Morphometric and Molecular Identification of *Eimeria bovis* and *Eimeria zuernii* on Beef Cattle in Lamongan, East Java, Indonesia

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Abstract

Lamongan Regency, located in East Java, Indonesia, is a significant center for beef cattle production. Despite its prominence, studies on the identification and differentiation of *Eimeria* spp. parasites in this region are notably lacking. This research aims to address this gap by evaluating the prevalence of *Eimeria* spp. and identifying two pathogenic species: *Eimeria bovis* and *Eimeria zuernii*. The study involved the collection of 250 fecal samples from beef cattle raised on smallholder farms across Lamongan. Sampling was conducted during the rainy season to optimize the detection of Eimeria infections. Using the sugar flotation method, Eimeria oocysts were isolated from 5–10 g of fecal matter per sample. Molecular identification employed polymerase chain reaction (PCR) assays targeting ribosomal RNA's internal transcribed spacer 1 (ITS-1) region to detect the pathogenic species. Fecal examination using the Whitlock test revealed a prevalence of *Eimeria* spp. at 44.45% (111/250). PCR analysis further identified *E. bovis* with a 238 bp amplicon in Solokuro District and *E. zuernii* with a 344 bp amplicon in Tikung District, highlighting the presence of these two pathogenic species in distinct geographic areas. The findings underline the need for further research that includes expanded sampling from different regions and cattle breeds and the application of varied diagnostic methods. Such studies will contribute to a more comprehensive understanding of the genetic diversity, distribution, and epidemiology of *E. bovis* and *E. zuernii* in Indonesia, supporting improved cattle health and management practices in the region.

Keywords: disease, *Eimeria bovis, Eimeria zuernii*, molecular, morphometric

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INTRODUCTION

Lamongan, located in East Java, is recognized as a key region for beef cattle production (Kusumaningtias and Munir, 2022). Health management plays a pivotal role in ensuring the success of beef cattle development enterprises (Kurniawan *et al.,* 2023). Among the health challenges, protozoan parasites, known for their significant pathogenic potential, represent a considerable threat (Ekaswati *et al.,* 2019). Gastrointestinal infections caused by protozoa, such as *Eimeria* spp., are commonly called coccidiosis (Zefanya *et al.,* 2021). Coccidiosis in cattle is clinically characterized by bloody or watery diarrhea, lethargy, weight loss, reduced

appetite, and ultimately stunted growth (Ekawasti *et al.,* 2019; Deb *et al.,* 2022). Although cases of bovine coccidiosis have been documented across various regions in Indonesia, encompassing identification at both the genus and species levels, specific reports on *Eimeria* spp. in Lamongan remain scarce. In particular, studies that include morphometric, morphological, and species-level identification of *Eimeria* spp. are lacking for this important cattle-producing region.

Cattle worldwide are reported to be susceptible to gastrointestinal parasites, including protozoa, as noted by Hastutiek *et al.* (2022). Among these parasites, the genus Eimeria comprises numerous species that infect cattle, including *Eimeria alabamensis*, *E. auburnensis*, *E. bovis*, *E. ellipsoidalis*, *E. canadensis*, *E. brasiliensis*, *E. bukidnonensis*, *E. cylindrica*, *E. pellita*, *E. subspherica*, *E. wyomingensis*, and *E. zuernii* (Alcala-Canto *et al.,* 2020). Ekawasti *et al.* (2019) identified *E. auburnensis*, *E. alabamensis*, *E. zuernii*, *E. ellipsoidalis*, and *E. bovis* as pathogenic species capable of causing clinical symptoms. Among these, *E. bovis* and *E. zuernii* are particularly notable for their significant impact, as they are major contributors to elevated calf mortality rates globally, especially in animals under one year old (Hiko *et al.,* 2016; Ekawasti *et al.,* 2019; Saravia *et al.,* 2021).

Although the frequency of coccidiosis varies depending on the nation or farm management system, calves are more susceptible to *Eimeria* spp. infections than adult cattle due to increased vulnerability (Ekawasti *et al.,* 2019). Prevention is emphasized over treatment in managing coccidiosis because subclinical production losses and the potential for irreversible damage often render treatment ineffective and costly (Sriasih *et al.,* 2018; Cruviel *et al.,* 2021). Effective preventive measures include mitigating overcrowding, maintaining proper sanitation of feeding and drinking utensils, ensuring adequate sun exposure, and promoting desiccation to reduce environmental oocyst contamination (Keton and Narre, 2017). Diagnosis of coccidiosis in field settings primarily relies on parasitological techniques, particularly fecal examination using the Whitlock test (Ananta *et al.,* 2014; Hamid *et al.,* 2016; Makau *et al.,* 2017). Although microscopy is a rapid and cost-effective standardized method, it is limited in diagnostic capability, as it only allows identification up to the genus level (Yang and Rothman, 2004; Ekawasti *et al.,* 2019).

