Identification of Gastrointestinal Protozoa of Swine (*Sus* sp.) In Surabaya Slaughterhouse in Dry Season

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Abstract

Surabaya slaughterhouse provides pork cuts from several swine farms. Prior to slaughter, the swine placed in a temporary pen, which allows the transmission of gastrointestinal protozoa to fellow swine and to humans at the Surabaya slaughterhouse. This research conducted to identify gastrointestinal protozoa of swine that slaughtered in Surabaya slaughterhouse. 100 fecal samples was observed using native method, sedimentation method, Fulleborne floating method and Ziehl neelsen method. Based on the results, there were 47 samples positive. The protozoa found included *Balantidium* sp. with 46% prevalence, *Eimeria* sp. / *Isospora* sp. with 32% prevalence, *Entamoeba* sp. with 24% prevalence, and *Blastocystis* sp.with 12% prevalence.

Keywords: Swine, *Balantidium* sp., *Eimeria* sp., *Entamoeba* sp., *Blastocystis* sp.

Introduction

Swine (*Sus* sp.) are omnivorous animals that bred to meet human consumption needs. Swine farming spread in almost all regions in Indonesia, including in East Java. In order to meet the needs of porks in East Java, especially Surabaya, the slaughterhouse provides pork cuts from swine farms in Mojokerto, Blitar, Tulungagung, Situbondo, and several other areas in East Java Province. Prior to slaughter, the swines placed in a temporary pen at the Surabaya slaughterhouse. This allows the transmission of gastrointestinal protozoa to fellow swines and to humans at the Surabaya slaughterhouse. Smith *et al*., (2011) stated that swines are a potential reservoir for several diseases, including zoonotic diseases.

Based on research conducted by Ziemer *et al*., (2010), stated that there was a protozoan parasite infects swines. This research supported by the results obtained by Agustina *et al*., (2016) from 250 samples of piglet feces taken from farms in Bali province, it was known to be infected with protozoa with a prevalence of 91.6%. Protozoa that infect, among others, *Amoeba* sp. (82.4%), *Balantidium* sp. (61.2%) and *Eimeria* sp. (54.8%). In addition, data on the prevalence of swine gastrointestinal protozoa also obtained from Papua, namely the Baliem Valley with a prevalence of 60% and the Arfak Mountains with a prevalence of 83.3%. Protozoa found in the Baliem Valley include *Eimeria* sp. (50%), *Isospora* sp. (20%), *Entamoeba* sp. (20%), and *Balantidium* sp. (10%) while from 22 samples examined in the Arfak Mountains the protozoan found were *Eimeria* sp. (83.3%), *Isospora* sp. (33.3%), *Entamoeba* sp. (33.3%), and *Balantidium* sp. (58.3%) (Yuliari *et al*., 2013). In the city of Denpasar, it recorded that from 300 samples from various farms in Denpasar, 46% infected by digestive protozoa consisting of *Coccidia* sp. (40.3%) and *Balantidium* sp. (18.3%) (Kurniawan, 2003). Meanwhile, from a study conducted by Widyasari *et al*., (2018) in a Denpasar slaughterhouse, it was reported that the prevalence of protozoa was 48% consisting of *Eimeria* sp (43%), *Isospora* sp (1%) and *Giardia* sp (4%). In another study conducted in Fujian Province, Southeast China, it was found that 370 samples of swine feces from total of 668 samples were infected by protozoa of the genus Entamoeba. Entamoeba polecki was 45.2%, *Entamoeba suis* was 13% and *Entamoeba histolytica* was not found (Ji *et al.*, 2019). Some of the protozoa found in swines can be zoonotic so that they can pose a health hazard to humans.

One of the protozoa is *Isospora* sp. is an agent that causes coccidiosis in swines. According to Laszlo (2018), the incidence of coccidiosis in swines will cause diarrhea and reduce body weight in swines, even a secondary infection by the bacterium *Clostridium perfringens* can cause death, resulting in economic losses for farmers if the protozoa in the digestive tract of swines were not identified properly. Therefore, it is necessary to identify the protozoa in the digestive tract of swines.

There are several species of the genus Entamoeba that can infect swines and cause digestive diseases. There are three species, namely *Entamoeba histolytica, Entamoeba polecki* and *Entamoeba suis*. Of the three species, it known that *Entamoeba histolytica* can infect humans and cause colitis (Matsubayashi *et al*, 2016).

There are several factors that influence disease transmission, including disease agents, hosts, and environmental factors (Agustina *et al*., 2016). Environmental factors that can affect the prevalence of gastrointestinal protozoa are contamination of feed and water, maintenance management, malnutrition, and climatic conditions (Giarratana *et al*., 2012). Humid climatic conditions can increase the chances of swine infected by parasites (Agustina, 2013). This opinion supported by the results of research by De Souza *et al*. (2015) in Brazil, which stated that the prevalence and the degree of infection of *Eimeria* sp. in sheep raised in the rainy season is higher than in sheep raised in the dry season.

