Eimeria SPECIES COMPOSITION AND FACTORS INFLUENCING OOCYSTS SHEDDING IN DAIRY FARM, BANDUNG, INDONESIA

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Received 15 July 2015/Accepted 25 May 2017

ABSTRACT

Coccidiosis is one of the most widely distributed parasitic diseases of cattle throughout the world. Coccidiosis infection in ruminants was caused by *Eimeria* spp. The objective of this study was to determine *Eimeria* species composition and various factors influencing *Eimeria* oocysts shedding in dairy farm. This study was conducted with a cross-sectional study design in dairy farm in South Bandung District from July 2014 to January 2015. Samples were obtained from 400 dairy cattle (196 cattle at age < 6 months, 37 cattle at age 6 - 12 months and 167 cattle at age > 12 months). Fecal samples were collected, examined and counted for *Eimeria* species composition and numbers of oocysts per gram of feces (OPG) using McMaster technique. A questionnaire was completed for individual dairy cattle farmer to record information about cattle's health and husbandry. The effect of cattle's sex, age and type of pen flooring to OPG values were analyzed using Mann-Whitney and Kruskal-Wallis tests. The Kruskal-Wallis test was performed followed by Dunn test as a multiple comparison test. Ten species of *Eimeria* were identified in all infected cattle. Among the *Eimeria* identified species, *Eimeria bovis* was found to have the highest prevalence (42.5%), followed subsequently by *Eimeria wyomingensis* (39.1%), *Eimeria bukidnonensis* (32.4%), *Eimeria pellita* (26.3%), *Eimeria auburnensis* (19.6%), *Eimeria zuernii* (17.3%), *Eimeria cylindrica* (3.9%), *Eimeria canadensis* (3.9%), *Eimeria brasiliensis* (3.4%) and *Eimeria alabamensis* (1.1%). The numbers of oocysts shed was correlated significantly ($p < 0.05$) with cattle's sex and age as well type of pen flooring which influenced the infection pressure. Younger calves aged less than 6 months shed the highest amount of *Eimeria* oocysts than older cattle. Many factors may cause the increasing number of OPG in fecal samples. Therefore, it is important to keep good sanitation and control of *Eimeria* among dairy cattle in the KPBS Pangalengan dairy farm.

Keywords: Coccidiosis, cross-sectional, dairy farm, *Eimeria*, OPG

INTRODUCTION

Coccidiosis is a protozoic disease in cattle caused by *Eimeria* spp. More than twelve different species of *Eimeria* have been described in cattle; most of which are considered harmless. Of these many species, *E. bovis* and *E. zuernii* are highly pathogenic causing mortality and morbidity by disturbing absorption mechanisms (Rehman *et al.* 2011). Infection occurs on calves caused by large numbers of oocysts of *E. bovis* or *E. zuernii,* may result in severe diarrhea with feces containing blood, fibrin and intestinal tissue. However, coccidiosis in cattle commonly occurs as subclinical disease without signs of the disease and involving great economical losses due to reduced appetite, reduced body weight, impaired feed conversion, unthriftness, diarrhea, dysentery, anemia and increased susceptibility to other diseases (Abebe *et al.* 2008). A poorer body condition score (BCS), potentially as the result of a disease, can be connected to a lower milk production in dairy cattle (Lassen 2009). Coccidiosis is estimated to cause annual economic loss in excess of USD 400 million in the US (Bruhn *et al.* 2011).

The development of clinical coccidiosis in cattle mainly depends on factors such as species

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of *Eimeria*, age of infected animal, number of oocysts ingested, presence of concurrent infections, as well as type of production system and management practices (Abebe *et al.* 2008). The aim of this present study was to determine the *Eimeria* species composition and factors influencing the incidence of *Eimeria* oocysts shedding in Cooperation of South Bandung Dairy Farm (KPBS) Pangelangan, District of Bandung, Indonesia. Thus, determination of species composition and evaluation of factors influencing the shedding of *Eimeria* oocysts per gram (OPG) is very useful in designing efficient control strategies.