With advancements in science, biomolecular techniques such as the polymerase chain reaction (PCR) have been developed to enhance the detection and identification of *Eimeria* spp. (Yang and Rothman, 2004). While microscopy remains a valuable diagnostic tool, it has limitations, particularly in species differentiation. In contrast, PCR provides superior sensitivity by amplifying Eimeria DNA, enabling the detection of infections even at low parasitemia levels. Additionally, it allows for identifying mixed infections that are often difficult to distinguish using microscopic examination (Kusnadi and Arumingtyas, 2020; Vandendoren *et al.,* 2022). Morphological identification of *Eimeria* spp. is often unreliable, especially when species exhibit similarities, such as *E. bovis* and *E. zuernii*. Consequently, PCR analysis is crucial for accurate species determination (Ekawasti *et al.,* 2019; Nurdianti *et al*., 2023). DNA markers, particularly those derived from nuclear genomes, provide greater consistency in establishing phylogenetic relationships than morphological traits. Ribosomal DNA (rDNA) is a commonly employed molecular marker in phylogenetic studies, with the Internal Transcribed Spacer-1 (ITS-1) gene frequently used as a target for differentiating *E. bovis* and *E. zuernii* through conventional PCR (Kawahara *et al.,* 2010; López-Osorio *et al.,* 2018).

Conventional identification of Eimeria species primarily relies on morphological observations of sporulated oocysts. However, morphological similarities among certain species, as highlighted by Ekawasti *et al.* (2019), can make accurate identification challenging. No studies have been conducted to identify the morphology, morphometry, or species of *Eimeria* spp. in the Lamongan region. This underscores the importance of molecular characterization to ensure accurate species identification. Detecting *E. bovis* and *E. zuernii*, two highly pathogenic

species, further emphasizes the need for comprehensive research.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Number: 1.KEH.167.12.2022).

Study Period and Location

This research was conducted between December 2022 and February 2023, focusing on smallholder beef cattle farms in Lamongan Regency, East Java, Indonesia. Sampling was conducted in three districts: Tikung, Laren, and Solokuro. A single fecal sample was collected from each animal, with sampling timed to coincide with the beginning of the rainy season. Fecal samples were collected and placed in sterile plastic containers, carefully labeled, and preserved using a 2.5% potassium bichromate solution to inhibit the growth of other microorganisms during transit. To maintain sample quality and integrity, they were stored in refrigerated containers until delivery to the Protozoology Laboratory, Veterinary Parasitology Division, Faculty of Veterinary Medicine, Universitas Airlangga, where subsequent analyses were performed.

Collection of Fecal Samples

250 fecal samples were collected from beef cattle in Lamongan Regency. An overview of the sampling locations is presented in Figure 1. Samples were obtained directly from the cattle's rectum using rectal gloves to ensure sterility. After collection, the feces were carefully transferred into sterile plastic containers to preserve their shape and consistency. The samples were then preserved with a 2.5% potassium bichromate solution to prepare them for further laboratory analysis. Notably, none of the animals exhibited clinical symptoms during the sampling process.

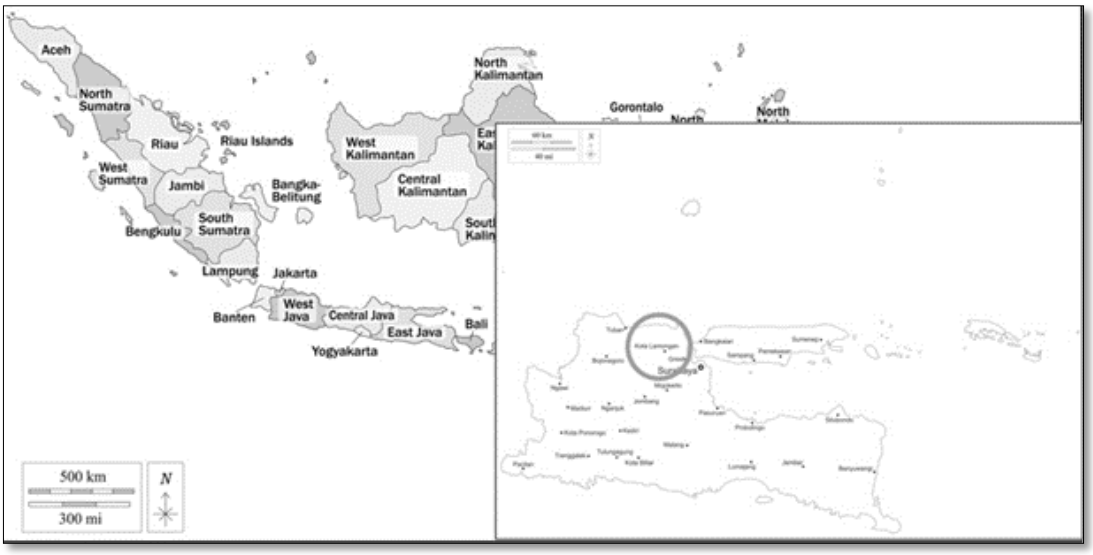

Figure 1. Location in Lamongan, Indonesia.