Based on previous studies conducted by Widyasari *et al*., (2018) have proven that there are gastrointestinal protozoa in swines kept in Denpasar slaughterhouse, but identification of digestive tract protozoa in Surabaya slaughterhouse in dry season is still limited. In this study, the identification of the digestive tract protozoa of swines slaughtered at the Surabaya slaughterhouse in dry season will be carried out. The results obtained in this study expected to be used as an effort to prevent the transmission of zoonotic diseases from swines to humans and prevent economic losses for both swine farmers and pork sellers.

Methods

This study is a descriptive type of research with a cross-sectional design by conducting an observational survey to identify digestive protozoa in slaughtered swine at the Surabaya slaughterhouse. This study used samples in the form of feces from swine slaughtered at the Surabaya slaughterhouse. Fecal samples taken purposively with the criteria of solid, brown and/or black feces. These criteria were chosed because feces with these criteria found mostly in Surabaya slaughterhouse. Fecal sampling carried out within a period of four cuts in August 2021. The time was chosed because based on data by BMKG Surabaya, August is the month with the lowest rainfall throughout the year. Each sampling carried out in 23 different swine pens. The feces taken were feces on the floor of the cage with the appropriate criteria.

The fecal samples that taken then examined in the laboratory of the Veterinary Parasitology division, Faculty of Veterinary Medicine, Airlangga University. Stool samples examined using the native method, the sedimentation method, the floating method, and the Ziehl Neelsen method. Protozoa found in the gastrointestinal tract of swine identified based on the presence of oocysts, cysts, and/or trophozoites found in faecal samples. Protozoa identification according to the literature by Schuster and Avila (2008), Tan (2008), Lastuti *et al*. (2012), El-Dib (2017), and Gordo and Katerina (2018). The results of the identification of protozoa in the digestive tract of swine presented in tabular form and described descriptively.

Results and Discussion

The results of the examination conducted on 100 samples of Landrace swine feces, ages older than 6 months that slaughtered at the Surabaya Slaughterhouse at August 2021, it was found that 47 out of 100 stool samples were positive for protozoa infection. The protozoa found included *Eimeria* sp. / *Isospora* sp., *Balantidium* sp., *Entamoeba* sp., and *Blastocystis* sp. (Table 1.). The presence of other protozoa such as *Cryptosporidium* sp., and *Giardia* sp. not found in this study. On microscopic examination, it was found that all positive stool samples infected by two or more different types of protozoa.

Table 1. The Results of the Examination of the Gastrointestinal Protozoa of Swine (Sus sp.) in Surabaya Slaughterhouse in Dry Season

No.	Types of protozoa	Positive sample	Prevalence (%)
1.	*Balantidium* sp.	46	46%
2.	*Eimeria* sp. / *Isospora* sp.	32	32%
3.	*Entamoeba* sp.	24	24%
4.	*Blastocystis* sp.	12	12%

On examination using the sedimentation method and the fullborne floating method, *Balantidium* sp. cysts found. The cyst is round and there is no visible cilia on the cyst. The cyst is the infective phase of *Balantidium* sp. In addition, on examination using the native method, sedimentation method, and fulleborne floating method, unsporulated *Eimeria* sp. / *Isospora* sp. oocysts found. Unsporulated oocysts of *Eimeria* sp. / *Isospora* sp. are oval shaped and contains one sporoblast with size approximately 18-20 µm in length. *Blastocystis* sp. with a granular form seen on examination by the sedimentation method and the fulleborne floating method. *Blastocystis* sp. appeared on this study were round, have granules in the middle and have a thin cytoplasmic wall, sizing approximately 10- 15µm. While the immature cysts of *Entamoeba* sp. seen on examination using the sedimentation method. The immature cysts are spherical in shape with a single nucleus located at the periphery.