MATERIALS AND METHODS

Study Area

The study was conducted in KPBS Pangalengan, South Bandung District, West Java Province from July 2014 to January 2015. KPBS Pangalengan Bandung covers 27.3 ha of highland area with an altitude of 1,000 to 1,420 m asl, 12 – 28 °C mean annual temperature and 60 – 70% relative humidity. The average annual rainfall was > 2,000 mm during the study period.

Study Design

Cross-sectional study design was applied based on Thrusfield (2007). A total of 400 dairy cattle (n) from a total population of 14,000 dairy cattle distributed on 37 cooperation service area (TPK) and 225 farmers groups were selected for the study with 95% confidence interval, 50% expected prevalence (P) and 5% accepted error (L). The relevant formula for a 95% confidence interval is based on Thrusfield (2007):

$$n = 4PQ/L^2$$

where: n = total of cattle
P = expected prevalence
Q = 1 – P
L = accepted error

Samples obtained from dairy cattle farmers covered all age groups i.e. calves (age < 6 months); weaners (6 - 12 months); and adults (age > 12 months). From the total of 225 farmers groups, 10 farmers groups consisted of 80 farmers were randomly selected because these farmers had complete cattle age groups. From each of these farmers, five fecal cattle samples were collected including all calves in the selected farmers, while the remainder fecal samples were taken from weaners and adults.

Sample Collection

Fecal samples of 20 - 50 g were collected from each age group. The feces was directly obtained from cattle's rectum or immediately collected after cattle defecation. Fecal samples were stored in respective plastic bag and preserved in refrigerator at 4 °C before being tested in the laboratory for the occurrence of *Eimeria* spp. oocysts. Fecal consistency was assessed immediately after sampling and classified as normal or diarrheal without any additional differentiations (Bangoura *et al.* 2011). The plastic bags containing fecal samples were labeled with sampling information such as sample number, sampling date, sampling location, cattle's age group, and farmer's name. Practices of animal health management were recorded by the participating farmers in a questionnaire. In addition, record of cattle's sex and age (in months) were documented for each cattle from which a sample was taken.

Counting of Oocysts Per Gram of Feces (OPG)

Fecal samples were examined and oocysts numbers were counted using the McMaster method. Two grams of fecal material was mixed thoroughly with 28 mL of sugar-salt solution and filtered through a 200 µm mesh wire sieve or tea strainer. The suspension was equally poured into two McMaster counting chambers (2 x 0.15 mL) and was let still for 5 minutes. The counting was carried out using light microscope with 100x magnification. The numbers of oocysts in the two chambers were multiplied by dilution factor (50) to obtain the number of oocysts per gram of feces (OPG). This protocol was modified from Dong *et al.* (2012), Lucas *et al.* (2006) and Soulsby (1986).

Eimeria Species Identification

The remainder of each positive samples were centrifuged at 1,500 rpm for 5 minutes at room temperature of 25 °C. For identification, a

flotation of the *Eimeria* oocysts in sugar-salt solution was performed. Oocysts were measured under ocular eye piece that was calibrated with micrometer under 40x objective lense of a light microscope. Identification of *Eimeria* species was based on the morphological features of the oocysts (size, index, shape, color and texture of oocyst's wall, presence or absence of micropyle and polar cap) with the aid of taxonomic keys (Soulsby 1986; Daugschies & Najdrowski 2005). Measurement of oocysts index value were performed by dividing the length and width of *Eimeria* oocysts. The shape of oocysts were examined for each sample and classified as round (length/width = 1), ovoid (length/width between 1 - 1.5) and ellips (length/width > 1.5) (Cahyaningsih & Supriyanto 2007).

Data Analysis

The effect of cattle's sex and age as well as type of pen flooring to *Eimeria* OPG counts were analyzed using Mann-Whitney and Kruskal-Wallis tests. The Kruskal-Wallis test was performed followed by Dunn test as multiple comparison test.

RESULTS AND DISCUSSION

Species Identification and Composition of *Eimeria*.

