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Morphological variations of *Eimeria* spp., in beef cattle in Bangkalan District, East Java, Indonesia

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Abstract. *Hastutiek P, Lastuti NDR, Suwanti LT, Kurniawati DA, Efendi MH. 2022. Morphological variations of* Eimeria *spp., in beef cattle in Bangkalan District, East Java, Indonesia. Biodiversitas 23: 3457-3461. Eimeria* spp. infection in cattle can cause high economic losses and increase the susceptibility to infection with other infectious diseases, so the government needs attention to coccidiosis in cattle. Coccidiosis can easily be found in farms that are managed with poor management systems, housing sanitation, and poor environment. Cases of coccidiosis often do not show clinical symptoms and cause sudden death of livestock. This study aimed to identify *Eimeria* spp., morphologically the cause of coccidiosis in beef cattle. Fresh feces were collected from 120 beef cattle from smallholder farms in Bangkalan District. The results of stool examination revealed that 70 (58.33%) positive samples containing *Eimeria* spp. oocysts. The six *Eimeria* species obtained from the samples were: *Eimeria bovis*, *Eimeria zuernii*, *Eimeria ellipsoidal*, *Eimeria subspherica*, *Eimeria aubernensis*, and *Eimeria canadinensis*. Calculation of oocysts per gram (OPG) of feces showed that most cows were infected with mild to moderate coccidiosis. Morphological identification can be used to diagnose *Eimeria* species in coccidiosis in beef cattle.

Keywords: Beef cattle, coccidiosis, *Eimeria*, infectious diseases, morphological variations, OPG

INTRODUCTION

Beef is one of the main sources of meat in Madura. Data from the Livestock Service Office of Bangkalan District shows that the population of beef cattle in Bangkalan District in 2016 was 200,279 tails (Bangkalan District Livestock Service Office 2016). Cattle are generally kept as savings by farmers with small capital and are kept in a traditional cage system. Cattle often suffer from malnutrition and are infected with parasites, which have the potential to inhibit meat production and reduce livestock reproduction. Coccidiosis caused by infection with *Eimeria* spp., has been reported to attack cattle, causing acute diarrhea and in young cattle can cause death. *Eimeria* spp. is a protozoan that lives in the digestive tract and is the most difficult to control in cattle farming. Coccidiosis is easily found in farms managed with poor management systems, housing sanitation, and poor environment. Cattle are infected with *Eimeria* spp., because they ingest sporulated oocysts that contaminate water and animal feed as a source of infection. The prevalence of coccidiosis varies widely, generally being high in developing countries. Infection with *Eimeria* spp. in cattle can cause high economic losses and can increase susceptibility to infection with other infectious diseases so coccidiosis in cattle needs attention from the government. Cases of coccidiosis often do not show clinical symptoms and cause sudden direct death of livestock (Ibrahim et al. 2015).

Coccidiosis in Madura cattle was reported with a prevalence of *Eimeria* spp., of 75.07% of the total 357 (71.4%) stool samples that were positive for protozoa infection, either single infection or mixed with other protozoa, Coccidiosis-infected cattle were reared with traditional systems, the condition of the cage is damp and the floor is from the ground (Hastutiek et al. 2019). In cattle, it has been reported that 76 stool samples tested with oocysts per gram (OPG) of feces contained 201-300 *Eimeria* spp. (Marskole et al. 2016). In cattle, there are 20 species, but only three species are pathogenic and often cause clinical symptoms, namely *Eimeria bovis*, *E. zuernii*, and *E. aubernensis* (Ekawasti and Wardhana 2010; Kawahara 2010). *Eimeria bovis* and *E. zuernii* are the most common pathogenic species in the world, causing morbidity and mortality in cattles with intestinal damage with clinical symptoms of diarrhea containing blood and fibrin (Eckert et al. 2008; Ekawasti and Wardhana 2010; Malek and Kuraa 2018). Although *Eimeria* spp. are non-pathogenic species that do not cause death, but these species cause tissue damage, thereby increasing sensitivity to infection with other infectious diseases (Ekawasti and Wardhana 2010).

