



# Epidemiological Survey on *Eimeria* spp. Associated with Diarrhea in Pre-weaned Native Korean Calves

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**Abstract:** Bovine coccidiosis is one of the most important parasitic diseases affecting calf productivity. Here, we investigated the prevalence of *Eimeria* spp. in pre-weaned native Korean calves and determined the correlation between diarrhea and *Eimeria* spp. Fecal samples were collected from individual calves (288 normal and 191 diarrheic) in 6 different farms. Of the 479 samples, *Eimeria* oocysts were detected in 124 calves (25.9%). Five *Eimeria* spp. were identified; *E. zuernii* (18.8%) was the most prevalent, followed by *E. auburnensis* (12.5%), *E. bovis* (7.5%), *E. subspherica* (5.8%), and *E. bukidnonensis* (1.0%). A significant correlation was observed between diarrhea and mixed infection with more than 2 *Eimeria* spp. (odds ratio [OR]=2.21; 95% confidence interval [CI]: 1.09-4.49;  $P=0.03$ ) compared to single infection (OR=1.29; 95% CI: 0.77-2.15;  $P=0.33$ ). Of the 5 *Eimeria* spp. identified, *E. subspherica* (95% CI: 1.24-5.61;  $P=0.01$ ) and *E. bukidnonensis* (95% CI: 825.08-1,134.25;  $P=0.00$ ) strongly increased the risk of diarrhea by 2.64-fold and 967.39-fold, respectively, compared to other species. Moreover, mixed infection with *E. auburnensis* and *E. bukidnonensis* was significantly associated with diarrhea (OR=2,388.48; 95% CI: 1,009.71-5,650.00;  $P<0.00$ ) in pre-weaned native Korean calves. This is the first report to demonstrate the importance of *E. bukidnonensis* associated with diarrhea in pre-weaned native Korean calves. Further epidemiological studies should investigate the prevalence of *E. bukidnonensis* and the association between *E. bukidnonensis* and diarrhea.

**Key words:** Coccidiosis, *Eimeria*, pre-weaned calves, diarrhea

Bovine coccidiosis is caused by *Eimeria* spp. and is one of the most important parasitic diseases, leading to substantial economic losses in the livestock industry worldwide. Of more than 20 *Eimeria* species, the most prevalent species are *E. bovis*, *E. zuernii*, and *E. auburnensis* [1,2]. In particular, *E. bovis* and *E. zuernii* are highly pathogenic and can cause mortality and morbidity by disturbing absorption mechanisms in calves and young animals [3-5]. The clinical manifestation of coccidiosis in calves is characterized by watery to hemorrhagic diarrhea, abdominal pain, fever, dehydration, malnutrition, anorexia,

and weight loss [4,6-9], while in adult cattle, coccidiosis occurs in subclinical forms [10]. Coccidiosis infections with more than one species of *Eimeria* are generally observed in the field; however, single species infections have been reported in some cases [8,11].

In cattle, *Eimeria* spp. are transmitted via the fecal-oral route and disease incidence is associated with several factors such as animal age; fecal contamination of feed, water, or soil; housing conditions (indoor or grazing); overcrowding; and poor hygiene [1,12,13]. Coccidiosis mainly causes problems in young animals, especially between 4 to 7 weeks after birth, and infected calves can spread the infection to other animals through feces containing infective oocysts in the environment and are also more susceptible to secondary bacterial and viral infections [13,14]. Despite the importance of coccidiosis, awareness of this disease is relatively very low in the Republic

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of Korea (ROK). Currently, there is little information available regarding *Eimeria* spp. circulating in cattle in the ROK. Therefore, the objective of this study was to examine the prevalence of *Eimeria* spp. in pre-weaned native Korean calves and to determine the association between diarrhea and the *Eimeria* spp. identified in this study.

All pre-weaned native Korean calves used in this study were sampled with the approval of the animal ethics and welfare committee of Chonbuk National University (approval number 2017-00026). Consent was obtained from the participating cattle owners.

A total of 479 fecal samples (191 diarrheic samples and 288 normal feces) were individually collected from pre-weaned native Korean calves (1-60 days) raised in 6 different locations (Gimje, Samrye, Youngju, Sangju, Mungyung, and Asan) in the central region of the ROK, between April and November 2017. Each animal's identification number, age, gender, and the number of animals in its herd were recorded. The fecal samples were directly collected from the rectum by practicing veterinarians and then suspended in a solution of 2.5% potassium dichromate and transported to the laboratory for diagnosis. All fecal samples were recorded and classified as normal, pasty, watery, or hemorrhagic, according to their physical characteristics.