Results of this study showed that the shedding of *Eimeria* oocysts were commonly occurred in KPBS Pangalengan dairy farm in Bandung. From

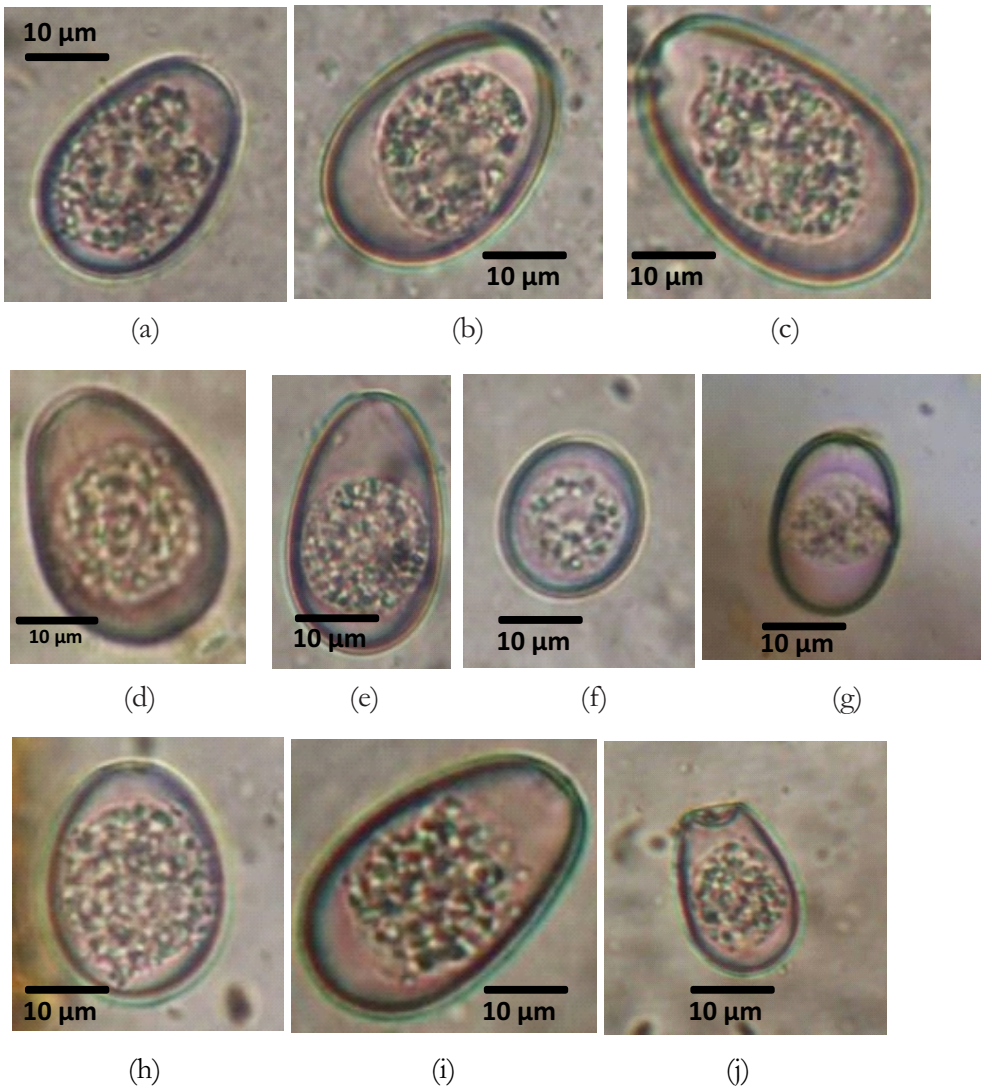

Figure 1 The results of *Eimeria* species oocysts in dairy cattle in KPBS Pangalengan, Bandung
(a) *Eimeria bovis*; (b) *Eimeria wyomingensis*; (c) *Eimeria bukidnonensis*; (d) *Eimeria pellita*; (e) *Eimeria auburnensis*; (f) *Eimeria zuernii*; (g) *Eimeria cylindrica*; (h) *Eimeria canadensis*; (i) *Eimeria brasiliensis*; (j) *Eimeria alabamensis*