Table 1. Primer set used for the PCR

Species	Primer sequences		Expected product size (bp)
	Forward	Reverse	
E. bovis	TCATAAAACATCACCTCCAA	ATAATTGCGATAAGGGAGACA	238
E. zuernii	AACATGTTTCTACCCACTAC	CGATAAGGAGGAGGACAAC	344

Fecal Examination

The fecal examination was performed using the sugar centrifugal flotation method. A 1-gram fecal sample was mixed with 9 mL of distilled water, filtered through a steel mesh, and centrifuged at 800 ×g for 5 minutes. The supernatant was discarded, and the sediment was mixed with 10 mL of a sugar solution with a specific gravity of 1.2, prepared by dissolving 100 g of sugar (Gulaku Indonesia, Lampung, Indonesia) in 120 mL of distilled water. The mixture was centrifuged at 800 ×g for 5 minutes, and the resultant material was transferred onto a glass slide. The entire smear was then examined under a light microscope to identify any findings (Ekaswati *et al.,* 2019).

Sporulation

The sporulation process for the samples was conducted by maintaining an ambient temperature of 16°C to 39°C for 2 to 3 days. This temperature range ensured the optimal development of *Eimeria* spp. oocysts, particularly *E. bovis* and *E. zuernii*, under open conditions with adequate oxygen supply (Alcala-Canto *et al.,* 2020).

Purification of Eimeria Oocysts

Eimeria oocysts were extracted from positive fecal samples (approximately 5–10 g) following the previously described procedure using the sugar flotation method. The feces were diluted with distilled water, filtered through steel mesh or gauze, and centrifuged at 800 ×g for 5 minutes. A sugar solution was then added to the sediment, followed by centrifugation at 1200 ×g for 10 minutes. The floating Eimeria oocysts were carefully collected using a Pasteur pipette and washed with distilled water. The purified oocysts were resuspended in 1–2 mL of PBS, and 15–20 µl of the solution was placed on a glass slide for microscopic examination to count the number of oocysts. OPG values (oocysts per gram of feces) were calculated, and the samples were stored at 4°C until molecular identification analysis (Ekaswati *et al.,* 2019).

Molecular Identification of *Eimeria* spp.

During DNA extraction, 400 µl of purified Eimeria oocysts were used. To release genomic DNA, the oocysts underwent five freeze-thaw cycles (Ekaswati *et al.,* 2019). After centrifugation at 5400 ×g for 3 minutes, 200 µl of the supernatant was processed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). A PCR assay targeting the internal transcribed spacer 1 (ITS-1) region of ribosomal RNA was then performed to identify the two pathogenic Eimeria species, *E. bovis* and *E. zuernii*, following previously described protocols (Kawahara *et al.,* 2010). The PCR products were separated by electrophoresis on agarose gels, stained with ethidium bromide, and visualized using a UV transilluminator. The primer sequences utilized in the study are listed in Table 1.

Data Analysis

The results of this study are presented descriptively in the form of figures and tables.

RESULTS AND DISCUSSION

Fecal examinations conducted in three Districts of Lamongan Regency—Tikung, Solokuro, and Laren—using the sugar flotation method and microscopic analysis revealed the presence of *Eimeria* spp. The results of the microscopic analysis, presented in Table 2, showed varying prevalence rates across the districts: Tikung had 33 positive samples with a prevalence of 40%, Solokuro had 43 positive samples with a prevalence of 56.17%, and Laren had 31 positive samples with a prevalence of 37.45%. Of the 250 samples examined, 111 tested positive, resulting in an overall prevalence rate of *Eimeria* spp. infection of 44.45% (111/250) in the three districts. The next phase of the study involved the sporulation process of the samples for molecular testing. Sporulation of *Eimeria* spp. requires optimal environmental conditions, specifically temperatures between 16°C and 39°C. Sporulation cannot occur if the temperature exceeds 39°C or falls below 12°C. Under these conditions, sporulation for *E. bovis* and *E. zuernii*

typically occurs within 2 to 3 days in open environments with adequate oxygen supply (Alcala-Canto *et al.,* 2020).

