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PROTOZOOLOGY - ORIGINAL PAPER

Prevalence and molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned native calves in the Republic of Korea

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Abstract

Cryptosporidium spp. and *Giardia duodenalis* are protozoan parasites that cause diarrhea in humans and animals. Molecular data on *Cryptosporidium* spp. and *G. duodenalis* in calves in the Republic of Korea (ROK) is limited; thus, we investigated the prevalence of *Cryptosporidium* and *Giardia* in pre-weaned calves, analyzed the association between these parasites and diarrhea, and identified their zoonotic potential for human infection. Fecal samples were collected from 315 pre-weaned calves aged 1–60 days from 10 different regions in the ROK and screened for *Cryptosporidium* spp. and *G. duodenalis* using PCR. Overall prevalence of *Cryptosporidium* spp. and *G. duodenalis* was 4.4% (n = 14) and 12.7% (n = 40), respectively. Co-infection was not detected. All *Cryptosporidium*-positive samples were identified as *C. parvum* after sequence analysis of a small subunit rRNA fragment and further subtyped into zoonotic IIaA15G2R1 (n = 13) and IIaA18G3R1 (n = 1) by DNA sequencing of the 60-kDa glycoprotein gene. To our knowledge, this is the first report of *C. parvum* IIaA15G2R1 subtype in calves in the ROK. Based on β-giardin (*bg*) gene, *G. duodenalis*–positive samples belonged to assemblages E (n = 36) and A (n = 4), with the latter belonging to subtype A1, the zoonotic genotype. Six subtypes of assemblage E were identified at the *bg* locus: E1 (n = 6), E2 (n = 3), E3 (n = 13), E5 (n = 1), E8 (n = 1), and E11 (n = 1). The occurrence of *C. parvum* and *G. duodenalis* was not associated with diarrhea in pre-weaned Korean native calves. The present results suggest that the prevalence of *C. parvum* is not related to calf age; in contrast, the prevalence of *G. duodenalis* was significantly higher in 41–50-day-old calves (odds ratio = 9.90, 95% confidence interval 2.37–41.34; P = 0.001) than in 1–10-day-old calves. Therefore, calves are a potential source of zoonotic transmission, which may have significant public health implications.

Keywords *Cryptosporidium parvum* · *Giardia duodenalis* · Pre-weaned calves · Zoonotic potential

Introduction

Cryptosporidium and *Giardia* are important protozoan parasites that cause diarrhea and other clinical symptoms in humans and numerous animals, including livestock, companion animals, and wild mammals (Huang et al. 2014). Infections with these pathogens occur via the fecal-oral route, by either direct or indirect contact with infected individuals or by the ingestion of infective oocysts or cysts from contaminated water or food (Feng and Xiao 2011; Xiao 2010). *Cryptosporidium* and *Giardia* have attracted attention as the major cause of human infections (Maddox-Hyttel et al. 2006; Monis and Thompson 2003). Cattle, especially calves, may be considered the most important reservoir of zoonotic infections (Fan et al. 2017). *Cryptosporidium* and *Giardia* infections in these animals could lead to significant economic losses owing to high morbidity and mortality (Olson et al. 2004).

To date, four *Cryptosporidium* species, namely *C. andersoni*, *C. bovis*, *C. parvum*, and *C. ryanae*, have been identified as the most common and frequently occurring in cattle (Slapeta 2013), and the distribution of these species varies with age (Xiao 2010). *Cryptosporidium parvum* is usually found in pre-weaned calves and is the main pathogenic species that affects humans in certain countries (Snel et al. 2009; King et al. 2019); on the other hand, *C. bovis* and *C. ryanae* usually infect post-weaned calves and

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yearlings, and *C. andersoni* is mainly observed in adult cattle (Santin et al. 2004; Zhao et al. 2013). Based on sequence analysis using the 60-kDa glycoprotein (*gp*60) gene, IIa and IId subtypes of *C. parvum* have been found in both humans and calves and can cause zoonotic cryptosporidiosis (Avendano et al. 2018; Chalmers and Giles 2010). Of the IIa family members, IIaA15G2R1 is most commonly reported as the major zoonotic subtype in cattle (Ryan et al. 2014). Other IIa subtypes such as IIaA16G1R1 and IIaA18G3R1 have different distributions according to region (Xiao 2010).