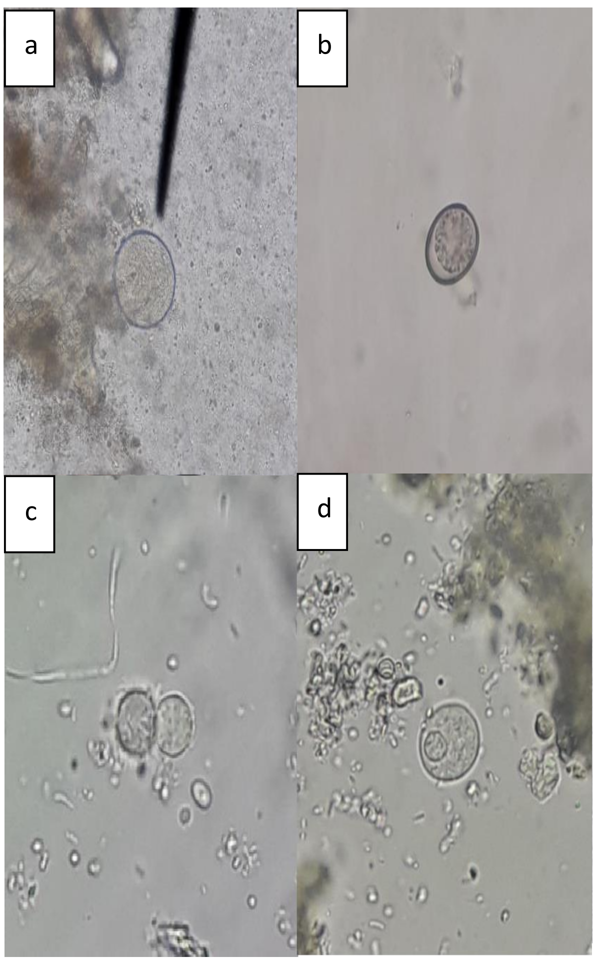

Figure 1. Cysts of *Balantidium* sp. (a), unsporulated cysts of *EImeria* sp. / *Isospora* sp. (b), cysts of *Blastocystis* sp. (c) and immature cysts of *Entamoeba* sp.

Discussions and Conclusion

On the results of fecal examination, found the presence of *Balantidium* sp. in the form of a cyst. Cysts are round, have one cell surrounded by a thin wall, and no cilia are visible. This is in accordance with the literature by Gordo and Katerina (2018), which states that *Balantidium* sp. It is round in shape, has a thin cyst wall, and sometimes cilia can be found.

Balantidium sp. is the cause of Balantidiasis in humans and swine. Balantidiasis disease categorized as foodborne or waterborne disease because its transmission occurs through ingestion of food or water contaminated with *Balantidium* sp. cysts. *Balantidium coli* cysts can be found in the feces of swine or humans infected by this protozoan. Transmission can also occur through coprophagy, namely the habit of eating feces (Schuster and Avila, 2008). Raised swine are the main reservoir for *Balantidium coli*, while other animals such as rodents, goats, wild boars, camels, and horses can also transmit *Balantidium* sp. to humans (Gordo and Katerina, 2018).

Balantidium coli infection in swine does not show any symptoms because in swine, this protozoa is a non-invasive and non- pathogenic protozoa (Schuster and Vivesvara, 2004). While in humans, most *Balantidium coli* infections are asymptomatic, in humans with a weaker immune system, perforation of the intestines or lesions of the lungs can occur (Maino *et al*., 2010). Humans infected with *Balantidium coli* can also show symptoms such as abdominal pain, headache, and diarrhea (Figueriredo, 2012). Swine known as the reservoir hosts of *Balantidium coli* and humans could be infected through direct or indirect contact with swine (Schuster and Avila, 2008).

The results of the examination conducted at the Surabaya slaughterhouse in August 2021 showed that the samples infected *by Balantidium sp.* with a prevalence of 46%. This prevalence is higher than the study conducted by Kurniawan (2003) in Denpasar, where in this study the prevalence of Balantidium sp. only 16.8%. Research by Yuliasari *et al*. (2013) in the Baliem Valley, which shows a prevalence of 10%. However, the results of the examination also showed a lower prevalence when compared to the study conducted by Agustina *et al*. (2016), the results of the study conducted in traditional market in Bali showed a prevalence of 61.2%. Higher prevalence of *Balantidium* sp. in Surabaya slaughterhouse may affected by temperature and humidity, Surabaya has higher temperature and humidity, which is good for the development of protozoa compared to Baliem Valley. Meanwhile, the lower prevalence of infections compared to the study by Agustina *et al.* (2016), could be due to the difference in the age of the swine at the time of sampling. Research by Agustina *et al.* (2016) used fecal sample from piglets, where piglets are generally more sensitive to protozoan infections.

Eimeria sp. / *Isospora* sp. also found in faecal samples taken from the Surabaya slaughterhouse. On examination, the oocysts of *Eimeria* sp. / *Isospora* sp., which has not sporulated has an oval shape. In this observation, the oocysts of *Eimeria* sp. / *Isospora* sp. not yet sporulated, so it appears to have only one sporoblast and it can not determined whether it was *Eimeria* sp. or *Isospora* sp. The unsporulated oocyst of *Eimeria* sp. / *Isospora* sp. is not infective.

Eimeria sp. and *Isospora* sp. is the cause of coccidiosis in swine. Coccidiosis characterized by diarrhea and weight loss, especially in piglets, but it is possible that adult swine can also get this disease (Das, 2019). Clinical symptoms of coccidiosis appear on day 2-4 after infection in piglets aged 2-3 weeks. The mortality rate due to coccidiosis is low, but the presence of secondary infection by bacteria, especially Clostridium perfringens can cause death (Laszlo, 2018). Environmental conditions play a role in the transmission of Eimeria sp. and Isospora sp. because these protozoa usually transmitted through ingestion of food or water contaminated by oocysts that cause Coccidia (Das, 2019).