The number of oocysts, calculated using the McMaster method, revealed a moderate OPG value with an average of <1,306.18955. Based on this value, the infection was classified as mild. Observations of the cows' feces showed a liquid and mushy consistency, indicating a disturbance in the digestive tract, although the infection level was still considered mild. Microscopic species identification confirmed the presence of two *Eimeria* species: *E. zuernii* (Figure 2) and *E. bovis* (Figure 3). Data on the size and shape of the oocysts of both species, including morphometric parameters such as length, width, and the characteristics of the oocyst wall, are presented in Table 3 and Table 4. Continued monitoring and implementing preventive measures are essential to prevent the infection from worsening and minimize its potential negative impact on livestock health.

Table 2. Identification results of *Eimeria* spp.

Sample Quantity	Sample (+)	Sample (–)	Prevalence (%)	Location
83	33	50	40	Tikung District
84	47	37	56.17	Solokuro District
83	31	52	37.45	Laren District

Table 3. Microscope measurement results

Type	Species	Shape	Size (µm)
Pathogenic species	*E. zuernii*	Subspherical	17–22 × 17–23
	E. bovis	Subspherical/Ellips	16–29 × 13–19
Non-pathogenic species	*E. albamensis*	Subspherical	17–18 × 16–17
	E. aburnensis	Ovoid	32–46 × 21–30
	E. brasilensis	Ellips	42–46 × 28–41
	E. canadensis	Ellips	20–33 × 20–24
	E. cylindrical	Cylindrical	22–27 × 18–19
	E. ellipsoidalis	Ellips	19–31 × 15–24

Table 4. Microscope measurement results

Type	Species	Shape	Size (µm)	External wall (µm)	Inner wall (µm)	Microfil (µm)	Sporozoit (µm)	Sporocyst (µm)
Not yet sporulated	*E. zuernii*	Subspherical	23–24 × 17–23	0.4	0.2	–	–	–
	E. bovis	Subspherical/Ellips	12–30 × 10–21	0.8	0.7	4.66	–	–
Already sporulated	*E. zuernii*	Subspherical	23–24 × 17–23	0.4	0.4	4.73	7–10 × 4–5	1–2 × 4
	E. bovis	Subspherical/Ellips	12–30 × 10–21	0.5	0.4	4.96	9–12 × 5–7	3–4 × 6–7

Table 5. Molecular identification results

Location	*Eimeria* spp.	Molecular detection	
		E. bovis	*E. zuernii*
Tikung District	+	–	+
Solokuro District	+	+	–
Laren District	+	–	–

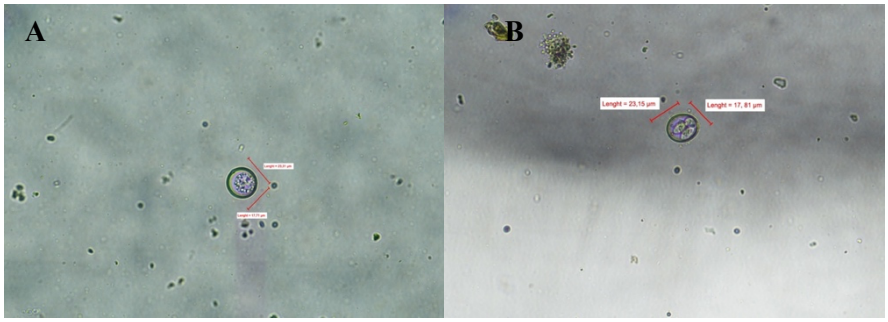

Figure 2. Not sporulated *E. zuernii* (A) and sporulated *E. zuernii* (B) with 400× magnification microscope.

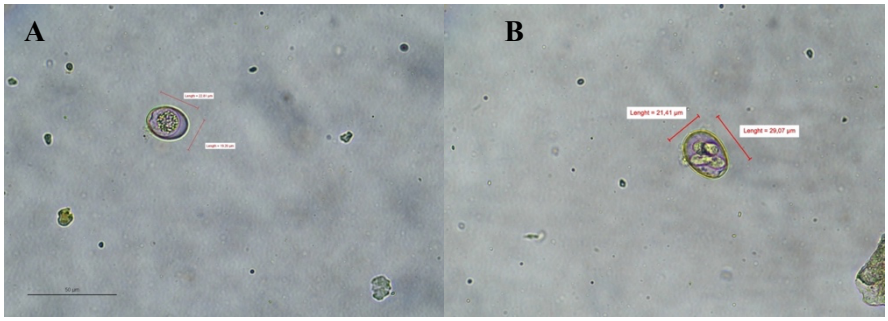

Figure 3. Not sporulated *E. bovis* (A) and sporulated *E. bovis* (B) with 400× magnification microscope.

