



## Bovine coccidiosis cases of beef and dairy cattle in Indonesia

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### ABSTRACT

The number of bovine coccidiosis cases is generally high and distributed worldwide. It can reach up to 100% in the early weeks of age in calves and has a corresponding impact on further cattle development and the economic outcome. For these reasons, this study was conducted to detect the presence of *Eimeria* spp. oocysts followed by morphologically species characterization in different breeds of beef and dairy cattle. These breeds were reared by rural farmers in 9 provinces in Indonesia from March to October 2017. A total of 2150 fecal samples were collected, along with farmer knowledge about coccidiosis through interviews. Results show that prevalence varied as indicated by the following regions: Riau (68.73%), South Sumatera (78.34%), Banten (63.73%), Special Region of Yogyakarta (54.69%), East Java (63.89%), Bali (83.34%), West Nusa Tenggara (78.43%), Central Kalimantan (72.46%), and South Sulawesi (85.07%). The overall prevalence of bovine coccidiosis in all provinces sampled was 72.07%. Eight species of *Eimeria* spp. were identified, namely, *E. auburnensis* (52.11%), *E. bovis* (81.89%), *E. bukidnonensis* (18.44%), *E. canadensis* (38.22%), *E. cylindrica* (31.67%), *E. zuernii* (60%), *E. ellipsoidalis* (10.11%), and *E. alabamensis* (11.67%). The highest prevalence was found in *E. bovis* compared with any other *Eimerias* ( $P \leq .0001$ ) followed by *E. zuernii*. Most of the interviewed farmers in this study did not know about coccidia, especially *Eimeria* spp. in cattle. They were more familiar with worm infections because of both their experience and knowledge. The distribution of cattle with ages < 6 months, 6–18 months, and > 18 months exhibited 97%, 81%, and 38% infection, respectively. Based on these results, the authors saw the necessity to inform farmers about bovine coccidiosis as well as the veterinarian regarding better preventive and curative treatments.

### 1. Introduction

Bovine coccidiosis or eimeriosis is caused by Apicomplexan parasites, *Eimeria* spp. Until now, 20 different bovine *Eimeria* species have been reported to occur worldwide (Lucas et al., 2014). The most pathogenic species in bovine coccidiosis are *E. bovis* and *E. zuernii* that cause severe diarrhea and emaciation, and *E. alabamensis*, the etiologic agent of 'pasture coccidiosis' (Faber et al., 2002). Cases of bovine coccidiosis are generally high, distributed worldwide, and can reach up to 100% during the early weeks of life (Cornelissen et al., 1995; Faber et al., 2002; Fox, 1985). Infected animals with well-developed immune responses may suffer from diarrhea and then self-heal, but sometimes cases lead to high mortalities because of gastrointestinal disorders (Dauguschies and Najdrowski, 2005). *Eimeria* spp. infect bovine orally with the infective stage, sporulated oocysts that are ubiquitously found in the environment.

The costs associated with morbidity, impaired performance,

mortality, and anticoccidial treatment result in considerable economic losses (Dauguschies and Najdrowski, 2005; Fitzgerald, 1980; Hermosilla et al., 2006). It is assumed that losses because of subclinical disease exceed those resulting from clinical coccidiosis (Bürger, 1983; Faber et al., 2002). Subclinical cases occur more frequently and may impair intestinal physiology, feed conversion, and animal growth consistently for long periods of time compared with clinical cases, which can be quickly diagnosed and treated (Cornelissen et al., 1995; Fox, 1985). Also, animals that survived severe clinical coccidiosis always show retarded growth and will not become profitable again since the intestinal damage permanently impairs nutrient absorption (Dauguschies and Najdrowski, 2005).

In Indonesia, many beef and dairy cattle farmers are not familiar with infectious gastrointestinal protozoa in bovine. Almost all treatments related to intestinal parasites are performed using anthelmintic drugs. This treatment is inefficient since coccidian parasites have a very different mode of action compared with helminths and adversely affect

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the host. This outcome was implied since only a few farmers had already performed anticoccidial treatments. On the other hand, the lack of treatment may be because coccidiosis usually occurs in a subclinical form. Sporadic treatment, however, is not recommended since it does not efficiently reduce the environmental contamination with oocysts and the risk of parasite transmission. Precise diagnosis is key in both determining treatment and understanding specific epidemiological conditions within livestock. The aforementioned information compels the authors to both portray and describe *Eimeria* spp. diversity, which circulated in Indonesia. We want to provide additional information to farmers and veterinarians and emphasize the implied importance of bovine coccidiosis for further efficient and effective treatment and possible prevention.