*Giardia duodenalis*consists of eight distinct assemblages, A–H(Feng and Xiao 2011). Among these, assemblages A and B can infect both humans and other animals including companion animals, livestock, and wild mammals, whereas the remaining assemblages (C–H) appear to be more host-specific, with assemblages C and D observed in dogs and other canids, assemblage E mainly observed in ungulates including cattle, sheep, and goats, assemblages F and G observed in cats and rodents, and assemblage H observed in marine mammals (Ryan and Caccio 2013). In particular, assemblages A, B, and E have been detected in cattle, with assemblage E being predominant in many countries (Feng and Xiao 2011; Li et al. 2016). Recently, several studies have also identified assemblage E in humans (Abdel-Moein and Saeed 2016; Zahedi et al. 2017). There is no difference in the distribution of *G. duodenalis* assemblage among cattle of different age groups (Naguib et al. 2018).

Despite the threat of cryptosporidiosis and giardiasis to humans and livestock, the importance of these diseases (particularly in cattle) is often overlooked, and information on these diseases is limited in the Republic of Korea (ROK). Recently, our group revealed the presence of assemblages A and E as well as *G. duodenalis* in pre-weaned Korean native calves. Moreover, assemblage A has been detected in calves with normal feces (Lee et al. 2018). Only one recent study on the molecular epidemiology of *Cryptosporidium* spp. in the ROK has identified the presence of *C. parvum*, *C. bovis*, and *C. ryanae* in young calves with diarrhea (Lee et al. 2016b). Thus far, little is known about the subtype and genotype of *C. parvum* and *G. duodenalis* among the calves in the ROK (Lee et al. 2016a, b, 2018). Therefore, this study aimed to investigate the prevalence of *Cryptosporidium* and *Giardia* in pre-weaned calves, analyze the association between these parasites and diarrhea, and identify the zoonotic potential of *C. parvum* and *G. duodenalis* subtypes/assemblages.

Materials and methods

Sample collection

Between January and October 2018, 315 fecal samples were collected directly from the rectum of pre-weaned Korean native calves aged 1–60 days from 10 different farms (Anseong, Geochang, Gimje, Gyeongju, Jeongup, Mungyeong, Naju, Sangju, Yecheon, and Yeongju) in the ROK. The collected samples were placed into a disposable plastic bag and immediately transported to the laboratory for subsequent DNA isolation and molecular testing. The fecal consistency of each calf was categorized as normal or diarrheic according to its physical characteristics. For each animal, the sampling date, age, sex, fecal consistency, farm location, and farm management system were recorded.

DNA extraction and PCR amplification

Genomic DNA was extracted from 200 mg of each fecal sample using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations and subsequently frozen at – 20 °C until use. Small subunit (SSU) rRNA gene was used to identify *Cryptosporidium* spp. (Xiao et al. 1999). *C. parvum* was subtyped by targeting the 60-kDa glycoprotein (*gp60*) gene using nested PCR (Alves et al. 2003). *G. duodenalis* assemblage types were analyzed using the triose phosphate isomerase (*tpi*) gene (Sulaiman et al. 2003), β-giardin (*bg*) gene (Caccio et al. 2002; Lalle et al. 2005), and glutamate dehydrogenase (*gdh*) gene (Read et al. 2004). In this study, only samples showing a good sequencing result were considered to be positive for *C. parvum* and *G. duodenalis*.