Fecal samples were analyzed for the presence of oocysts using the flotation technique with Sheather's solution (saturated sugar solution; specific gravity 1.28) and examined microscopically ( $\times 400$  magnification) for parasitological objects. Identification of *Eimeria* species was based on the morphological features of the oocysts (including size, shape, color, and texture of the oocyst wall and the presence or absence of a micropyle and polar cap) [15,16].

Statistical analysis was performed to assess the association between diarrhea and the following variables: *Eimeria* infection, *Eimeria* spp. single- or mixed infection. Binary univariate logistic regression models were constructed using the generalized linear mixed model (GLMM) in SPSS 24.0 software package (SPSS, Chicago, Illinois, USA), with farm as a random ef-

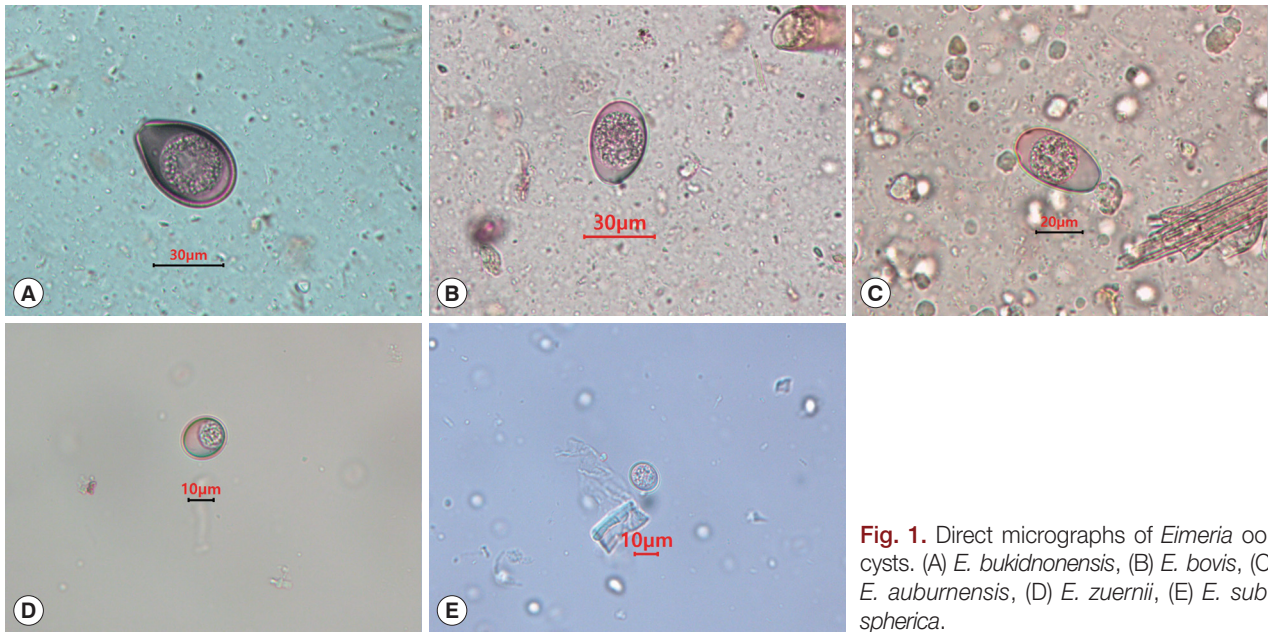
fect. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the likelihood of association. A value of  $P < 0.05$  was considered significant.

Of a total of 479 fecal samples, *Eimeria* oocysts were detected in 124 fecal samples (25.9%, 124/479). As shown in Table 1, *Eimeria* infection was detected at a relatively higher level in diarrheic feces ( $P = 0.03$ ) than in normal feces, indicating that *Eimeria* infection was significantly associated with diarrhea. *Eimeria* infection increased the occurrence of diarrhea by 1.76-fold compared with non-infection (OR = 1.76; 95% CI: 1.06-2.91;  $P = 0.03$ ). When comparing the relationship between *Eimeria* spp. single- and mixed infection and diarrhea, no association was observed between *Eimeria* single infection and diarrhea ( $P = 0.33$ ); however, the occurrence of diarrhea was significantly increased by 2.21-fold in mixed infections compared to single infection (95% CI: 1.09-4.49;  $P = 0.03$ ; Table 1). These results suggest that the occurrence of diarrhea observed in pre-weaned native Korean calves is closely associated with mixed infections involving more than 1 species of *Eimeria*, but not with single infection. As *Eimeria* infection was not detected in fecal samples from calves with diarrhea, the possibility of infections with other pathogens, such as bacteria, viruses, and protozoa (*Cryptosporidium* and *Giardia*), cannot be ruled out.