Research in Geger subdistrict, Bangkalan district, showed that the soil around cages and pastures for Madura

cattle identified the presence of *Eimeria* spp., *Isospora* sp., and *Blastocystis* sp., (Putri et al. 2021), this indicates that soil is the main source of infection that can contaminate animal feed, and can later infect cattle orally. Coccidiosis has the potential to open the door to other disease agents, such as viruses, bacteria, fungi, or other parasites. Therefore, coccidiosis control strategies in cattle farms must be considered, especially identification of the *Eimeria* species present on the farm. Improper control strategies can increase coccidiosis cases because oocysts can continue to pollute the environment and have the potential to become a source of transmission for livestock, especially young livestock (Ekawasti et al. 2021; Gupta et al. 2016). Coccidiosis can be detected by conventional parasitology techniques (native and floating) by observing the morphology under a microscope.

However, the morphological characteristics between species are almost similar, so they have almost the same shape and size (Sanshez 2008). *Eimeria* species that attack cattle in Madura have so far only been identified to the genus level using conventional methods by observing microscopic morphology which actually cannot be used as a reference in identifying *Eimeria* species, because the morphological characteristics of *Eimeria* spp. have similar structures, shapes and sizes between species, and identification has not been carried out to determine morphological variations. An appropriate diagnostic method is required to control coccidiosis disease, therefore research should be done on the identification of different *Eimeria* species that infect cattle because each species has different pathogenicity.

MATERIALS AND METHODS

Ethical clearance

Fresh fecal samples were used in this study. Hence ethical clearance was not necessary. Fresh fecal samples were collected from beef cattle in Keleyan Village, Socah subdistrict, Bangkalan District, East Java province, Indonesia.

Samples collection

More than 120 samples were collected during the rainy season and the age categories of beef cattle were as follows: age < 6 months, 17 samples; ages 6 months to 2 years, 41 samples; and age > 2 years, 62 samples. One to five fecal samples were collected randomly at each farm. Most of the stools were normal, except 4 diarrhea and 7 which were soft. Samples were placed in plastic bottles and then stored in an icebox and immediately brought to the laboratory, and stored at 4°C until analyzed (Forslid et al. 2015).

Microscopic examination

The morphology of oocysts of *Eimeria* spp., was observed using conventional, sedimentary, and floating methods (the sample was declared positive if one of the examination methods found *Eimeria* spp.). Examination of fecal samples was performed using the floating by adding saturated sugar (Matsubayashi et al. 2005). 1 g of stool sample was centrifuged 800 × g for 5 minutes. The supernatant was discarded, and a sugar solution with a specific gravity of 1.2 was added, followed by centrifugation. The cover glass was placed at the mouth of the tube, transferred to an object-glass, and then examined with a microscope. The detected protozoan species were identified based on Soulsby (1986). A qualitative microscopic examination was carried out to determine the positive and negative presence of *Eimeria* spp. oocysts with a Nikon® E100 microscope, Japan at 100-400x magnification. A quantitative examination was done by counting the number of *Eimeria* spp. oocysts by using Oocysts Per Gram (OPG) feces.

RESULTS AND DISCUSSION

Results

Eimeria spp., were identified from beef cattle feces samples using the sugar flotation method. The morphology of *Eimeria* spp. oocysts can be seen in Figure 1.