Nucleotide sequencing and phylogenetic analysis

All secondary PCR products were purified using the AccuPower PCR Purification Kit (Bioneer, Daejeon, ROK) and used for direct sequencing (Macrogen, Daejeon, ROK). The nucleotide sequences obtained in this study were analyzed using BioEdit (version 7.2.5) and the Basic Local Alignment Search Tool available from the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). To determine the subtype and genotype of *C. parvum* and *G. duodenalis*, nucleotide sequences were aligned using ClustalX and analyzed by performing a direct comparison with reference sequences from GenBank. A phylogenetic tree was constructed based on nucleotide alignments by employing the maximum-likelihood method. Bootstrap analysis was conducted with 1000 replicates using MEGA version 7 software (Kumar et al. 2016). Nucleotide sequences obtained in this study were submitted to the GenBank database under the accession numbers MK732491–MK732504 and MK720232–MK720271 for *C. parvum* and *G. duodenalis*, respectively.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 25 software package for Windows (SPSS Inc., Chicago, IL,

USA). Chi-square (χ^2) and Fisher's exact tests were used to compare the infection rates of *C. parvum* and *G. duodenalis*. In addition, the association between prevalence and specific variables (age and fecal consistency) was determined using binary univariate logistic regression models. Odds ratio (OR) with 95% confidence interval (CI) was calculated to assess the likelihood of association. A P value of ≤ 0.05 was considered to be statistically significant.

Results

Prevalence of C. parvum and G. duodenalis

The overall prevalence of *C. parvum* and *G. duodenalis* was 4.4% (14/315) and 12.7% (40/315), respectively. Co-infection with *C. parvum* and *G. duodenalis* was not detected in these calves. Among the 10 farms examined, *C. parvum* was detected in only three farms (Geochang, Gimje, and Gyeongju), whereas *G. duodenalis* was detected in all but two farms (Jeongup and Naju) (Table 1). Interestingly, a mixed infection with both assemblages A and E was found in two farms (Table 1). Infection with *C. parvum* (χ^2 = 0.004, P = 0.952) and *G. duodenalis* (χ^2 = 0.053, P = 0.817) was not associated with diarrhea in pre-weaned Korean native calves (Table 2). *C. parvum– and G. duodenalis*–positive samples were compared according to different age groups of the calves. As shown in Table 2, infection with *C. parvum* was observed only in calves aged ≤ 20 days. The prevalence of *C. parvum* was the highest in calves aged 11–20 days, but these differences were not significant (Table 2). On the contrary, the percentage of positive calves to *G. duodenalis* was significantly highest in calves aged 41–50 days (Table 2). Thus, the risk of being positive to *G. duodenalis* was 9.9- and 4.4-fold higher in calves aged 41–50 days (95% CI: 2.37–41.34; P = 0.00) and 31–40 days (95% CI: 0.70–7.77; P = 0.03) respectively, than in those aged 1–10 days (Table 2).

Distribution of *C. parvum* subtypes

All *Cryptosporidium*-positive samples were identified as *C. parvum* using SSU rRNA sequence analysis. Based on *gp60* sequence analysis, the *C. parvum* isolates were identified to belong to the IIa family and were further subtyped into IIaA15G2R1 (n = 13) and IIaA18G3R1 (n = 1) (Table 3). IIaA15G2R1 was the predominant subtype in pre-weaned Korean native calves and was detected in both diarrheic and normal feces, whereas IIaA18G3R1 was only found in the feces of a 14-day-old calf with diarrhea. Thirteen isolates of IIaA15G2R1 showed 89.4–99.5% similarity with each other and were closely related to a human isolate (EF576979) obtained from the Netherlands. Our isolate belonging to IIaA18G3R1 showed 100% identity with another Korean isolate (KX342059).