400 fecal samples, there were 179 fecal samples (44.8% prevalence; Confidence Interval (CI) 95%; 40.0 - 49.6) contained *Eimeria* oocysts. A total of 10 *Eimeria* species i.e. *E. bovis*, *E. wyomingensis, E. bukidnonensis, E. pellita, E. auburnensis, E. zuernii, E. cylindrica, E. canadensis, E. brasiliensis* and *E. alabamensis* were identified from the 179 positive fecal samples collected from KPBS Pangalengan dairy farm (Fig. 1, Table 1, Table 2).

Our study showed that *E. bovis* was the most prevalent species (42.5%; CI 95%: 37.1 - 47.8) and had the highest mean OPG number (538.2 OPG (Oocysts Per Gram of feces); CI 95%: 191.5 - 884.8) followed by *E. wyomingensis* (39.1; CI 95%: 33.8 - 44.4 and 285.0; CI 95%: 136.0 - 434.0) and *E. bukidnonensis* (32.4; CI 95%: 27.3 - 37.5 and 237.1; CI 95%: 128.3 - 345.9). *E. zuernii* had the second highest mean OPG counts (509.7; CI 95%: 46.1 - 973.3). The lowest prevalent species was *E. alabamensis* (1.1; CI 95%: 0.0 - 2.3 and 75.0; CI 95%: 0.0 - 392.7) (Table 3).

A number of authors reported that *E. bovis* was the most prevalent species in cattle, but clinical coccidiosis was not observed in the calves or in adults cattle (Kennedy & Kralka 1987; Lucas *et al.* 2006; Heidari & Gharekhani 2014). The results of those studies were in close agreement with the results of our study. According to Waruiru *et al.* (2000), the mere present of pathogenic *Eimeria* spp. did not necessarily indicate clinical disease.

Eimeria species were classified as highly pathogenic (*E. bovis* and *E. zuernii*), low pathogenic (*E. ellipsoidalis, E. alabamensis, E auburnensis* and *E. subspherica*) and non-pathogenic (*E. brasiliensis, E. bukidnonensis, E. canadensis, E. cylindrica, E. pellita* dan *E. wyomingensis*) (Lassen & Jarvis 2009). In other tropical areas such as Brazil, several species of *Eimeria* in cattle were also found (Floriao *et al.* 2015). *E. bovis* was recorded as the highest prevalent coccidian species which was in accordance with reports of Arslan and Tuzer (1998), Lucas *et al.* (2006) and Abebe *et al.* (2008).

Table 1 Oocyst morphology of *Eimeria* species of cattle has been identified in fecal samples obtained from KPBS Pangalengan, Bandung

Length (µm)	Width (µm)	Index	Form	Color	Other characteristics	*Eimeria* species
26.25-33.00 *29.77±1.37	18.75-22.50 *21.58±0.73	1.17-1.48 *1.38±0.05	Ovoid	Greenish-brown	Bilayered wall, micropyle	*E. bovis*
37.50-43.50 *42.67±2.04	26.25-30.75 *29.58±1.20	1.22-1.49 *1.44±0.04	Ovoid	Greenish-brown	Single layered wall, micropyle	*E. wyomingensis*
47.25-49.50 *48.70±0.68	33.75-37.50 *35.72±1.88	1.26-1.47 *1.37±0.08	Ovoid	Yellowish-brown	Bilayered wall, micropyle	*E. bukidnonensis*
36.00-37.50 *37.43±0.28	26.25-29.25 *29.16±0.51	1.26-1.37 *1.28±0.02	Ovoid	Dark brown	Thick wall, micropyle	*E. pellita*
37.50-45.75 *38.74±2.73	22.50 *22.50±0.00	1.67-2.03 *1.72±0.12	Ellips	Yellowish-brown	Bilayered wall, micropyle	*E. auburnensis*
15.75-20.25 *19.87±1.19	15.00-17.25 *16.65±1.01	1.05-1.35 *1.20±0.08	Ovoid	Pale yellow	Thin wall, no micropyle	*E. zuernii*
25.50-27.00 *25.93±0.73	15.00 *15.00±0.00	1.70-1.80 *1.73±0.05	Ellips	Pale yellow	Single layered wall, no micropyle	*E. cylindrica*
30.00-33.75 *30.42±1.25	25.50-26.25 *26.00±0.38	1.14-1.32 *1.17±0.06	Ovoid	Yellowish	Bilayered wall, micropyle	*E. canadensis*
41.25-42.75 *41.50±0.53	25.50-26.25 *25.67±0.33	1.60-1.63 *1.62±0.007	Ellips	Brownish-yellow	Bilayered wall, micropyle	*E. brasiliensis*
15.00-18.75 *16.88±2.65	11.25-15.00 *13.13±2.65	1.25-1.33 *1.29±0.06	Ovoid	Pale yellow	Smooth wall, no micropyle	*E. alabamensis*