## 2. Materials and methods

### 2.1. Sample collection

This study was performed for eight months, from March to October 2017. A random sampling method was performed in the following 9 Indonesian provinces: Riau (0°29'17.6"N 101°25'51.2"E), South Sumatera (3°01'15.7"S 104°34'06.9"E), Banten (6°21'10.8"S 106°05'37.8"E), Special Region of Yogyakarta (7°43'57.0"S 110°20'11.0"E), East Java (7°36'02.2"S 112°14'11.0"E), Bali (8°20'33.3"S 114°39'02.8"E), West Nusa Tenggara (8°43'10.1"S 117°12'17.4"E), Central Kalimantan (1°47'05.5"S 113°33'18.0"E), and South Sulawesi (3°28'05.6"S 120°02'33.1"E). All the necessary methods for sample collection have been ethically approved according to the ethical clearance certificate number 00091/04/LPPT/VII/2017 issued by Universitas Gadjah Mada, Yogyakarta, Indonesia.

In total, 2150 stool (fecal) samples were collected directly from the rectum and feces of different cattle breeds. In Indonesia, Ongole and Ongole crossbred (PO), Simmental, Limousin, Simmental-PO crossbred, Limousin-PO crossbred, Brahman, Angus, Angus Brahman crossbred, and Bali cattle were reared for beef production purposes. In contrast, Friesian Holstein (FH) were commonly reared for dairy production. In this study, cattle breed identification was based on the dominant phenotype characteristic, which can be observed. Following collection, stool samples were stored in plastic containers and refrigerated at 4 °C until the time of examination. Since some free-ranged yearling cattle are difficult to handle, samples were collected from fresh feces that fall onto the ground.

### 2.2. Farmers questionnaire

The farmers of the sampled bovine were asked about coccidiosis symptoms instead of their knowledge about helminthiasis. They were asked about the occurrence of common diarrhea and the treatment, which they might have given to their cattle. The information provided by the farmers would be used as supporting explanations for our findings in this study.

### 2.3. Fecal samples processing

Samples were prepared by sedimenting the fecal material by centrifugation (400 rpm, 5 min), followed by water discharge. Then, saturated NaCl was added to concentrate the oocysts. Parasitological objects were microscopically observed using 400× magnification. Oocysts per gram of feces (OPG) were determined for samples using a modified McMaster technique (Koutny et al., 2012). Identification of *Eimeria* species used oocysts from the remaining material from McMaster for sporulation in 2.5% potassium dichromate. After 7–10 days at room temperature, samples were identified under a light microscope at 400× magnification. Identification was determined by the morphological appearance of sporulated oocysts (Eckert et al., 1995). Species composition was morphologically determined after sporulation in

potassium dichromate. However, we did not count the number of each species within the fecal sample. Total OPG values were counted for total *Eimeria* spp. oocysts found. Therefore, there was no differentiation about the quantity of pathogenic and non-pathogenic *Eimeria* spp. in a mixed infection.

### 2.4. Data analysis

Observed parameters in this study were *Eimeria* spp. prevalence, species variation, the distribution of infected cattle, and severity indicator of infection. The prevalence was determined in percentage by dividing the positive observed sample with the total number of collected sample. Species identification was determined morphologically and compared among species prevalence using an analysis of variance. At this point, the *P*-value was set at < 0.05. The infected distribution was clustered according to cattle age groups of ≤6, 6–18, and ≥ 18 months. Meanwhile, the severity indicator of infection was counted at ≤1000, ≤5000, and ≤10,000 OPG of total *Eimeria* spp.

## 3. Results

*Eimeria* spp. oocysts were detected in all 9 provinces in Indonesia. The prevalence varied among provinces as follows: Riau (68.73%, 193/281), South Sumatera (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) (Fig. 1). Overall, *Eimeria* spp. were detected in all sampled provinces, and the prevalence was 72.07% (1510/2150).

Eight species of *Eimeria* spp. were identified in this study: *E. auburnensis* (52.11%), *E. bovis* (81.89%), *E. bukidnonensis* (18.44%), *E. canadensis* (38.22%), *E. cylindrica* (31.67%), *E. zuernii* (60%), *E. ellipsoidalis* (10.11%), *E. alabamensis* (11.67%) (Fig. 2A). The highest prevalence was *E. bovis* (*P* ≤ .0001) compared with any other Eimerias followed by *E. zuernii*. Co-infection between species was present in positive bovine (75%) with 2 to 5 species in a specimen (example in Fig. 2B).