Genotypes and subtypes of *G. duodenalis*

Sequence analysis of the *bg* gene revealed that two assemblages, A (n = 4) and E (n = 36), were identified (Fig. 1). All isolates belonging to assemblage A were identified as subtype A1 (Table 4) and detected in diarrheic feces. All nucleotide sequences were identical to those (MH104712 and MH104713) obtained from healthy calves previously identified by our group. Of the 36 assemblage E isolates, 17 and 19

Table 1 Prevalence of *Cryptosporidium parvum* and *Giardia duodenalis* in pre-weaned Korean native calves

Farm	Sample number	*Cryptosporidium parvum*		*Giardia duodenalis*	
		No. of positive (%)	subtype	No. of positive (%)	Assemblage
Mungyeong	83	0		14 (16.9%)	Assemblage A (1) Assemblage E (13)
Yeongju	33	0		6 (18.2%)	Assemblage E (6)
Sangju	2	0		1 (50%)	Assemblage E (1)
Yecheon	1	0		1 (100%)	Assemblage A (1)
Gimje	71	7 (9.9%)	IIaA15G2R1, IIaA18G3R1	3 (4.2%)	Assemblage E (3)
Geochang	78	5 (6.4%)	IIaA15G2R1	8 (10.3%)	Assemblage E (8)
Naju	1	0		0	
Jeongup	1	0		0	
Gyeongju	6	2 (33.3%)	IIaA15G2R1	1 (16.7%)	Assemblage E (1)
Anseong	39	0		6 (15.4%)	Assemblage E (4) Assemblage A (1)
Total	315	14 (4.4%)		40 (12.7%)	

Table 2 Prevalence of *Cryptosporidium parvum* and *Giardia duodenalis* considering the age of the animals and the fecal consistency

Variables	Categories	Sample number	*Cryptosporidium parvum*				*Giardia duodenalis*			
			No. of positive (%)	OR	95% CI	*P* value	No. of positive (%)	OR	95% CI	*P* value
Diarrhea	No (Ref)	155	7 (4.5%)				18 (11.6%)			
	Yes	160	7 (4.4%)	0.97	0.3–2.8	0.952	21 (13.8%)	1.1	0.56–2.1	0.817
Age	1~10 (Ref)	70	5 (7.1%) *D, 5; *N, 0	1.00	–	–	4 (5.7%) D, 3; N, 1	1.00	–	–
	11~20	104	9 (8.7%) D, 2; N, 7	1.23	0.39–3.84	0.72	13 (12.5%) D, 5; N, 8	2.36	0.74–7.55	0.15
	21~30	81	0	0.00	–	0.99	10 (12.3%) D, 7; N, 3	2.32	0.10–7.77	0.17
	31~40	33	0	0.00	–	0.99	7 (21.2%) D, 4; N, 3	4.44	1.20–16.46	0.03
	41~50	16	0	0.00	–	0.99	6 (37.5%) D, 3; N, 3	9.90	2.37–41.34	0.00
	51~60	11	0	0.00	–	0.99	0	0.00	–	0.99

D, positive sample from diarrheic feces; *N*, positive sample from normal feces

isolates were identified from diarrheic and normal feces, respectively. Sequence analysis of the *tpi*, *gdh*, and *bg* genes revealed the genetic diversity within assemblage E. Among the 36 *G. duodenalis bg*-positive samples, the *tpi* and *gdh* loci were amplified and sequenced in 6 and 8 specimens, respectively. At the *bg* locus, the following six subtypes of assemblage E were observed: E1 (MK252651, $n = 16$), E2 (MK252652, $n = 4$), E3 (MK252653, $n = 13$), E5 (MK252649, $n = 1$), E8 (MK252650, $n = 1$), and E11 (KY769089, $n = 1$) (Table 4). Furthermore, at the *tpi* locus, the three subtypes, E1 (MF671900, $n = 2$), E3 (KT92259, $n = 3$), and E10 (KT710746, $n = 1$), were identified, whereas at the *gdh* locus, the three known subtypes, E1 (MK252654, $n = 4$), E3 (MK252657, $n = 3$), and E13 (MH621339, $n = 1$), were found. Altogether, two samples were successfully subtyped at all three genetic loci (Table 4). E1 was the most common subtype in pre-weaned Korean native calves (Table 4).