In this study, 5 *Eimeria* spp. were identified based on morphological criteria (Fig. 1). *E. bukidnonensis* has a micropyle and is the largest of the *Eimeria* spp. (Fig. 1A). The oocyst is over 40  $\mu\text{m}$  in size, has a very distinct dark brown color, and is very thick, thus making it easy to distinguish it from other species. *E. bovis* has microtubules and a smooth and homogeneous oocyst wall. It is commonly found in cattle in the ROK and has a typical ovoidal shape (Fig. 1B). The size of the oocyst is approximately  $\geq 30 \mu\text{m}$ . *E. auburnensis* has a micropyle and is yellowish-brown in color. It has an elongated shape and is  $\geq 35 \mu\text{m}$  in size (Fig. 1C). There is a lot of space on both sides of the oocyst because the zygote is nearly in the center. *E. zuernii* has no micropyle and is the most common *Eimeria* species in cattle. Its size ranges between 15-20  $\mu\text{m}$  (Fig. 1D); slightly larger than *E. subspherica*. Moreover, as the zygote exists

**Table 1.** Association between *Eimeria* spp. infection and diarrhea determined using the logistic regression models with random farm effect

Variable	Category	No. examined (n=479)	No. of diarrheic feces (n=191)	No. of normal feces (n=288)	P-value	OR (95% CI)
<i>Eimeria</i> infection (%)	No (Reference)	355 (74.1)	132 (69.1)	223 (77.4)	-	-
	Yes	124 (25.9)	59 (30.9)	65 (22.6)	0.03	1.76 (1.06-2.92)
	Single infection	54 (11.3)	22 (11.5)	32 (11.2)	0.33	1.29 (0.77-2.15)
	Mixed-infection	70 (14.6)	37 (19.4)	33 (11.5)	0.03	2.21 (1.09-4.49)



**Fig. 1.** Direct micrographs of *Eimeria* oocysts. (A) *E. bukidnonensis*, (B) *E. bovis*, (C) *E. auburnensis*, (D) *E. zuernii*, (E) *E. subspherica*.

**Table 2.** Association between the different *Eimeria* infections and diarrhea determined by the logistic regression models with random farm effect

<i>Eimeria</i> species	Total No. (n=479)	No. of diarrheic feces (n=191)	No. of normal feces (n=288)	P-value	OR (95% CI)
<i>E. zuernii</i> (total)	90 (18.8)	42 (22.0)	48 (16.7)	0.10	1.76 (0.89-3.46)
<i>E. auburnensis</i> (total)	60 (12.5)	26 (13.6)	34 (11.8)	0.35	1.22 (0.80-1.87)
<i>E. bovis</i> (total)	36 (7.5)	19 (9.9)	17 (5.9)	0.14	2.12 (0.78-5.76)
<i>E. subspherica</i> (total)	28 (5.8)	16 (8.4)	12 (4.2)	0.01	2.64 (1.24-5.61)
<i>E. bukidnonensis</i> (total)	5 (1.0)	5 (2.6)	0 (0)	0.00	967.39 (825.08-1,134.25)

only in part of the oocyst, there is a lot of space. *E. subspherica* is the smallest and is approximately 10  $\mu\text{m}$  in size (Fig. 1E). It does not possess a micropyle, the oocyst wall is thin, and the zygotes nearly fill the interior.

According to our results, *E. zuernii* (18.8%) was the most frequently detected species, followed by *E. auburnensis* (12.5%), *E. bovis* (7.5%), *E. subspherica* (5.8%), and *E. bukidnonensis* (1.0%). *E. wyomingensis* was not identified in this study. Although the infection rates of *E. subspherica* and *E. bukidnonensis* were relatively low compared to other species, both showed a significant correlation with diarrhea in pre-weaned native Korean calves ( $P=0.01$  for *E. subspherica* and  $P=0.00$  for *E. bukidnonensis*; Table 2). In addition, the occurrence of diarrhea was strongly increased in all animals infected with *E. subspherica* (OR=2.64; 95% CI: 1.24-5.61;  $P=0.01$ ) and *E. bukidnonensis* (OR=967.39; 95% CI: 825.08-1,134.25;  $P=0.00$ ) compared to those not infected with *E. subspherica* and *E. bukidnonensis*, respectively. However, we could not determine for certain whether the origi-

nal infection rate of *E. subspherica* and *E. bukidnonensis* was low or whether the positive rate was low because of the small number of samples collected. Further studies are thus necessary to investigate the prevalence of *E. subspherica* and *E. bukidnonensis* through a larger epidemiological survey.

