Incidence of *Eimeria spp*. in Fat-Tailed Sheep Breed in Malang, Indonesia

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Abstract

Coccidiosis caused by *Eimeria spp*. is a parasitic disease that affects various animal species, including sheep. This study aimed to detect *Eimeria spp*. and the degree of infection in the fat-tailed sheep breed in Malang. This study used fecal samples from 62 fat-tailed sheep. Detection of *Eimeria spp*. was performed by using the flotation method and then observed the morphology of the oocysts found in the fecal samples of fat-tailed sheep. The positive sample was followed by the McMaster test to calculate oocysts per gram (OPG). The results showed that 20 (32%) samples were positively infected by *Eimeria spp*. with the degree of infection in the severe category.

INTRODUCTION

Sheep are a type of livestock that have a variety of uses and advantages, including producing meat that can satisfy the community's need for animal protein. According to Saifudin *et al*. (2018), Indonesia has seen an annual growth in the production of lamb meat. One of the major challenges is the decline in productivity brought on by ruminant intestinal parasite diseases (Win *et al*., 2020).

Although coccidiosis is frequently found in sheep, the condition is frequently disregarded. An important illness that can harm farmers is subclinical coccidiosis. Small ruminant coccidiosis has been documented in numerous parts of the world, however there are currently no data that can validate the precise geographic distribution of coccidia species, despite surveys showing variable prevalence rates (Engidaw *et al*., 2020). The geographical conditions in Indonesia, which include a tropical environment supported by hot, humid air, contribute to the protozoa's rapid rate of transmission (Efendi *et al*., 2019).

Eimeria are the culprits behind coccidiosis. According to Prakashbabu *et al*. (2017), the subclinical and clinical impacts of coccidiosis cause malabsorption, a decreased rate of feed conversion, and weight loss in addition to increased mortality and susceptibility to secondary infections. In sheep, *E. parva*, *E. pallida*, *E. marsica*, *E. ovinoidalis*, *E. crandallis*, *E. weybridgensis*, *E. faurei*, *E. granulosa*, *E. ahsata*, *E. intricata*, and *E. ovina* (syn. *E. bakuensis*) have been identified. Biological characteristics such as oocyst size and morphology, presence or absence of oocyst residues, micropylar cap, sporulation time, prepatent period, and colonization site have been used to distinguish between species primarily when examining oocysts under a microscope (Trejo-Huitrón *et al*., 2019). According to Ekawasti *et al*. (2019), the oocysts of *Eimeria spp*. are elliptical, ovoid, and spherical in shape and have a smooth, homogenous, and transparent wall surface. The objective of this study was to investigate the prevalence of coccidiosis, specifically in fat tail sheep in Lawang, Malang, caused by *Eimeria spp*.

MATERIALS AND METHODS

Samples

The sample size was calculated using the Slovin formula, i.e., $n=N/(1+e^2)$ which is "n"

stands for number of samples, "N" for population size, and "e" for Percentage of error (10%). The sample used was 62 male fat-tailed sheep. Fecal samples were collected randomly. A total of 34 samples were collected from farm A and 28 samples were collected from farm B in Lawang, Malang. Feces collected directly with sterile gloves from the sheep's rectum were collected in a sample container and stored in a cooler box containing ice packs. The samples were then transferred to the Animal Reproduction and Health Laboratory, Malang Agricultural Development Polytechnic. The sample was then added to a 2,5% potassium dichromate solution and stored at room temperature until sporulation occurred.

Fecal Evaluation using Floating Method

Feces were examined using the flotation concentration method using a saturated sugar solution. The feces sample was put into a centrifuge tube, and centrifuged at a speed of 1500 rpm within 5 minutes. The centrifuge tube was removed from the centrifugator, the supernatant was discarded by pouring it off. The next step was to add saturated sugar float solution to ¾ of the tube, stir until homogeneous, then centrifuge again at a speed of 1500 rpm within 5 minutes. The centrifuge tube was then carefully removed from the centrifuge and placed on the test tube rack in an upright position. The floating fluid was then added slowly by dripping it using a Pasteur pipette until the surface of the liquid was convex, provided that the additional floating fluid must not spill, wait 5 minutes to give the oocysts a chance to float to the surface. The cover glass was placed on the surface of the floating liquid and then attached to the object glass. The examination was carried out using a 400x objective magnification microscope and identified based on the morphology and morphometry of the oocysts (Simamora *et al*., 2017; Mohamaden *et al*., 2018).

Fecal Evaluation using McMaster Method

Quantification of oocysts per gram of sample (OPG) was carried out using the McMaster technique (Reginato *et al*., 2020). The feces sample was weighed at 2 grams and put into a plastic cup, and then 58 ml of floating solution (saturated sugar) was added. Mortar was used to crush solid sheep feces. The fecal suspension was filtered with a filter to reduce debris that interferes with visibility. The feces suspension was homogenized and a sample was taken with a pipette to be filled into the counting chamber. The suspension was homogenized while a sample was taken to fill the counting chamber. The counting chamber was observed under a microscope with 100 times magnification. Both counting chambers were observed and only oocysts within the chamber boundaries were counted. The OPG value was calculated using the formula, i.e., Number of oocysts/0,3 ml x 60 ml/2 gram = number of oocysts x 100 (Winarso, 2019).