Discussion

In the present study, we assessed the prevalence of *Cryptosporidium* spp. and *G. duodenalis* among different age groups as well as the association with diarrhea in pre-weaned Korean native calves aged ≤ 60 days. The prevalence of *C. parvum* infection was lower than that of *G. duodenalis* infection. Recent studies have reported infection rates of 6.1% for *C. parvum* and 10–13.1% for *G. duodenalis* in young calves (< 3 months) in the ROK (Lee et al. 2016a, b, 2018). In this study, the prevalence of *C. parvum* was lower than that

Table 3 Distribution of *Cryptosporidium parvum* subtypes in pre-weaned Korean native calves identified in this study

Specimen	Subtype	Farm	Age (days)	Fecal consistency	GenBank accession number
GJHong	IIaA15G2R1	Gimje	7	Diarrhea	MK732491
GJSeok	IIaA18G3R1	Gimje	14	Diarrhea	MK732492
GJHong3	IIaA15G2R1	Gimje	20	Normal	MK732498
05GJSong	IIaA15G2R1	Gimje	3	Diarrhea	MK732499
08GJSong	IIaA15G2R1	Gimje	8	Diarrhea	MK732500
GJParkJK	IIaA15G2R1	Gimje	20	Normal	MK732501
GJParkSN	IIaA15G2R1	Gimje	10	Diarrhea	MK732502
GC2-1	IIaA15G2R1	Geochang	15	Normal	MK732593
GC2-5	IIaA15G2R1	Geochang	15	Normal	MK732594
GC2-6	IIaA15G2R1	Geochang	15	Normal	MK732595
GC2-8	IIaA15G2R1	Geochang	15	Normal	MK732596
GC2-9	IIaA15G2R1	Geochang	14	Normal	MK732597
GJ5	IIaA15G2R1	Gyeongju	12	Diarrhea	MK732503
GJ6	IIaA15G2R1	Gyeongju	9	Diarrhea	MK732504