Note: * = mean; ± = standard deviation

Table 2 Oocyst morphology of *Eimeria* species of cattle based on references

Species	According to Soulsby (1986)						According to Daugschies and Najdrowski (2005)					
	Length (μm)	Width (μm)	Index	Form	Color	Other characteristics	Length (μm)	Width (μm)	Index	Form	Color	Other characteristics
E. bovis	23-34	17-23	1.35-1.48	Ovoid	Greenish-brown	Smooth wall, micropyle	23-34	17-23	1.35-1.48	Ovoid	Brownish-yellow	Bilayered wall, micropyle
E. wyomingensis	37-44.9	26.4-30.8	1.40-1.46	Ovoid	Greenish-brown	Micropyle	37-45	26-31	1.42-1.45	Ovoid	Yellowish-brown	Single layered wall, micropyle
E. bukidnonensis	47-50	33-38	1.32-1.42	Oval	Yellowish-brown	Bilayered wall, micropyle	47-50	33-38	1.32-1.42	Pear-shaped	Brownish-yellow	Bilayered wall, micropyle
E. pellita	36.1-40.9	26.5-30.2	1.35-1.36	Egg-shaped	Dark brown	Thick wall, micropyle	36-41	26-30	1.37-1.38	Ovoid	Dark brown	Thick wall, micropyle
E. auburnensis	32-46	20-25	1.60-1.84	Ellips	Yellowish-brown	Smooth wall, micropyle	32-46	20-25	1.60-1.84	Ellipsoid	Yellowish-brown	Bilayered wall, micropyle
E. zuernii	15-22	13-18	1.15-1.22	Sub-spherical	Pale yellow	Thin wall, no micropyle	15-22	13-18	1.15-1.22	Subovoid	Colorless	Single layered wall, no micropyle
E. cylindrica	16-27	12-15	1.67-1.80	Cylindrical	Colorless	Thin wall, no micropyle	16-27	12-15	1.67-1.80	Elipsoid	Colorless	Single layered wall, no micropyle
E. canadensis	28-37	20-27	1.37-1.40	Ovoid/ ellips	Yellowish-brown	Smooth wall, micropyle	28-37	20-27	1.37-1.40	Ovoid/ ellipsoid	Yellowish	Bilayered wall, micropyle
E. brasiliensis	34.2-42.7	24.2-29.9	1.64-1.65	Ellips	Yellow	Smooth wall, micropyle	34-43	24-30	1.65-1.67	Ellipsoid	Brownish-yellow	Bilayered wall, micropyle
E. alabamensis	13-24	11-16	1.18-1.44	Pear-shaped	Colorless	Thin wall, no micropyle	13-24	11-16	1.18-1.44	Ovoid	Colorless / pale yellow	Thin wall, no micropyle

Table 3 *Eimeria* species identified, the prevalence and mean OPG counts in dairy cattle in KPBS Pangalengan dairy farm, Bandung