Data Analysis

Data from the study in the form of morphology were processed qualitatively and presented descriptively, then the data was presented in the form of figures and tables.

RESULTS AND DISCUSSION

A total of 20 (32%) samples of 62 fecal samples reported the presence of *Eimeria spp*. in fat-tailed sheep feces (Table 1). The morphology of the oocysts being ovoid in shape, with a smooth wall surface, and there were 4 sporocysts within the sporulating oocysts (Figure 1). General characteristics of *Eimeria spp*. those that have sporulated have 4 clearly visible sporocysts, have two layers of walls, the outer layer is colorless while the inner layer is dark (Efendi *et al*., 2019). Oocysts of *Eimeria spp*. round, ovoid and elliptical with a smooth, homogeneous and transparent oocyst wall surface. The length of the oocysts of *Eimeria spp*. ranges from 10 to 50 μm (Ekawasti *et al*., 2019). In study conducted by Awaludin *et al*. (2021) *Eimeria spp*. 8% were detected in sheep feces samples taken in Jember. Several regions of other countries also detected *Eimeria spp*. in sheep as per study, i.e., 46,39% in Al-Diwaniyah, Iraq (Majeed *et al*., 2020), 87,31% in Tigray, Northern Ethiopia (Etsay *et al*., 2020), 57,70% in Suez, Egypt (Mohamaden *et al*., 2018),

Table 1. Incidence of *Eimeria spp.* in farm A and B

Farm	n	Positive of *Eimeria spp.*	(%)
A	34	9	26
B	28	11	39

Table 2. Evaluation of oocysts per gram (OPG) from fecal samples

Farm	Sample Code	OPG	Category
A	A1	8300	severe
	A2	10000	severe
	A6	7000	severe
	A11	4000	severe
	A14	3900	severe
	A15	11100	severe
	A17	8400	severe
	A29	4800	severe
	A34	27900	severe
B	B2	1300	severe
	B3	12000	severe
	B4	21700	severe
	B5	16500	severe
	B6	7600	severe
	B8	3800	severe
	B9	12700	severe
	B11	2300	severe
	B12	13600	severe
	B13	5600	severe
	B22	7700	severe
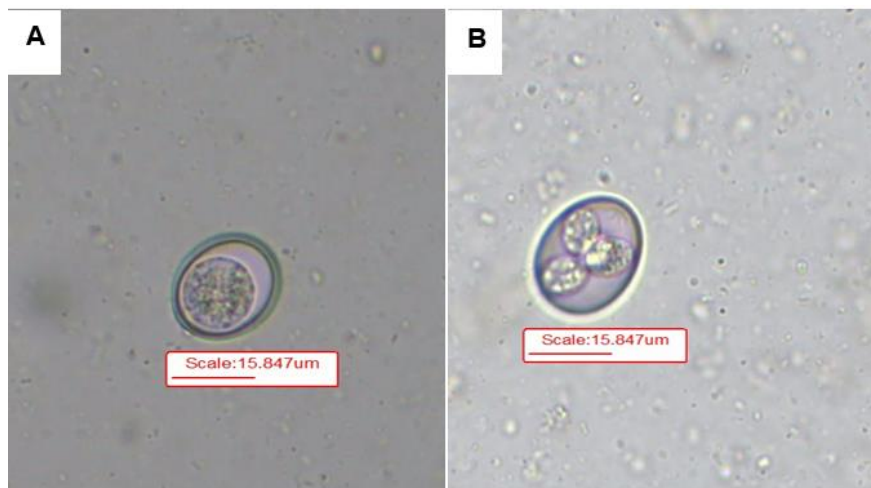
Figure 1. Morphology of *Eimeria spp.* oocysts using 400x magnification.
(A) oocysts without sporulated, (B) sporulated oocysts.

49,36% in Erzurum, Turkey (Akyüz *et al*., 2019), 56,25% in Tikrit, Iraq (Hasan and Mahmood, 2021), and 47,8% in Brazil (de Macedo *et al*., 2020).

Parasitic infections are one of the causes of health problems in livestock, gastrointestinal parasites such as worms and protozoa are often found in sheep (Awaludin *et al*., 2021). This parasitic infection has the potential and can cause a decrease in livestock productivity (Tiuria and Noviara, 2020). Disease can interfere with sheep's weight growth (Daryanto and Aziz, 2019). Protozoan parasites cause ulceration in the intestinal epithelium, thereby reducing the

intestine's ability to digest and absorb food substances and reducing the production of enzymes that lead a role in the digestive process (Efendi *et al*., 2019). The diagnosis of coccidiosis is based on history, clinical signs, presence of oocysts in feces, and intestinal lesions at necropsy (Arifin *et al*., 2019). A more specific diagnosis of parasites can be made by identifying oocyst morphology with sugar solution, oocyst morphometry, and sporulation of oocysts in 2,5% potassium dichromate solution (Debela *et al*., 2020). Diagnostic methods for the detection of *Eimeria spp*. with conventional methods mainly based on the morphological characteristics of oocysts by microscopic examination (Bawm *et al*., 2020). Detection of *Eimeria spp*. oocysts can be done using various techniques ranging from conventional to molecular. The conventional method is still a popular method and is widely used in laboratories by observing the morphology, color and size of oocysts in fecal samples (Ekawasti *et al*., 2019; Hafeez *et al*., 2015).

