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The Occurrence of Ovine Coccidiosis in Domestic Sheep in Kulon Progo, Yogyakarta

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Abstract

Ovine coccidiosis is a gastrointestinal disease caused by parasites of the genus *Eimeria* spp. Disease caused by coccidia in domestic sheep needs to be explored since associated with economic detrimental worldwide. An appropriate detection method would be beneficial for the accurate diagnosis and treatment of ovine coccidiosis properly. This study aims to investigate the occurrence of ovine coccidiosis in domestic sheep in Sentolo, Kulon Progo. A total of 104 fecal samples were collected randomly from smallholder farmers in Sentolo, Kulon Progo. Individual fecal samples were investigated by flotation quantitatively using the FLOTAC method. The number of oocysts per gram of feces was determined morphologically by a combination of fill-FLOTAC and mini-FLOTAC devices. It was found that 17/104 (16.3%) domestic sheep were infected by *E. ovinoidalis*, whereas 10/104 (9.6%) were infected by *E. intricata*. Statistical analysis showed no statistically significant difference between females, males, and age. Data reported in the present study indicate an updated report of ovine coccidiosis in Sentolo, KulonProgo. Finally, these findings are essential for better understanding the ovine coccidian infection in this area. Additionally, this report would benefit the ovine coccidiosis control strategy in the sheep population in this area.

Keywords: coccidiosis; domba; *Eimeria*; Sentolo

Introduction

Coccidiosis is a global ovine health problem caused by the parasite of the genus *Eimeria*. This parasite is an intracellular parasite of the intestinal epithelial cells. They cause disease and economic loss (Underwood et al., 2015). Under most sheep production systems, animals are susceptible exposed by some *Eimeria* spp., although most infections are inapparent. At least 13 species of *Eimeria* were reported previously to infect sheep as follows: *Eimeria ahsata, E. bakuensis, E. crandallis, E faurei, E. gilruthi, E. granulosa, E. intricata, E. marsica, E. ovina, E. ovinoidalis, E. pallida, E. parva*, and *E. weybridgensis*. However, only two are considered pathogenic: *E crandallis* and *E. ovinoidalis* (Gondipon & Malaka, 2021). Except for *E. gilruthi*, all coccidian sheep species are considered host-specific (stenoxenous parasite), and they are not able to infect cattle, goats, or poultry (Ammar et al., 2019). Often concurrent mixed *Eimeria* spp. exists in the host and may associate with the severity of any symptoms. There is no cross-immunity between ovine *Eimeria* species as described previously by Shivaramaiah et al. (2014).

The life cycle of sheep *Eimeria* species is complicated and takes 2 to 3 weeks to complete (prepatent period). Oocysts released into the environment are only infectious once they sporulate. Infective oocysts can appear throughout the year in Indonesia (Pamungkas et al.). Oocysts resist

environmental conditions and disinfectants but can become damaged by heating or temperatures > 55º–60º C (Gondipon & Malaka, 2021; López-Osorio et al., 2020). Almost *Eimeria* species infect cells in the small or large intestine. For instance, *E. crandallis* can infect the ileum and *E. ovinoidalis* not only infects the small intestine but can also infect the cecum, causing bloody diarrhea (Olmos et al., 2020). In this case, there is decreased water absorption and severe bleeding where the colon mucosa is denuded (Mohamaden et al., 2018; von Samson-Himmelstjerna et al., 2006).

In Indonesia, the disease often occurs during transition throughout the year. Intensive grazing areas and feedlots are at the most significant risk due to topping, ration change, crowding stress, and contamination of the environment with infected oocysts. Cattle pens can be contaminated with feces containing oocysts, especially when not cleaned. If a female has recently given birth, the udders and teats can cause early infection in the lambs. Coccidia infection in adult cattle is usually subclinical. Most clinical cases occur between 1 and 6 months of age (Ekawasti et al., 2021; El-Alfy et al., 2020). Most clinical disease occurs in 4–8 weeks old lambs reared in a contaminated environment. When lambs are all a similar age, most may show clinical signs. Acute infections result in sudden diarrhea, anorexia, dullness, and abdominal pain. Dehydration may result in a marked loss of weight and body condition (Ekawasti et al., 2021; Pamungkas *et al.*). Severe acute infections may lead to diarrhea before oocyst shedding, especially *E. ovinoidalis*, which may be bloody. Chronic infections reduce feed consumption, feed conversion, and growth rates (Olmos et al., 2020).