The mixed infection patterns associated with diarrhea were also analyzed. Although *E. bovis* was not associated with diarrhea as a single infection, it demonstrated a significant relationship with diarrhea in mixed infections with *E. subspherica*, *E. zuernii*, and *E. auburnensis* ( $P<0.00$ ). Similar to *E. bovis*, *E. zuernii* did not cause diarrhea as a single infection; however, a mixed infection of *E. zuernii* with *E. auburnensis*; *E. subspherica*; or *E. bovis*, *E. auburnensis*, and *E. subspherica* significantly increased the occurrence of diarrhea (2.5-fold and 5.1-fold, respectively) compared with *E. zuernii* single infection (Table 3). Moreover, mixed infection with *E. auburnensis* and *E. bukidnonensis* was strongly associated with diarrhea (OR=2,388.48; 95% CI: 1,009.71-5,650.00;  $P<0.00$ ; Table 3). Importantly, to

**Table 3.** Concurrent *Eimeria* spp. significantly associated with diarrheic feces determined by the logistic regression model with random farm effect

Reference	Concurrent <i>Eimeria</i> spp.	P-value	OR (95% CI)
<i>E. bovis</i>	<i>E. subspherica</i>	<0.00	Not tested
	<i>E. zuernii</i> + <i>E. auburnensis</i> + <i>E. subspherica</i>	<0.00	Not tested
<i>E. zuernii</i>	<i>E. auburnensis</i> + <i>E. subspherica</i>	0.01	2.46 (1.28-4.74)
	<i>E. bovis</i> + <i>E. auburnensis</i> + <i>E. subspherica</i>	<0.00	5.10 (2.40-10.82)
<i>E. auburnensis</i>	<i>E. bukidnonensis</i>	<0.00	2,388.48 (1,009.71-5,650.00)

date, there have been no reports that mixed infection with *E. auburnensis* and *E. bukidnonensis* is associated with diarrhea. Taken together, we conclude that although *Eimeria* is not related to the occurrence of diarrhea in pre-weaned calves as a single infection, mixed infection with more than 2 *Eimeria* spp. appears to have an important effect on the occurrence of diarrhea. Consequently, of the 5 *Eimeria* spp. identified in the present study, our results suggest that mixed infections with *E. auburnensis* and *E. bukidnonensis* are significantly associated with the occurrence of diarrhea in pre-weaned native Korean calves and that the impact of *Eimeria* infection on calf diarrhea cannot be ignored.

In the present study, the overall prevalence of *Eimeria* was 25.9% in pre-weaned native Korean calves regardless of diarrhea. The prevalence of *Eimeria* spp. in this study was lower than previously reported [1,2,4,6,17]. These differences may be attributed to calf age, breed, the sampling time, and geographical location. The risk of *Eimeria* infection increases until 3 months of age; however, problems are most common in young animals (3 weeks to 6 months) [9]. Therefore, further studies are necessary to investigate the prevalence of *Eimeria* in calves of various ages.

Several studies have shown that *E. zuernii* and *E. bovis* are the most prevalent species known to cause clinical coccidiosis in calves [1,6,10,18,19]. However, our results demonstrate that *E. bovis* was detected less frequently than *E. auburnensis* and that both *E. zuernii* and *E. bovis* were not associated with diarrhea ( $P=0.1$  and  $P=0.14$ , respectively), while *E. bukidnonensis* was significantly associated with diarrhea in pre-weaned native Korean calves. To date, there have been no reports of *E. bukidnonensis* causing diarrhea in calves. Based on our results, *E. bukidnonensis* is strongly associated with diarrhea in mixed infection with *E. auburnensis* ( $P<0.00$ ), as well as a single infection ( $P=0.00$ ). To the best of our knowledge, this is the first study to demonstrate that *E. bukidnonensis* is associated with diarrhea. Although mixed infection with *E. bukidnonensis* and *E. subspherica* was not observed in the present study, it may play an

important role in causing diarrhea in pre-weaned calves, as *E. bukidnonensis* and *E. subspherica* were strongly associated with diarrhea as a single infection.

In conclusion, the present study identified the 5 *Eimeria* spp. in pre-weaned native Korean calves and showed that *Eimeria* appears to strongly cause diarrhea in mixed infections with 2 or more species ( $\geq 2$ ), but not in single infection. Contrary to previous studies, *E. zuernii* and *E. bovis* were not related to diarrhea. Further studies are necessary to identify the importance of *Eimeria* infection in calf diarrhea in the ROK via epidemiological surveys and to investigate other factors affecting the development of coccidiosis.

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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