Meanwhile, the results of the examination showed that the swine infected by *Eimeria* sp. / *Isospora* sp. with a prevalence of 32%. This prevalence is lower than the study by Yuliari *et al.* (2013) in the Baliem Valley, which found *Eimeria* sp. with 50% prevalence and Widyasari *et al.* (2018) in Denpasar slaughterhouse, which found *Eimeria* sp. with 43% prevalence. The lower prevalence of infections could be due to differences in rearing methods, Yuliari *et al.* (2013) stated that swine rearing management in the Baliem Valley still uses traditional methods, where the food given to swine is leftovers from the kitchen. This is in line with Roy *et al.* (2011), that differences in prevalence influenced by differences in maintenance management.

Blastocystis sp. has several forms, so it can sometimes lead to misinterpretation of the results. In the research results, identification of *Blastocystis* sp. in the granular shape was adapted to the literature by Tan (2008), where the protozoa looked spherical, with granules in the cytoplasm, and sized around 5-20μm. *Blastocystis* sp. is a protozoan that can infect humans and animals. Symptoms in the form of diarrhea and abdominal pain are usually seen in humans infected by this protozoa (Kaya *et al.*, 2007).

According to Palasuwan *et al.* (2016), animals can act as intermediary hosts for *Blastocystis hominis* infection to humans. Swine are one of the domestic animals that can transmit this parasitic infection (Roberts *et al.*, 2013). Incidence of infection by *Blastocystis* sp. more common in countries with poor sanitation (Cruz et al., 2016). Meanwhile, close contact with infected animals can also be a cause of transmission of *Blastocystis sp.* in humans (Pintong et al., 2018).

In this research, *Blastocystis* sp. infection occured with a prevalence of 16%, lower than the study conducted by Widisuputri *et al.* (2020) in the Province of Bali and Cruz *et al.* (2016) in Laguna, Philippines. The lower prevalence could be due to differences in observation methods, where the research conducted by Cruz *et al.* (2016) and Widisuputri *et al.* (2020) used Iodine staining method followed by PCR. Iodine staining proved good in detection of trophozoites and oocysts of protozoa (Wolf *et al.*, 2014). Meanwhile, PCR methods were the most sensitive method with highest specificity to diagnose *Blastocystis* sp. compared to other method (Widisuputri *et al.*, 2020).

In the observations found *Entamoeba* sp. cyst phases. *Entamoeba* cysts appear to be spherical in shape with one nucleus. Cysts usually found in solid feces, while trophozoites usually found in soft stools. Cysts can live for several weeks outside the host's body (Menchaca *et al.*, 2014).

These protozoa have zoonotic potential. *Entamoeba* sp. can cause Entamoebiasis and cause diarrhea, especially in children because the transmission related to poor sanitation (Mohammadi and Petri, 2006). Transmission occurs due to ingestion of infective cysts secreted by feces. These cysts are able to survive outside the host's body because protected by the cyst wall (Agustina *et al.*, 2017).

Entamoeba sp. found in this study with 24% prevalence. The prevalence is higher but not much different from the research of Yuliari *et al.* (2013) in the Baliem Valley, Papua. Higher prevalence of *Entamoeba* sp. infection in Surabaya slaughterhouse may affected by climate factors. Jaco

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et al. (2013), stated that *Entamoeba sp.* cysts morphologically developed at tropical temperature and it can survived at 37ºC temperature. Based on the data by BMKG Surabaya, in dry season, the temperature is around 32 ºC. Meanwhile, Baliem Valley, Papua was located at an altitude of 1600 m above sea level, with temperature around 14-25 ºC and it is difficult to distinguish the dry season from the rainy season in this area, because rainfall is quite high throughout the year (Hastanti, 2017).

Gastrointestinal protozoan infections in swine related to sanitation and rearing management. Swine reared with maintenance management and good hygiene practices can reduce the risk of severe parasitic infections, but there is still the possibility of gastrointestinal parasitic infections, especially protozoal infections (Nishi *et al*., 2000). In the Surabaya slaughterhouse there are 23 swine pens that are used as temporary cages for swine sent from various farms in East Java before slaughter. In this observation, parasites can be found in samples taken from approximately 10 cages out of a total of 23 cages. The type of cage used is a double cage, where two rows of cages placed opposite each other. The cage has a wall of approximately 1 meter high and the base of the cage is made of cement. In the cage there is a container that is used as a place to eat and drink. There are 7-8 swine each cage, so swine infected with gastrointestinal protozoa has a potential to transmit infection to other swine in the same cage.

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