Figure 1. Oocysts of *Eimeria* spp., at 400 x magnification

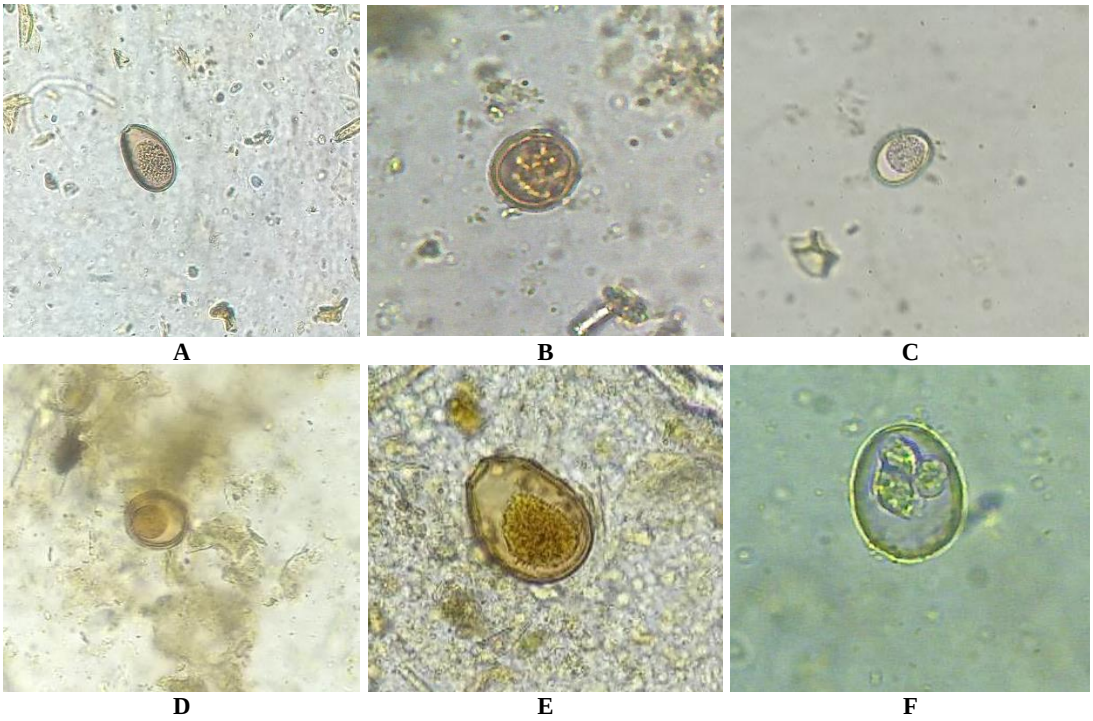

Figure 2. Morphology of six *Eimeria* species at 100 x magnification: A. *Eimeria bovis* oocysts. B. *Eimeria zuernii*. C. *Eimeria ellipsoidal*. D. *Eimeria subspherica*. E. *Eimeria aubernensis*, F. *Eimeria canadinensis*

Table 1. Identification of *Eimeria* species from beef cattle feces samples in Bangkalan based on age and prevalence

Cow age	Number of samples	Number of positive samples (%)	Name of *Eimeria* species					
			Eimeria bovis	*Eimeria zuernii*	*Eimeria ellipsoidal*	*Eimeria subspherica*	*Eimeria auburnensis*	*Eimeria canadiensis*
<6 month	17	8 (47.06)	6	5	1	-	1	-
6 month-2 year	41	27 (65.85)	10	4	3	1	2	-
>2 year	62	35 (56.45)	17	6	7	1	4	2
Total	120	70 (58.33)	33 (47.14)	15 (21.43)	11 (15.71)	2 (2.86)	7 (10)	2 (2.86)

Of the 120 stool samples, 70 samples were found to be positive for *Eimeria* spp., with a prevalence of 58.33%. A total of six *Eimeria* spp., were identified on the basis of morphology, namely *E. bovis*, *E. zuernii*, *E. ellipsoidal*, *E. subspherica*, *E. auburnensis* and *E. canadiensis* (Figure 2).