Species	Dairy cattle (n = 179)		Mean OPG (CI 95%)
	Positive no.	Mean Prevalence (%) (CI 95%)	
E. bovis	76	42.5 (37.1 - 47.8)	538.2 (191.5 - 884.8)
E. wyomingensis	70	39.1 (33.8 - 44.4)	285.0 (136.0 - 434.0)
E. bukidnonensis	58	32.4 (27.3 - 37.5)	237.1 (128.3 - 345.9)
E. pellita	47	26.3 (21.5 - 31.1)	177.7 (124.7 - 230.7)
E. auburnensis	35	19.6 (15.2 - 23.9)	397.1 (0.0 - 820.9)
E. zuernii	31	17.3 (13.2 - 21.4)	509.7 (46.1 - 973.3)
E. cylindrica	7	3.9 (1.8 - 6.02)	78.6 (42.2 - 115.0)
E. canadensis	7	3.9 (1.8 - 6.02)	121.4 (51.5 - 191.3)
E. brasiliensis	6	3.4 (1.4 - 5.3)	125.0 (45.4 - 204.6)
E. alabamensis	2	1.1 (0.0 - 2.3)	75.0 (0.0 - 392.7)

According to Daugschies and Najdrowski (2005), *E. bovis* and *E. zuernii* are the most pathogenic of the bovine coccidia. Furthermore, *E. alabamensis* causes disease at extremely large infective doses (> 10 million oocysts) only. *E. alabamensis* usually induces watery diarrhea without blood, dehydration, depression and reduced growth, whereas infection remains subclinical under moderate infection pressure.

Single or Mixed-Species Infection Caused by Different *Eimeria* Species

In this study, mixed-species infection experienced by a single cattle hosting several *Eimeria* species were commonly observed. The number of *Eimeria* species in single and mixed-species infection per examined fecal sample ranged from 1 to 5. Mixed-species infection caused by 2 to 5 *Eimeria* species were found in 55.9% of cases (CI 95%; 50.5 - 61.3). The remainder fecal samples had been infected with single infection of *Eimeria* species (44.1%; CI 95%: 38.7 - 49.5) (Table 4).

This finding is similar to the works of Kennedy and Kralka (1987) in Canada, which reported 5 *Eimeria* species. The results of mixed-species infection were lower than the observation of Arslan and Tuzer (1998) in Turkey who reported 6 *Eimeria* species, Abebe *et al.* (2008) in Ethiopia who reported 7 *Eimeria* species, Yu *et al.* (2011) in China who reported 10 *Eimeria* species and Dong *et al.* (2012) also in China who recorded 8 *Eimeria* species. Many previous studies indicated that under natural conditions, mixed-species infection cases were much more common than single species infection (Yu *et al.* 2011; Dong *et al.* 2012). The level of pathogenicity of *Eimeria* species increased to a higher level due to mixed-species infection with other pathogenic *Eimeria*, causing mortality in cattle (Daughschies & Najdrowski 2005).

Table 4 Single and mixed infection of *Eimeria* species

No. of *Eimeria* spp.	Dairy cattle (n = 179)	
	Positive no.	% of positive samples (CI 95%)
1	79	44.1 (38.7 - 49.5)
2	59	33.0 (27.8 - 38.1)
3	24	13.4 (9.7 - 17.1)
4	15	8.4 (5.4 - 11.4)
5	2	1.1 (0.0 - 2.3)
Total	179	100.0

Table 5 Results of Mann-Whitney and Kruskal-Wallis tests followed by Dunn test toward several factors and mean OPG counts

Categorical factors	No. Dairy cattle (n)	Mean OPG (CI 95%)	*p* value
Sex			
Male	80	855.6 (150.9 – 1,560.4)	0.000*
Female	320	144.5 (77.7 - 211.4)	
Age			
Calves (< 6 months)	196	537.0[a] (231.8 - 842.2)	0.000*
Weaners (6-12 months)	37	154.1[a] (79.8 - 228.3)	
Adults (> 12 months)	167	22.5[b] (11.0 - 33.9)	
Type of pen flooring			
Straw	13	53.9[a] (0.0 - 162.4)	0.000*
Wood	70	703.6[b] (0.0 – 1,450.2)	
Rubber	96	405.7[b] (116.9 - 694.6)	
Cement	221	116.7[a] (52.4 - 181.1)	