Cattle < 6 months old (calves) exhibited 97% infection, 6–18 months old 81% infection, and > 18 months old 38% infection (Fig. 3A). In this study, one severity indicator of infection was determined by an OPG count of total *Eimeria* spp., where ≤1000 was found in 55% of positive samples, ≤5000 was in 32%, and ≥ 10,000 was in 13%, which were mostly under 18 months old (Fig. 3B). In our investigation, the fecal condition was divided into three categories

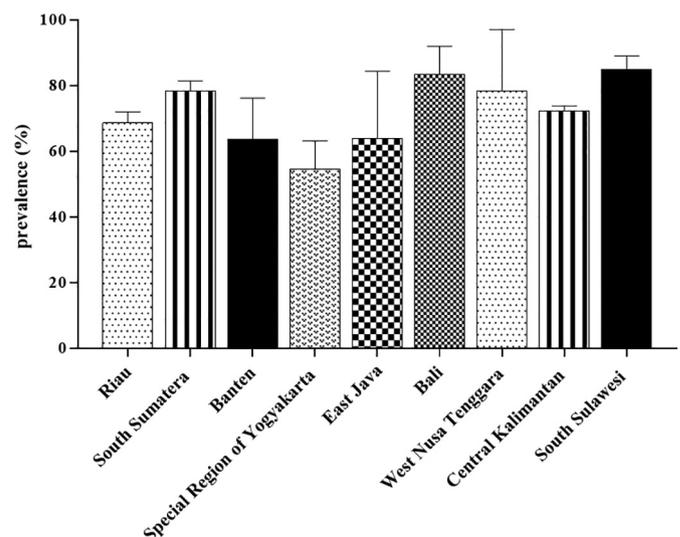


Fig. 1. Prevalence of bovine *Eimeria* spp. in Indonesia.

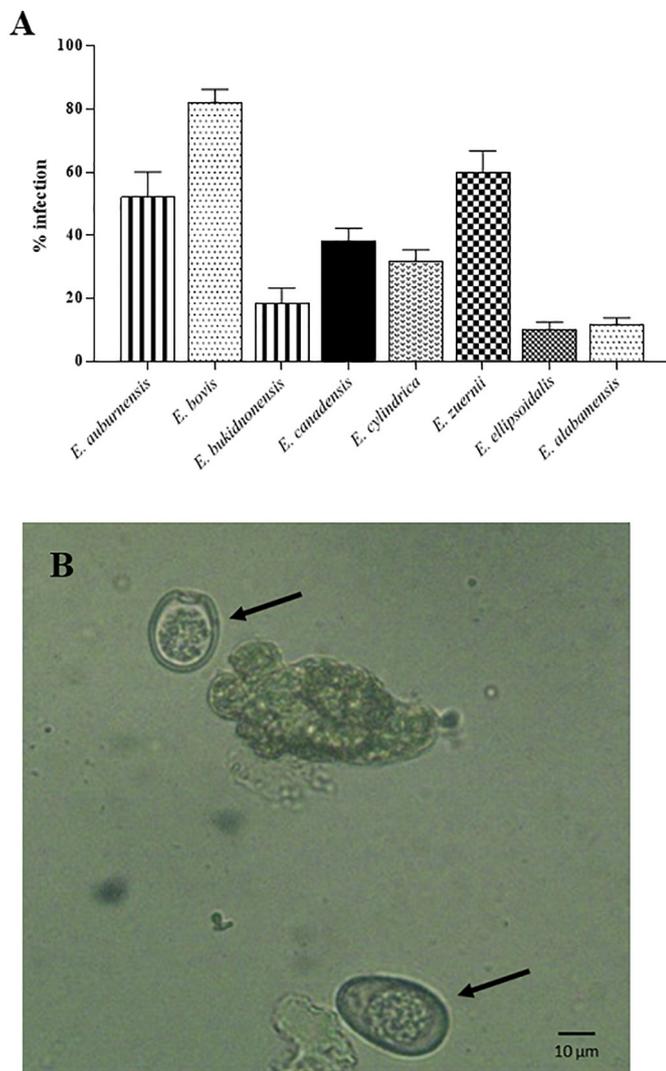


Fig. 2. A. Species distribution in *Eimeria* spp. infection. B. Example of co-infection between two different species (arrows) from the fresh sample, marked by different morphology of oocysts.

namely normal (45%), watery (52%), and bloody (3%). Fecal watery conditions were found in positive samples from calves < 6 months old (61%), 6–18 months old (45%) and > 18 months old (15%).