Diagnosis of coccidiosis is based on factors such as age, sex, environmental conditions, presence of oocysts from stool examination results, and postmortem findings after necropsy. Severe diarrhea in 4–8-week-old lambs indoors or on highly stocked pastures is an indication. Fecal samples should be taken from lambs with and without diarrhea (Andrews, 2013; Engidaw et al., 2015). Detection of oocysts in feces using the flotation method. A fecal oocyte count of more than 5,000 oocysts per gram with appropriate symptoms may be noteworthy, but speciation should be undertaken. Speciation should be conducted with 2% potassium dichromate to identify the species of *Eimeria*. Animal selection can complicate coccidian oocyst count, sampling timing, nonpathogenic *Eimeria* spp., and lack of agreement on the interpretation of noteworthy oocyst counts (Dorney, 1964; Dryden *et al.,* 2005).

Ovine coccidiosis in Egypt was investigated by Elkhatam et al. (2021). It was found that six *Eimeria* species were identified, and the most predominant was *E. parva (*38.4%). The prevalent *Eimeria s*pecies in the previous study in Iran were reported *E. ahsata, E. parva, E. pallida*, and *E. granulosa* with varying proportions. Multiple species co-infection was found in most positive samples (89.3%). This previous study highlights a notably high prevalence of coccidial infection in western Iran (Hashemnia *et al.,* 2014). Moreover, only a few studies have explored the occurrence of ovine coccidiosis in Kulon Progo, Yogyakarta. Therefore, this study aimed to investigate the presence of ovine coccidiosis in Sentolo, Kulon Progo, Yogyakarta, which never has been done previously. The finding of this study may shed light on the updated report of ovine coccidiosis detected by the FLOTAC method.

Materials and methods

Study design and sample collection

A cross-sectional study was conducted in this study in order to detect the presence of coccidiosis infection in domestic sheep, refer to the previous study (Ola-Fadunsin et al., 2018). According to unpublished data from the department of livestock in Kulon Progo district, the total sheep population in this area was reported to be 689 heads at the end of 2020. Sample size has been calculated based on disease detection in a finite population. The study was conducted from January to March 2021. A total of 104 fecal samples were collected randomly from domestic sheep in Sentolo, Kulon Progo, Indonesia. The sampling sites were located in Sentolo, Kulon Progo, and Yogyakarta. Fresh samples were taken directly from the rectum, put in a labeled plastic container, and stored at 4^0 C in a portable cooling box before being transferred to the refrigerator in the laboratory department of parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, for further analysis.

FLOTAC method

The FLOTAC method was carried out to detect coccidia infection in all fecal samples according to the manufacturing protocol. A total of 5 g of feces was added to the corresponding fill-FLOTAC device and homogenized with 45 mL of saturated natrium chloride (NaCl) solution (specific gravity 1.2). The fecal suspension was then transferred into the mini-FLOTAC reading chamber disc and left for 10 min before rotating clockwise (90^0) the lid of the reading chamber. Coccidia infection was determined based on the presence of coccidia oocyst. Coccidia oocyst was counted in each chamber under a microscope Olympus CX23 and documented with a DP12 Olympus camera (100 ×). Each chamber of the mini-FLOTAC device consists of 1 mL fecal suspension. Consequently, the number of oocysts counted in each chamber (2 chambers) was multiplied by five according to the ratio of fecal suspension (1:10) to determine the number of oocysts per gram. Morphological characterization was assessed based on the size, shape, and presence of the micropyle and polar cap. Oocyst dimensions were measured according to height (H) and width (W) and given as mean. Morphological identification was performed following morphological characteristics previously reported by (Christensen, 1938; El-Alfy et al., 2020; Macedo et al., 2019).