The Effect of Cattle's Sex, Age and Type of Pen Flooring to *Eimeria* OPG Counts

The effect of various categorical factors to OPG counts were first analyzed with the Mann-Whitney and Kruskal-Wallis tests. A Mann-Whitney test was used to compare mean OPG between 2 groups, whereas Kruskal-Wallis test was used to compare mean OPG among 3 or more groups. The Kruskal-Wallis test was performed followed by Dunn test as a multiple comparison test. A p value < 0.05 was required to indicate significance. Several categorical factors (cattle's sex, age and type of pen flooring) and mean OPG counts are presented in Table 5.

A highly significant (p = 0.000) effect was observed between different sexes of cattle. Intensity of *Eimeria* infections were shown by mean OPG found in cattle. This study showed that higher OPG counts were found in male cattle (855.6 OPG; CI 95%: 150.9 – 1,560.4) compared with females (144.5 OPG; CI 95%: 77.7 - 211.4). In this study, male cattle harbored more coccidia than female cattle. This situation might be caused by less care given to the male cattle as compared to the female cattle that were deemed to produce future cows. This result was consistent with reports from other researchers showing significant correlation (p < 0.05) between cattle's sex and coccidiosis infection (Heidari & Gharekhani 2014). Previous studies done on cattle reported higher prevalence of *Eimeria* in female than in male cattle. Nevertheless, this could be attributed to the greater physiological stress experienced by female cattle in relation to pregnancies and breeding as compared to male cattle (Rehman *et al.* 2011; Dawid *et al.* 2012; Alemayehu *et al.* 2013; Heidari *et al.* 2014).

Results of this study showed that calves shed much higher numbers of oocysts than those of weaners and adults. For instance, the mean OPG for the calves was 537.0 (CI 95%; 231.8 - 842.2), whereas mean OPG for the weaners and adults were 154.1 (CI 95%; 79.8 - 228.3) and 22.5 (CI 95%; 11.0 - 33.9), respectively. Analysis showed that there were statistically significant differences in OPG levels among different age groups (p < 0.05). Subsequently, Dunn test as a multiple comparison test showed that the OPG values for adult cattle were found to be significantly correlated (p < 0.05) to the weaners and calves, respectively. However, the weaners did not show significant effect (p > 0.05) to the calves in OPG levels caused by coccidiosis (Table 5).

Age is a major risk factor in coccidiosis spreading, while morbidity and risks of infections are greater in calves (Abebe *et al.* 2008) compared to other age groups. Calves had significantly higher oocysts counts (p < 0.05) than adults. These results were in agreement with Waruiru *et al.* (2000) in Kenya and Dong *et al.* (2012) in Shanghai, China who demonstrated that age strongly influenced the intensity of *Eimeria* OPG counts in cattle.

Coccidiosis is a self-limiting disease. Spontaneus recovery without specific treatment is common when multiplication stage of the coccidia has passed, which suggests that previous exposure may have contributed to the

development of a certain immunity level in the older cattle as compared to the younger ones that did not have previous exposure (Dong *et al.* 2012; Heidari *et al.* 2014; Kocis *et al.* 2015).

This study showed that weaners shed *Eimeria* oocysts with OPG counts of 154.1 (CI 95%; 79.8 - 228.3). Mean OPG for the weaners were lower than that for the calves ($p > 0.05$). In this study, range of OPG in calves and weaners were 0 - 24,450 and 0 - 900, respectively. OPG values over 5,000 indicate a clinical case (Arslan & Tuzer 1998). Results of *Eimeria* oocysts counts for calves and weaners in this study were not different from previous study by Bruhn *et al.* (2012) which reported that no difference was observed ($p > 0.05$) in EPG and OPG counts between calves in the pre-weaning and post-weaning phases.

Most cattle examined during this study had low OPG counts, suggesting that the infections were usually subclinical. This result concurred with other cross-sectional observational studies on *Eimeria* spp. in Iran, which also did not observe cases of clinical cases of clinical coccidiosis among infected cattle, probably due to low quantities of oocysts eliminated in the cattle's feces (Heidari *et al.* 2014; Heidari & Gharekhani 2014).