Result showed that all the six species of *Eimeria* had a different morphology. *Eimeria bovis* was oval in shape with a size of 25.4 × 17.2 µm and had a micropyle, *Eimeria zuernii* was spherical shape with a size of 18.8 ×17.9 µm but without micropyle, *Eimeria ellipsoidal* was elliptical shape with a size of 17.2 × 12.4 µm but lack of micropyle, *Eimeria subspherica* was slightly circular with a size of 11.7 × 11 µm and had no micropyle, *Eimeria auburnensis* was elliptical shape with a size of 33.9 × 20 µm and had a micropyle and *Eimeria canadinensis* was ellipse with a size of 29.4 × 20.3 µm and had a micropyle.

The prevalence of *E. bovis* was 47.14% (33 samples), followed by *E. zuernii* 21.43% (15 samples), *E. ellipsoidal* 15.71% (11 samples), *E. auburnensis* 10% (7 samples), and *E. auburnensis* and *E. subspherica* each 2.86% (2 samples). Four species of *Eimeria* were identified from cattle aged less than 6 months, namely *E. bovis*, *E. zuernii*, *E. ellipsoidal*, and *E. auburnensis*. Five species of *Eimeria* were identified from the cattle aged 6-2 years, namely *E. bovis*, *E. zuernii*, *E. ellipsoidal*, *E. subspherica* and *E. auburnensis*. There were six species of cattle over 2 years old, namely *E. bovis*, *E. zuernii*, *E. ellipsoidal*, *E.subspherica*, *E. auburnensis* and *E. canadinensis* were found associated with cattle over 2 years old (Table 1).

Discussion

In Java, *E. bovis* (10.4%) was reported as the species with the highest prevalence, other species were *E. ellipsoidalis*, *E. alabamensis*, *E. zuernii*, *E. auburnensis*, and *E. cylindrica*, cattle infected with *Eimeria* single or mixed (Ekawasti et al. 2019). Coccidiosis in cattle is usually caused by more than one *Eimeria* species (Lee et al. 2018; Das et al. 2015) also reported that 71.2% of cattle were infected with more than one *Eimeria* species (Morgoglione et al. 2020). This study showed different results with higher single infection than mixed infection. Many researchers have reported studies on the prevalence of *Eimeria* species in cattle from various countries (Heidari

and Gharekhani 2014). The prevalence varies from country to country, the prevalence of *Eimeria* species is 83.67% in Austria, 70% in UK, 96% in USA, 22.1% in South Korea, 75.5% in Colombia, and 47.09% in Pakistan, 11. 97% in India, 29.2% in Ethiopia, and 30% in Kenya (Rehman et al. 2011; Lee et al. 2018; Alemnew et al. 2017; Peter et al. 2015). This study showed the lowest (58.33%) prevalence, while the previous studies on Madura cattle feces samples taken from 10 sub-districts in Bangkalan, showed highest (75.07%) prevalence (Hastutiek et al. 2019). Ananta et al. (2014) and Hamid et al. (2016) reported a higher prevalence of 22.4% and 15.5% in west Java and central Java, respectively. Other investigators reported 52.3% prevalence of *Eimeria* spp., in cattle from the Java Island (Ekawasti et al. 2019). These differences may be due to differences in detection methods, sampling season, livestock management strategies, geographic conditions, feed sources, and feeding behavior (Eckert et al. 2008; Gupta et al. 2016). In ruminants, adequate protein-based food supplementation can enhance the animal's immune system so that it is more resistant to *Eimeria* spp., infection. The cow's body scoring condition was in the range of 3.0-3.5, which means that the cow had adequate nutrition. This lower prevalence may be due to a significant relationship with climate (atmospheric humidity, temperature, atmospheric ammonia, etc.) (Bangoura et al. 2012). Madura Island has high temperatures and low humidity, which may influence the development of *Eimeria* oocysts. The type of cage in Madura is also unique because almost all of them use dirt floors, there is no irrigation for cage sanitation, and feces and urine are scattered on the floor. Higher ammonia levels can reduce oocyst survival (Chapman et al. 2002). The lower prevalence may also be because Madura cattle have a higher tolerance for parasites. This is because Madura cattle are known to be genetically tolerant of hot climates and marginal environments, resistant to ticks, have high adaptability to low feed quality and lower feed requirements compared to other types of cattle (Nurgiartiningsih 2011).