Statistical analysis

The Clopper-Pearson method confidence interval method was used to calculate the proportion. The association of coccidia infection between males, females, and different ages was analyzed by the chi-square test. All data were evaluated with a statistical analysis tool implemented in an epidemiological calculator freely accessed at https://epitools.-ausvet.com.au/.

Results and discussions

The current study investigates the prevalence of coccidia using the FLOTAC method. Suspected E. ovinoidalis and *E. intricata* were identified, with higher occurrences of *E. ovinoidalis* (17/104) followed by *E. intricata* (10/104). The Clopper-Pearson confidence interval method evaluated the proportion, as presented in Table 1.

Table 1. Oocysts per gram feces evaluated by mini-FLOTAC

Coccidia	OPG (min-max)	Proportion (%)	lower-upper 95% CL
E. ovinoidalis	2300 (85-12995)	16.3	9.82-24.88
E. intricata	148 (10-695)	9.6	4.71-16.97

OPG: oocyst per gram feces, CL: confidence level

The oocyst of *E. intricata* was measured at an average height of 50.7 µm and width of 45.6 µm, whereas suspected *E. ovinoidalis* was measured at an average height and width of 19.8 µm and 20.5 µm, respectively. In the present study, ovine coccidia is depicted in Figure 1.

This study investigated the occurrence of ovine coccidiosis in Sentolo, Kulon Progo, Yogyakarta. The present findings show that the prevalence of *E. ovinoidalis* and *E. intricata* infection reached 16.3% and 9.6%, respectively. We found that the most predominant causative agent of coccidiosis in this area was *E. ovinoidalis.* As depicted in Figures 2a and 2b, *E. intricata* was identified as morphologically ellipsoid in shape, thin wall and indistinct cap over the micropyle.

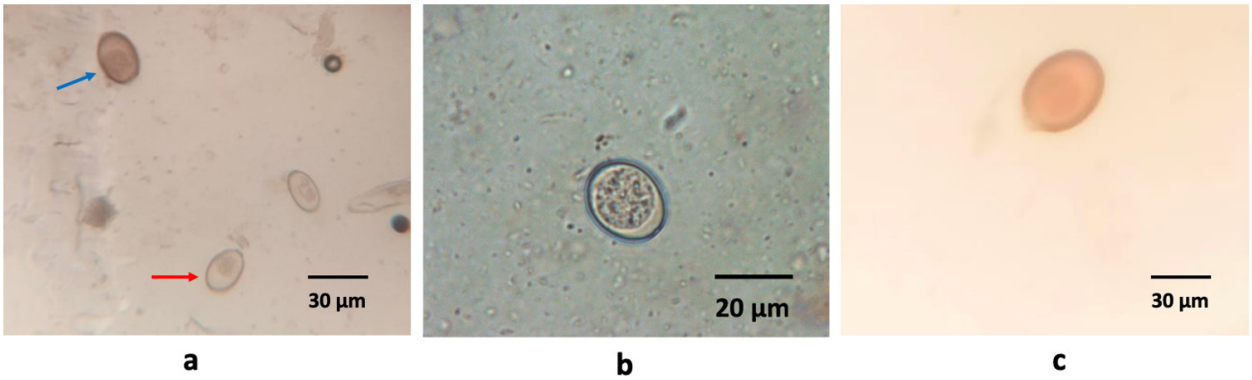

Figure 1. Unsporulated Eimeria oocysts in this study. (a) *E. ovinoidalis* (red arrow) and *E. intricata* (blue arrow) (b) *E. ovinoidalis* (c) *E. intricata*

This species was reported as pathogenic coccidian species in sheep (Al-Neama et al., 2021; El-Alfy et al., 2020; Mohamaden et al., 2018), which the infection could lead to epithelial intestine damage (Long, 199 0). It should be noted that all domestic sheep in the current study were asymptomatic even though coccidia was detected. This report corroborates the previous study by Field El-Alfy et al. (2020), which reported that *E. ovinoidalis* was detected in sheep without clinical signs. However, *E. ovinoidalis* have been investigated cause bloody diarrhea in lambs (Ghanem & Abd El-Raof, 2005).