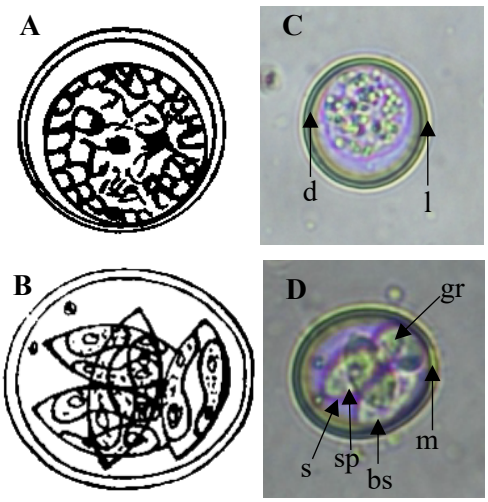

Figure 4. Composite line drawings (A and B) and photomicrographs (C and D) of *E. zuernii*. Micropyle (m); stida body (bs); refractile bubble (gr); sporocyst (s); sporozoite (sp); inner layer of oocyst wall (d); outer layer of oocyst wall (l). Scale bar: 10 µm.

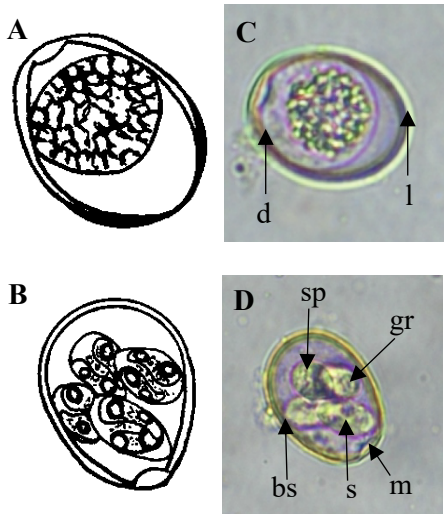

Figure 5. Composite line drawings (A and B) and photomicrographs (C and D) of *E. bovis*. Micropyle (m); stida body (bs); refractile bubble (gr); sporocyst (s); sporozoite (sp); inner layer of oocyst wall (d); outer layer of oocyst wall (l). Scale bar: 10 µm.

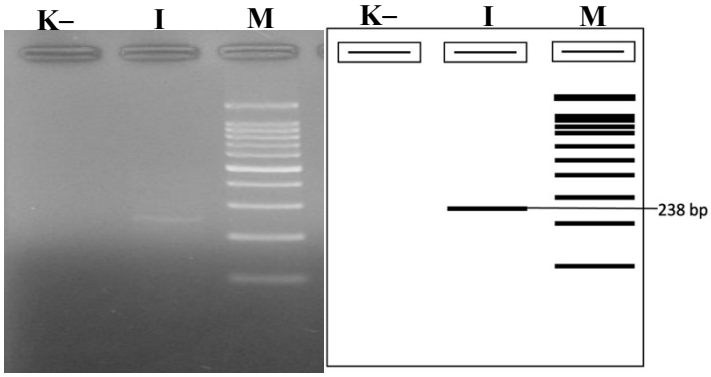

Figure 6. DNA visualization of ITS-1 gene of *E. bovis* in PCR. M = 1:100 bp standard molecular weight, K– = negative control, I = sample code.

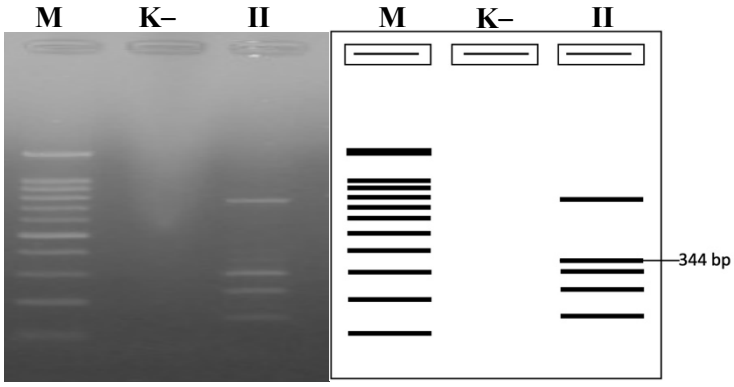

Figure 7. DNA visualization of ITS-1 gene of *E. zuernii* in PCR. M = 1:100 bp standard molecular weight, K– = negative control, II = sample code.

The findings revealed distinct morphological differences between the Eimeria species, *E. zuernii* and *E. bovis*. *E. zuernii* is round, with dimensions ranging from 17–22 × 17–23 µm, while *E. bovis* appears subspherical or elliptical, measuring 16–29 × 13–19 µm (Figures 4 and 5). PCR amplification produced 238 bp and 344 bp products for *E. bovis* and *E. zuernii*, respectively

(Figures 6 and 7). The annealing temperature for *E. bovis* was set at 50°C, whereas *E. zuernii* utilized an annealing temperature of 52°C. The molecular identification results from PCR are summarized in Table 5.