Based on OPG values of 1-499, 500-5,000, or more than 5,000 are classified as mild, moderate, and high infections, respectively (Bangoura et al. 2012), the average OPG in current study was 1 -50 indicates that most of the cattle examined were lightly infected with *Eimeria* spp. One sample had an OPG value of 5,000 in female cows aged < 6 months with clinical symptoms of diarrhea infected with *E. bovis. Eimeria bovis* was found as the dominant species in this study. *E. bovis* and E. *zuernii*, which are known pathogenic species of *Eimeria*, cause watery or bloody diarrhea (Fadly 2012; Enemark et al. 2013). Two fecal samples from bulls aged 6 months - 2 years with soft feces (1 sample infected with *E. bovis* and *E. zuernii* with OPG 3,000 and 1 sample with mixed infection with *E. bovis*, *E. zuernii*, and *E. auburnensis* with OPG 2500) and six fecal samples of cows > 2 years old with an OPG value of 500-25,000 (5 single infected samples and 1 mixed infection sample of *E. bovis* and *E. zuernii* with soft feces) there may be a relationship between abnormal stool shape and *Eimeria* infection. This shows that there is a positive correlation between fecal Oocysts Per Gram (OPG) and the occurrence of diarrhea. 48.6% of cases of diarrhea in calves are caused by a single infection and 51.4% were mixed infections (Bangoura et al. 2012). Also, clinical coccidiosis is more common in calves aged 3 weeks to 6 months (Taylor and Catchpole 1994). It should be noted that there is a possibility of severe diarrhea, if the handling is not done properly. *Eimeria* spp. transmitted by ingestion of sporulated oocysts and infected animals release *Eimeria* spp. oocysts, which are developed in the form of feces that is resistant to most disinfectants and environmental conditions. Parasites can survive for a long time and are a source of further infection in the environment (Lucas et al. 2014; Ekawasti et al. 2021). Thus, the presence of these *Eimeria* oocysts may indicate direct or indirect infection via the fecal-oral route. Bovine coccidiosis is an important parasitic disease of the genus *Eimeria* and is also one of the major livestock diseases worldwide. This disease is considered one of the five most economically important diseases in the livestock industry (Gupta et al. 2016). The biggest economic loss is usually caused by acute diarrhea which causes about 75% of the death loss. The highest prevalence of disease in calves less than one-year-old occurs in calves that are reared with conventional systems and can become infected early in life (Fadly 2012). *Eimeria* spp. very host-specific, and more than 20 species of *Eimeria* are defined in livestock (Heidari and Gharekhani 2014). *Eimeria bovis* and *Eimeria zuernii* are the most common pathogenic species in calves worldwide causing morbidity and death with impaired intestinal absorption and are often associated with diarrhea containing blood, and fibrin (Ekawasti et al. 2021). Calves can be affected by stress, challenge dose, and immune status in cases of coccidiosis. Several management issues must be addressed each time treatment, control, or prevention programs are instituted. Drinks, feeders, and equipments should be cleaned regularly to reduce fecal contamination. Dietary changes should be as gradual as economically feasible, and overcrowding should be avoided (Gupta et al. 2016).

Although the number of samples in the present study was limited, and the samples were identified to the species level by morphological analysis. The resistance to gastrointestinal parasites remains unknown, further studies are needed to clarify pathogenicity in local cattle herds and to increase productivity through better management practices. In conclusion, based on morphology, six species of *Eimera*, namely *E. bovis*, *E. zuernii*, *E. ellipsoidal*, *E. subspherica*, *E. auburnensis* and *E. canadiensis*, were identified, which infect Madura beef cattle. The prevalence of positive samples was 58.33%, which showed mild to moderate infection.

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