Calculation of OPG in sheep feces samples that were positive for *Eimeria spp*. carried out using the McMaster technique (Table 2). The McMaster technique is a quantitative method by counting the number of oocysts in fecal samples to obtain the amount of OPG which can help determine the severity of coccidiosis as a cause of disease (Ekawasti *et al*., 2019). The level of parasite infestation is classified as mild (50–799), moderate (800–1200), or severe (> 1200) (Gondipon and Malaka, 2021). In study conducted by Win *et al*. (2020) shows that sheep in Myanmar also have OPG values between 7350–29800 and are included in the severe category. Quantitative methods are used to provide an overview of the severity of the incidence of coccidiosis in an individual livestock (Muhamad *et al*., 2021). Quantitative methods have the advantage of being able to determine the severity of coccidiosis, but also e less sensitive and are only limited to identifying the direction of the genus (Suroiyah *et al*., 2018). This technique can only be used as a screening test for coccidiosis (Ekawasti *et al*., 2019).

In this study, the sheep did not show clinical symptoms even though the number of OPG was included in the severe category, so it can be declared that the sheep from which fecal samples were collected in Malang suspected subclinical coccidiosis. These results are the same as study conducted in several countries which also found that sheep whose fecal samples were examined had subclinical coccidiosis, such as in Suez, Egypt (Mohamaden *et al*., 2018), Tigray, Ethiopia (Etsay *et al*., 2020), and in Iraq (Albayati *et al*., 2020).

Coccidiosis in small ruminants is often found to be subclinical and chronic, thus disrupting livestock growth (Awaludin *et al*., 2021). Gastrointestinal protozoan infections show clinical symptoms depending on the number of infecting species, although the infecting species is pathogenic if only one species does not show clinical symptoms (Indraswari *et al*., 2017). *Eimeria spp*. can survive and multiply in the host without marked pathogenicity (Kurniawati *et al*., 2020). This balance between parasite and host is disturbed, especially when the host is exposed to stress, such as weaning, disease, changes in food, and climate changes, causing an increase in the number of parasites (Albayati *et al*., 2020). According to Mohamaden *et al*. (2018) Subclinical coccidiosis is common in small ruminants. Coccidiosis in sheep is caused by the genus Eimeria, but in general the animals do not show clinical signs called subclinical coccidiosis, which causes weight loss and increased susceptibility to several diseases (Albayati *et al*., 2020).

Coccidiosis has the potential to transmit to other disease agents, such as viruses, bacteria, fungi or other parasites (Chaerunissa *et al*., 2019). Management strategies for controlling coccidiosis in the livestock industry must be considered, especially carrying out detection to prevent the *Eimeria spp*. transmitted on the livestock (Ekawasti *et al*., 2019). Morbidity of coccidiosis in sheep is usually between 10–40%, but mortality is often more than 10%. *Eimeria spp*. can infect sheep of all ages (Albayati *et al*., 2020). Mixed infections with various *Eimeria spp*. are more common (Hasan and Mahmood, 2021). Life cycle of *Eimeria spp*. occurs in the host body and in the environment (Engidaw *et al*., 2020). Life

cycle of *Eimeria spp*. consists of schizogony, gametogony and sporogony. Once the oocyst is outside the host's body, it will experience sporulation. The presence of oxygen, temperature, humidity, and lack of direct contact with ultraviolet radiation, supports oocyst survival and sporulation (Purnama *et al*., 2021). This life cycle phase of *Eimeria spp*. starting from ingestion of infective oocysts by livestock through contaminated drinking water or feed (Indraswari *et al*., 2017).

Inappropriate control strategies will increase cases of coccidiosis because oocysts will continue to pollute the environment and have the potential to become a source of infection for livestock (Ekawasti *et al*., 2019). According to Muhammad *et al*. (2021) the prevalence of coccidiosis is closely related to several factors, including livestock population density, age of livestock, housing conditions, cage floor type, drinking water supply system, and feeding system. Parasitic infections in the digestive tract of sheep occur due to lack of attention to maintenance management and treatment programs, in particular in traditional breeders. Cage sanitation is also crucial to prevent the potential for the spread of parasites to become uncontrolled (Awaludin *et al*., 2021). Accurate identification of *Eimeria spp*. important not only for disease diagnosis and management of subclinical infections but also for epidemiological studies (Reginato *et al*., 2020).

CONCLUSION

This study reported 20 (32%) positive *Eimeria spp*. of 62 samples using microscopic examination of fat-tailed sheep in Lawang, Malang, the degree of infection was in the severe category.

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