Based on the microscope examination using the Whitlock sugar flotation method on fecal samples from beef cattle in smallholder farms in Lamongan, *Eimeria* spp. was identified. The examination results, detailed in Tables 3 and 4, consider the size and shape of the oocysts. The round-shaped *Eimeria* spp. corresponds to *E. zuernii*, while the subspherical or elliptical shape corresponds to *E. bovis*, consistent with the findings of Alcala-Canto *et al.* (2020). According to Alcala-Canto *et al.* (2020), *E. bovis* is round, measuring 23–24 × 17–23 µm, while *E. zuernii* is subspherical or elliptical, with a size of 12–30 × 10–21 µm. However, morphological examination using a microscope cannot definitively identify the species of *Eimeria* spp., even though some may resemble *E. bovis* and *E. zuernii* (Ekawasti *et al.,* 2019). This challenge is further emphasized by Hastutiek *et al.* (2022), who highlight the difficulties in species identification of *Eimeria* spp. based solely on oocyst morphology in stool examinations. Their research underscores the importance of molecular characterization for accurate species-level identification.

The PCR method is particular and sensitive, making it an essential tool for microorganism detection. PCR is a molecular technique used to synthesize DNA sequences by employing two oligonucleotide primers that hybridize to opposite strands, flanking the target DNA sequence. During each PCR cycle, the nucleotide base sequence is amplified, exponentially doubling the target DNA quantity (2n). A key development in PCR is optimizing the amplification of the target DNA sequence while minimizing the amplification of non-target sequences (Kalle *et al.,* 2014). Due to its precision, PCR can identify microorganisms with remarkable detail, even distinguishing between species and subspecies—an ability not achievable by conventional methods (Tiwari *et al.,* 2022).

Following the positive microscopy examination results, molecular PCR testing was performed using primers complementary to the 18S rRNA gene. The Internal Transcribed Spacer (ITS) region, a non-coding DNA segment, lies between the small subunit ribosomal gene (18S rRNA) and the large subunit (26S nrRNA) within the core ribosomal DNA (nrDNA). The ITS1 and ITS2 loci, located between coding regions (18S rRNA, 5.8S rRNA, and 26S rRNA), serve as spacers or non-coding DNA regions (Yang *et al.,* 2018). For this study, specific primers were employed: for *E. bovis*, the forward primer (TCATAAAACATCACCTCCAA) and reverse primer (ATAATTGCGATAAGGGAGACA), targeting a 238 bp amplification product. For *E. zuernii*, the forward primer (AACATGTTTCTACCCACTAC) and reverse primer (CGATAAGGAGGAGGACAAC) were used, with a target amplification of 344 bp (Kawahara *et al.,* 2010; Ekawasti *et al.,* 2022).

Based on data collected from Tikung District, 33 positive cases of *Eimeria* spp. were identified out of 83 fecal samples analyzed through microscopic examination, resulting in a prevalence rate of 40% (Table 2). The beef cattle population in this area typically ranges between 10 and 20 animals per farm. The relatively moderate prevalence of *Eimeria* spp. oocysts are attributed to the husbandry system employed, particularly the use of cages, which can help inhibit coccidiosis infections. In comparison, the prevalence of *Eimeria* spp. infection tends to be higher in cattle that graze freely than in those kept in enclosed systems. Several factors related to housing and maintenance management influence prevalence rates, including cage density, ventilation, lighting, sanitation, drainage, feeding systems, and the quality of drinking water sources (Tomczuk *et al.,* 2015; Muhamad *et al.,* 2021). Environmental factors, such as the proximity of feces disposal to the cages, also play a significant role. Fecal accumulation near cages promotes higher parasite infestation due to the abundance of viable oocysts, which can remain protected and infectious for several months, even under dry conditions. When exposed to rainwater, these feces may disintegrate and disperse, increasing the risk of infestation. Furthermore, certain farming practices, such as harvesting fresh feed

too early in the morning when the grass is still moist, can contribute to the spread of parasites (Gupta *et al.,* 2016; Hamid *et al.,* 2019; Nurany *et al.,* 2022).