Level of *Eimeria* oocyst shedding was significantly depended on different types of pen flooring ($p < 0.05$). When the types of flooring were compared, cattle kept on straw flooring exhibited lower OPG values (53.9; CI 95%: 0.0 - 162.4) than those kept on on wood flooring (703.6; CI 95%: 0.0 – 1,450.2) and on rubber flooring (405.7; CI 95%: 116.9 - 694.6) ($p < 0.05$). However, straw flooring did not show significant difference on OPG counts ($p > 0.05$) compared to cement flooring. Moreover, wood flooring did not show significant difference on OPG counts compared to rubber flooring ($p > 0.05$). Yet, *Eimeria* OPG counts was higher ($p < 0.05$) on wood flooring compared to cement flooring. Higher *Eimeria* oocysts count was recorded in cattle reared on rubber flooring compared to cement flooring ($p < 0.05$) (Table 5).

This finding was in agreement with a study by Bangoura *et al.* (2011) who described a significant association between different flooring types used for rearing cattle and the level of *Eimeria* oocysts shedding ($p < 0.05$). Rehman *et al.* (2011) stated that *Eimeria* infection was more prevalent ($p < 0.05$) in non-cement flooring type compared to partially cement flooring type. Lower *Eimeria* prevalence in cattle kept on cement flooring may be caused by the easiness of cleaning and disinfecting cement flooring, which resulted to less contaminated floor compared to non-cement floor.

In this study, the lowest level of *Eimeria* oocysts occurred in cattle kept on straw flooring than those kept on other flooring types, because solid flooring types (wood, rubber and cement) are dirtier than straw floors if the solid flooring types are not scraped and cleaned properly. Straw flooring type is usually temporarily used by calves aged 0 to 1 month to keep the calves warm. Daily removal and replacement of straw flooring with a new set of straws resulted in lower *Eimeria* OPG counts and reduced the occurence of *Eimeria* oocyst shedding. Previous studies concluded that cleaning was an important factor in preventing high counts of *Eimeria* oocysts (Bangoura *et al.* 2011; Rehman *et al.* 2011).

Clinical coccidiosis was observed in cattle pens having insufficient aeration leading to high concentration of ammonia, CO_2 and moisture. Also, clinical coccidiosis occurred in cattle pens where feces accumulated on the ground due to unsuited slatted floors (Daughschies & Najdrowski 2005). Local temperature, humidity, ammonia concentration and pH on pen flooring affected oocysts' survival (Gulliksen *et al.* 2009; Bangoura *et al.* 2011).

In intensive rearing having high population density, disease transmission is more readily occurred and oocysts are highly available within the environment. Thus, in dairy calves, coccidiosis occurs more frequently and appears with greater severity (Bruhn *et al.* 2011). It can be assumed that the cattle living in a group will be infected at the same time, or at around the same time, under field condition. Moreover, continuous oocysts shedding from subclinical infected calves contaminate the environment and the cattle's hair causing severe coccidiosis in highly susceptible new calves that are kept in these areas (Abebe *et al.* 2008; Alemayehu *et al.* 2013). Different hygiene conditions and farm management, animal breeding, study designs and methods, climates and different geographical regions may be the main cause of varied results (Yu *et al.* 2011).

CONCLUSIONS

E. bovis was the highest prevalent *Eimeria* species and had the highest level of mean OPG compared to other *Eimeria* spesies. Mixed infections in single cattle with 2 – 5 *Eimeria* species were commonly observed. Several species of *Eimeria* in cattle are found in other tropical areas. Various categorical factors (cattle's sex, age and type of pen flooring) influenced the number of OPG found in fecal samples. *Eimeria* has significant pathogenic potential. It is important to control the occurrence of *Eimeria*.

ACKNOWLEDGEMENTS

The authors thank all members of Protozoology Laboratory, Faculty of Veterinary Medicine, Institut Pertanian Bogor for facilitating this study with the necessary equipment.

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