*) captured in an urban

Received: 08-10-2023, Accepted: 01-12-2023, Published online: 10-05-2024
Available at https://e-journal.unair.ac.id/MKH/index

area of Japan. Parasitol Res.100:1417–1420.

Koehler, A, Wang T, Haydon S, and Gasser R. 2018. *Cryptosporidium viatorum* from the native Australian swamp rat Rattus lutreolus - an emerging zoonotic pathogen? Int. J. Parasitol. Parasites. Wildl. 7:18–26.

Livia, G, Fernández Á, Feliu C, Miquel J, Quilichini Y, and Foronda P. 2021. *Cryptosporidium* spp. in wild murids (Rodentia) from Corsica, France. Parasitology Research. 121:345-354.

Martin, W and Aitken I, 2000. Diseases of Sheep. 3rd Edn., Blackwell Science Ltd., UK.,pp: 153-159

Meerburg, B, Singleton G, and Kijlstra A. 2009. Rodent-borne diseases and their risks for public health. Crit. Rev. Microbiol. 35:221–270.

Mufa, R, Nunuk D, Fedik A, Lucia T, Endang S, Didik H, dan Mufasirin. 2020. Deteksi *Cryptosporidium canis* pada Anjing di Kota Surabaya. Jurnal Veteriner 21(2): 176-182.

Pantenburg, B, Cabada M, and White A. 2009. Treatment of cryptosporidiosis. Expert review of anti-infective therapy. 7:385.

Rekha, K, Puttalakshmamma G, and D'Souza P. 2016. Comparison of different diagnostic techniques for the detection of cryptosporidiosis in bovines. Veterinary world. 9. 211-215.

Smith, H, Caccio S, Cook N, Nichols R and Tait A , 2007. *Cryptosporidium* a n d *Giardia* as foodborne zoonoses. Vet Parasitol. 149: 29-40.

Tahvildar, B. and Salehi N. 2014. Detection of *Cryptosporidium* infection by modified ziehl-neelsen and PCR methods in children with diarrheal samples in pediatric hospitals in Tehran. Gastroenterol Hepatol Bed Bench. Spring. 7(2):125-30.

WHO (World Health Organization). 2006. Guidelines for Drinking Water Quality. Cryptosporidiosis. Microbiol Environ Health. 9-119.

Wijayanti T. 2017. Cryptosporidiosis in Indonesia. Balaba. 13(01):73-82.

Zahedi, A, Bolland S, Oskam C, and Ryan U. 2021. *Cryptosporidium abrahamseni n. sp*. (Apicomplexa: Cryptosporidiiae) from red-eye tetra (Moenkhausia sanctaefilomenae). Experimental parasitology, 223.2021 .108089

Zhao, Z, Wang R, Zhao W, Qi M, Zhao J, Zhang L, Li J, and Liu A. 2015. Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. Cambridge Univ Press.142(6):800–806.

Zhao, W, Zhou H, Huang Y, Xu L, Rao L, Wang S, Wang W, Yi Y, Zhou X, Wu Y, Ma T, Wang G, Hu X, Peng R, Yin F, and Lu G. 2019. *Cryptosporidium* spp. in wild rats (*Rattus* spp.) from the Hainan Province, China: Molecular detection, species/genotype identification and implications for public health. International journal for

Received: 08-10-2023, Accepted: 01-12-2023, Published online: 10-05-2024
Available at https://e-journal.unair.ac.id/MKH/index

Pratama *et al*. MKH (2024). 71-78
DOI: 10.20473/mkh.v35i2.2024.71-78

parasitology. Parasites and wildlife. 9:317–321

Received: 08-10-2023, Accepted: 01-12-2023, Published online: 10-05-2024
Available at https://e-journal.unair.ac.id/MKH/index