Based on data from the second location in Solokuro District, 47 positive cases of *Eimeria* spp. were identified out of 84 samples through microscopic examination, indicating a prevalence rate of 56.17%. Farms in this region typically house small cattle populations, ranging from 1 to 5 cows per farm. The area's dry and calcareous soil, enriched with high levels of magnesium carbonate ($MgCO_3$) and calcium carbonate ($CaCO_3$), results in an alkaline soil environment with a pH above 7 (Rahmawati *et al.,* 2018). These environmental conditions are conducive to the survival of *Eimeria* spp. oocysts, as protozoa, require an optimal pH range of 6.5 to 8 for survival in external environments (Alcala-Canto *et al.,* 2020). Consequently, the alkaline and calcareous nature of the soil in the Solokuro District plays a significant role in supporting the persistence of *Eimeria* spp. oocysts, thereby contributing to their high prevalence in the region.

Solokuro District exhibits the highest level of *Eimeria* spp. parasite infestation. Farms in this region are typically situated on small plots, with individual family units managing cage maintenance, including cleaning and excrement disposal. However, suboptimal management practices contribute to parasite breeding. Stalls are often dirty, with cleaning occurring only once daily, compromising the cleanliness of cows' resting areas. Environmental factors facilitating the presence of *Eimeria* spp. oocysts include stalls filled with cow feces and the proximity of feces disposal sites to the cages. Accumulated feces around barns provide an ideal habitat for oocysts, which can remain protected and viable for several months, even in dry conditions. Rainwater exacerbates the situation by breaking down fecal mounds and dispersing their contents, creating optimal conditions for oocyst respiration. Favorable factors such as oxygen availability, suitable temperature and humidity, and protection from ultraviolet (UV) radiation promote oocyst survival, increasing the risk of parasitic infestation (Indraswari *et al.,* 2017; Yanuartono *et al.,* 2019). Several factors influence the prevalence of protozoan infections, including animal age, sex, physiological condition, immunity, level of parasite contamination, maintenance management, medication, husbandry systems, sanitation, population density, geographical distribution, and regional conditions (Sriasih *et al.,* 2018; Rahmawati *et al.,* 2018; Purnama *et al*., 2019). *Eimeria* spp. transmission occurs directly via oocysts excreted by infected livestock, which contaminate forage, drinking water, and cage floors (Nugraheni *et al.,* 2015). Cages in infested environments are often characterized by unclean conditions, water puddles, and early consumption of fresh feed, where moist grass harbors parasites (Hamid *et al.,* 2019; Nurany *et al.,* 2022). To mitigate the risk of *Eimeria* spp. infestations, improved sanitation practices, proper excrement disposal, and better husbandry systems must be implemented. Regular cage cleaning, controlled feces disposal, and preventive measures against environmental contamination are critical for reducing the prevalence of this parasitic infection (Kartikasari *et al*., 2019).

Based on data from the third location in Laren District**,** 31 positive cases of *Eimeria* spp. were identified out of 83 samples through microscopic examination, indicating a prevalence rate of 37.45%**.** Farms in this area typically house beef cattle populations ranging from 10 to 20 animals. The lower prevalence compared to other locations is attributed to the maintenance systems implemented in cages, which help inhibit coccidiosis infections (Fikri *et al*., 2023). Notably, the prevalence of *Eimeria* spp. infections was higher in grazed cattle compared to those kept in cages, highlighting the protective role of controlled housing environments. Several factors related to maintenance and housing management influence prevalence rates, including cage density, oxygen availability, lighting, sanitation, drainage, feeding systems, and water sources (Tomczuk *et al.,* 2015; Muhamad *et al.,* 2021).

An important environmental factor contributing to the presence of *Eimeria* spp. oocysts is the proximity of feces disposal to the

cage. Accumulated feces around the cage create a favorable environment for parasite infestation, as oocysts within these fecal mounds can remain protected and viable for several months, even in dry conditions. Rainwater exacerbates this issue by breaking down fecal mounds and dispersing oocysts, significantly increasing the risk of parasite infestation. Furthermore, harvesting fresh feed too early in the morning often results in moist grass contaminated with parasites, further contributing to infection risks (Gupta *et al.,* 2016; Hamid *et al.,* 2019; Nurany *et al.,* 2022).

Sampling at the three research locations (Tikung District, Solokuro District, and Laren District) took place at the onset of the rainy season. During this period, grass grows abundantly around grazing lands, creating an ideal environment for *Eimeria* spp. oocysts to contaminate the grass. As a result, the oocysts can easily spread to cattle grazing in these areas, increasing the risk of infection (Bangoura *et al.,* 2012; Hamid *et al.,* 2019; Nurany *et al.,* 2022).