Almost no farmers in this study knew about coccidia, especially *Eimeria* spp. despite other coccidian parasites, bovine coccidiosis, or eimeriosis in cattle. From both their experience and knowledge, worm infections were more familiar and, therefore, they associated almost all gastrointestinal disorders with helminthiasis. They usually asked the veterinarian for deworming treatments that corresponded to all clinical signs, which were typically manifested with diarrhea.

#### 4. Discussion

Infection by *Eimeria* spp. worldwide generally occurs sub clinically without the presentation of clinical diarrhea (Fox, 1985). Bovine coccidiosis is responsible for economic losses because although animals appear healthy, many of the developmental stages such as damage to the intestinal lining result in reduced feed consumption, malnutrition, impaired growth, and weight loss (Bürger, 1983; Dausgschies et al., 1986). Their compromised immune system makes infected animals more susceptible to secondary infection by viruses and bacteria (Fox, 1985), which lead to many clinical symptoms. Although infections by *Eimeria* spp. are usually self-limiting, stressful environments can stimulate persistent infection and severe clinical manifestations (Foreyt, 1990). In addition, adult cattle serve as subclinical hosts and become carriers of *Eimeria* spp. to younger calves, which are more susceptible to infection.

Our investigation showed that the prevalence of *Eimeria* spp. detected in Indonesia was high (72.07%). To the best of our knowledge, we presented all areas where *Eimeria* spp. have been detected, and covered the islands in Indonesia completely. The prevalence of *Eimeria* spp. detected here corroborate other reports of *Eimeria* spp. infection in different countries worldwide such as Kenya (32.8%) (Makau et al., 2017), Denmark (96.2%) (Koutny et al., 2012), Germany (95.4%) (Bangoura et al., 2012), Poland (52.8%) (Tomczuk et al., 2015), Turkey (20.04%) (Cicek et al., 2007), and South Africa (29%–52%) (Matjila and Penzhorn, 2002). When compared with our previous finding (Hamid et al., 2016), the parasite prevalence was higher because in this study we added total sample collections and chose different age ranges of cattle. However, in our previous report, we collected samples mostly from abattoir with end-up cattle for consumption. The traditional farming system in Indonesia is performed by rural farmers. In some regions such as West Nusa Tenggara, cattle are free ranging in the wide savanna. In other regions, each family commonly rears 1 to 5 cattle

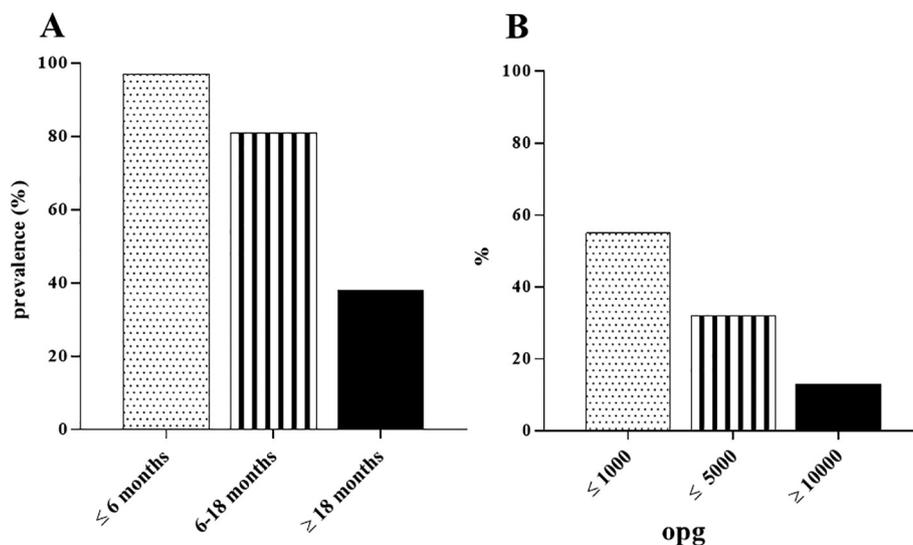


Fig. 3. A. Age distribution of bovine coccidiosis in cattle, B. Severity of *Eimeria* spp. infection as indicated by oocyst per gram of feces (OPG) of sampled cattle.

semi-intensively in the cattle house located in the backyard with soil-type, rough-cemented or latex-embedded flooring. In our study, both housed and free-ranging cattle have a high prevalence of *Eimeria* spp. with no significant difference ( $P > .05$ ). The warm and humid tropical climate in Indonesia may also be a factor of this coccidiosis since it occurred persistently. There are rainy and dry seasons, which are six months each, and our sampling period was from March to October, which intersects these two seasons. It is reported that the wet season significantly corresponds to a higher prevalence of bovine coccidiosis (Gorsich et al., 2014; Gupta et al., 2016). However, we found only a slightly higher tendency and higher prevalence in samples collected in August, September, and October 2017, but the difference was not significant ( $P > .05$ ). Since in 2017 rainfall occurred along year long, it was difficult to differentiate the seasonal effect.