In contrast, *E. intricata* have been reported as low pathogenic coccidia species in sheep (Šarić et al., 2015). We investigated *E. intricata* infection in this study, which was lower than *E. ovinoidalis.* This finding agrees with the previous study by Mohamaden et al. (2018). As presented in Figure 1c, *E. intricata* was identified morphologically as dark brown in color, ellipsoid shape, wall opaque and thick, and cap over micropyle is prominent. Interestingly, in the current study, *E. intricata* was observed co-infection with *E. ovinoidalis*. This finding suggested that *E. intricata* infection was likely not found in pure infection, as described previously by Christensen (1938).

This study also uses the chi-square method to evaluate the association between different ages, males and females, against coccidia infection. The statistical analysis indicated no statistically significant difference in coccidia infection between males, females, and ages in the present study (p-value >0.0 5). Table 2 presents the chi-square test of coccidia infection in domestic sheep in this study.

This study evaluated the association between sex and age against coccidiosis infection. There was no statistically significant difference between females, males, and age (p-value >0.0 5). Therefore, sex and age did not seem to influence the prevalence of infection with coccidiosis, particularly in this study. This result disagreed with a previous study by Elkhatam et al. (2020), which reported that females and lambs less than six months were more susceptible to coccidian infection. It is important to note that this study did not include the feces from lambs, and only three feces from males were collected. Therefore, further studies about ovine coccidiosis are recommended, particularly in lambs, and a large sample size is suggested to support statistical analysis. The species of *Eimeria* spp. identification in this study based on morphologically only. Despite being time-consuming and needing effort, the morphological detection and identification of *Eimeria* by the FLOTAC method employing saturated saline were effective. However, molecular characterization is necessary for ovine coccidiosis treatment properly and to prevent misdiagnosis.

Conclusion

In conclusion, this study focused on investigating the occurrence of ovine coccidiosis using the FLOTAC method. The prevalence of *E. ovinoidalis* and *E. intricata* infections in the examined area was determined as 16.3% and 9.6%, respectively. Notably, *E. ovinoidalis* was identified as the predominant causative agent of coccidiosis in the region. The morphological analysis depicted *E. intricata* as a pathogenic species, consistent with previous studies. Co-infection of

Table 2. The chi-square result of coccidia infection in this study

Sex	E. ovinoidalis		E. intricata	
	infected	uninfected	infected	uninfected
male	1	2	0	3
female	16	85	10	91
Chi-square (p-value)	1*		1*	
Age				
<1year	2	4	0	6
1-2 years	11	67	7	61
>2 years	4	16	3	17
Chi-square (p-value)	0.2*		0.2*	

*No significant difference (p-value greater than 0.05)

E. intricata with *E. ovinoidalis* was observed, suggesting mixed infections. Importantly, no significant association was found between coccidiosis infection and sex or age, diverging from certain previous findings. It's worth noting that the morphological identification of *Eimeria* spp. using the FLOTAC method proved effective, yet molecular characterization is advocated for accurate treatment and prevention strategies, especially to avoid misdiagnosis. This study shed light on the prevalence and characteristics of ovine coccidiosis and emphasizes the necessity for further research, mainly focusing on lambs and utilizing larger sample sizes to enhance the statistical robustness and comprehensiveness of the findings. This study also underlines an updated report of ovine coccidiosis in Sentolo, Kulon Progo. However, more molecular ovine coccidian studies are necessary to clarify species level and genetic analysis of different *Eimeria* in sheep.

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Conflict of interests

The authors declare that they have no conflicts of interest in this work.

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