As determined by microscopy, the prevalence of *Eimeria* spp. in beef cattle in Lamongan Regency was 44.45% (111/250). This finding is lower than the prevalence reported by Pinto *et al.* (2020), who found a rate of 48.5% (49/101) in dairy cows in India, and by López-Osorio *et al.* (2020), who reported a prevalence of 75.5% (1006/1333) in calves in Colombia. Similarly, Hamid *et al.* (2019) observed higher prevalence rates of *Eimeria spp.* in beef cattle across various regions of Indonesia, including Riau (68.78%, 193/281), South Sumatra (78.34%, 173/221), Banten (63.73%, 123/192), Special Region of Yogyakarta (54.69%, 211/380), East Java (63.89%, 155/270), Bali (83.34%, 190/227), West Nusa Tenggara (78.43%, 149/185), Central Kalimantan (72.46%, 118/163), and South Sulawesi (85.07%, 198/231). In addition, Cruvinel *et al.* (2021) reported a prevalence of 59.16% in beef cattle during the rainy season. However, the prevalence of *Eimeria spp.* in Lamongan was higher than the 21% (21/100) reported by Hastutiek *et al.* (2022) in Madura cattle, which also identified pathogenic Eimeria species, including *E. bovis* and *E. zuernii.*

Based on microscopic examination, 111 out of 250 fecal samples from beef cattle tested positive for *Eimeria* spp., resulting in an overall prevalence of 44.45% in Lamongan Regency. This variation in prevalence may be attributed to differences in farm management practices across the studied areas: Tikung District, Solokuro District, and Laren District. The positive samples from each district were combined into three composite samples, which were then analyzed using PCR for molecular identification. The PCR results revealed the presence of *E. bovis* with an amplification target of 238 bp in Solokuro District, and *E. zuernii* with an amplification target of 344 bp in Tikung District.

The prevalence of coccidiosis in Lamongan Regency is relatively low; however, the risk of transmission and an increase in cases remains, particularly during the rainy season. Rainy season weather conditions, characterized by high rainfall and increased humidity, significantly enhance the prevalence of coccidiosis. These conditions create an ideal environment for oocyst sporulation, accelerate their growth, and make them infect more rapidly (Keeton and Navarre, 2018). The resilience of *Eimeria* spp. oocysts to various environmental conditions underscores the importance of continuous surveillance and prevention efforts. Factors such as husbandry management, waste treatment, sanitation, and ecological conditions play a significant role in the spread of the disease (Sufi *et al.,* 2016; Sufi *et al.,* 2017; Rahmawati *et al.,* 2018). Ekaswati *et al.* (2019) further emphasized that low temperatures and high humidity during the rainy season accelerate oocyst sporulation, thereby increasing the risk of transmission. Additionally, *Eimeria* spp. oocysts in their infective phase exhibit high resistance and can survive under various environmental conditions (Indraswari *et al.,* 2017). The risk of infection is also higher in cattle housed on non-permanent floors, such as soil, compared to those housed on permanent floors, such as cement or plaster. Therefore, proper pen design and adequate sanitation are crucial in preventing the development and spread of coccidiosis, particularly during the rainy season

(Bangoura *et al.,* 2012; Gupta *et al.,* 2016; Nurany *et al.,* 2022).

The use of molecular techniques has not yet been reported for any *Eimeria* spp. species in cattle in Lamongan. This may be attributed to the limited number of oocyst infections, posing a challenge for PCR detection. DNA extraction was performed using the reference method of isolates containing 10,000 oocysts/mL. Ekawasti *et al.* (2019) reported minimal oocysts (about 20 of each species) as insufficient for species identification using PCR. In the fecal examination, the mean number of *Eimeria* spp. oocysts was 1,306,189.55. Previous reports categorized OPG values of 1–499, 500–5000, or >5000 as indicative of mild, moderate, and high infection, respectively. Additionally, cattle identified through microscopy did not exhibit clinical symptoms. Given that *E. bovis* has the highest incidence of all the Eimeria species, the positive cattle in this investigation could be regarded as weakly infected.

CONCLUSION

This study showed that the prevalence of coccidiosis in beef cattle in Lamongan Regency reached 44.45%, with *E. bovis* and *E. zuernii* as the most pathogenic dominant species. Both species are the main cause of severe clinical disease, characterized by hemorrhagic diarrhea, which has the potential to cause death and is a significant challenge in the livestock industry worldwide. Although the prevalence is not high, continued sanitation and environmental management efforts are needed to prevent further increase and spread. The results of this study can serve as a foundation for developing more effective prevention strategies in controlling coccidiosis in beef cattle in the Lamongan Regency to improve livestock health and farm productivity.

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AUTHORS' CONTRIBUTIONS

M'AK, KK, and PH: Conceived, designed, and coordinated the study. ES, MM, and LTS: Designed data collection tools and supervised the field sample and data collection, laboratory work, and data entry. IBM and KHPR: Validation, supervision, and formal analysis. YP and ANMA: Contributed reagents, materials, and analysis tools. BPP and ARK: Carried out the statistical analysis and interpretation and participated in preparing the manuscript. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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