Our investigation also showed that co-infection between species occurred (75%), which is typical (Hamid et al., 2016; Koutny et al., 2012; Stewart et al., 2008). During *in vitro* culture, mixed infections of the most pathogenic species, *E. bovis* and *E. zuernii*, were also reported and able to produce progenies/merozoites under artificial conditions (López-Osorio et al., 2018). The detected prevalence of the most pathogenic species, *E. bovis* (81.96%) and *E. zuernii* (60%), was high in the investigated samples. Since we did not separate the OPG count of the pathogenic from the non-pathogenic oocysts in mixed infections, we could not analyze the correlation of the species composition with fecal samples condition (i.e., normal, watery, and bloody). It is known that presence of *E. bovis* and *E. zuernii* have pathogenic effects and are associated with diarrhea as the clinical manifestation (Bangoura et al., 2012; Dausgchies and Najdrowski, 2005; Enemark et al., 2013). Bangoura et al., (2012) showed a significant association between fecal condition with *E. bovis* and *E. zuernii* oocyst detection. A significant correlation was observed between total *Eimerias* oocyst load (OPG) and diarrheic fecal condition (Enemark et al., 2013). However, in the sub-clinical condition, two pathogenic *Eimerias* were also detected (Autzen et al., 2002). It is still debatable whether the total OPG load or the pathogenic *Eimerias* deposition has more impact in clinical coccidiosis or maybe the factors have an additive effect. In addition, clinical diarrhea is present due to secondary infection by viral and bacterial infections, protective immune responses, and a combination of environmental factors.

Several reports of local domestic-animal breeds found that these animals have more immunity against diseases. For example, thin-tail sheep have higher resistance to fasciolosis compared with other sheep (Roberts et al., 1997). In chickens, Sentul chickens show a lower prevalence of avian coccidiosis compared with broilers and other local chicken breeds in Indonesia (Hamid et al., 2018). However, the field study in this paper showed no differences in oocyst detection cases between investigated breeds regarding resistance to *Eimeria* spp. In this study, age was the only factor related to *Eimerias* oocyst detection with older cattle that were less infected. The prevalence of coccidiosis in calves (< 12 months old) was significantly higher ( $P < .05$ ) than older ages. Both watery and bloody feces in positive samples were also higher (61%) compared with older groups, which are usually normal in consistency. This finding is similar to previous reports that clearly show a correlation between young cattle with the severity of coccidiosis (Tomczuk et al., 2015). This correlation can be explained by considering that severity correlates with immature gut-associated lymphoid tissue (GALT), which is an essential immunological barrier against gastrointestinal diseases.

Moreover, GALT contains a variety of immune cell types from the innate and acquired immune systems (Wershil and Furuta, 2008). Because of the proximity to the microbiome and immediate contact with food, it is continually exposed to both 'normal' and potentially dangerous antigens. Accordingly, GALT develops in a manner that allows non-pathogenic substances, such as commensal bacteria, to survive and enables tolerance to food antigens while protecting the host from pathogenic organisms and other potentially toxic substances (Wershil and

Furuta, 2008).

In this study, we also found that farmers did not know or hear about coccidiosis cases. They only knew that their cattle suffered from a gastrointestinal disorder (e.g., diarrhea was the clinical sign), then they usually asked the veterinarian for deworming medication. Nevertheless, the incidence of helminthiasis is also high (Hamid et al., 2016; Purwati et al., 2017). In this case, the authors see the necessity to inform farmers about coccidiosis in cattle, which is different from helminth infections, and reintroduce the veterinarian to curative treatments specifically.

## 5. Conclusions

According to our findings, the prevalence of *Eimeria* spp. in beef and dairy cattle in Indonesia is high. Eight different species of *Eimeria* spp. were found, and cross-species contamination occurred. Calves were mostly infected, and farmers did not know about coccidiosis in cattle. Based on these findings, a dissemination program regarding coccidiosis, which corresponds to the results of this study, needs to be delivered to farmers and veterinarians for effective and efficient action to prevent or cure the disease.

## Conflict of interest statement

There is no conflict of interest for this work.

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