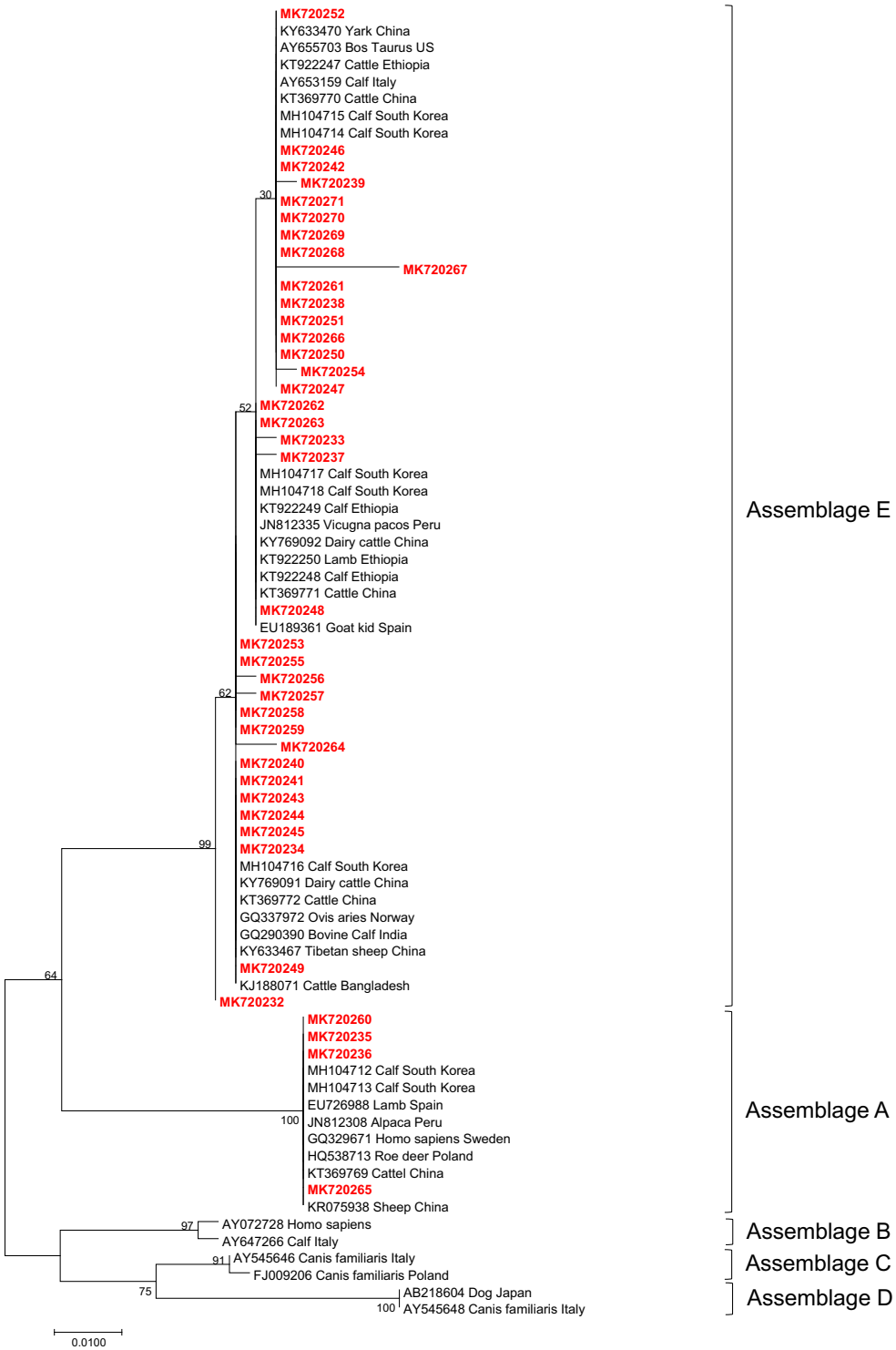

Fig. 1 Phylogenetic analysis based on the β-giardin gene of *Giardia duodenalis* reference strains/isolates and Korean isolates identified in this study. The phylogenetic tree was constructed using MEGA7 software by employing the neighbor-joining method. The numbers over branches indicate bootstrap values as a percentage of 1000 replicates that support each phylogenetic branch. The boldface type indicates the sequences determined in this study

reported in a previous study (Lee et al. 2016b). The differences between the two groups can be explained by the number of analyzed specimens, farm location, and season at the time of specimen collection. The percentage of *C. parvum* positive samples found in the present study was lower than that reported in several investigations (Smith et al. 2014; Ichikawa-Seki et al. 2015; Lee et al. 2016b; Naguib et al. 2018; Ouakli et al. 2018; Santoro et al. 2019). This may be due to low oocyst shedding when sampling, since oocyst excretion in calves is intermittent (Rieux et al. 2013; Santoro et al. 2019); so, resampling would allow us obtaining more accurate results. Moreover, we did not concentrate oocysts and did not apply freeze-thaw cycles before DNA extraction, resulting in lower DNA recovery and consequently decreasing PCR sensitivity.

Table 4 Multilocus sequence genotypes of *Giardia duodenalis* in pre-weaned Korean native calves identified in this study based on nucleotide sequence analysis of the beta-giardin (*bg*), triosephosphate isomerase (*tpi*), and glutamate dehydrogenase (*gdh*) genes

Specimen	Farm	Genotype		
		bg	*tpi*	*gdh*
0305MG33	Mungyeong	E1	E1	-
0326MG7		E1	-	-
0411MG2		E1	-	-
0411MG3		E3	-	-
0411MG4		E1	-	-
0411MG5		E3	-	-
0411MG7		E3	-	-
0411MG12		E3	-	-
0411MG13		E3	-	-
0411MG15		E3	-	-
0423MG5		A1	-	A1
0423MG6		E1	-	-
0428MG8		E2	-	-
0428MG12		E2	-	-
0313YJ1	Yeongju	E1	-	E1
0605YJ2		E1	-	-
0626YJ1		E1	-	E1
0828YJ1		E1	-	-
0828YJ3		E1	-	-
0912YJ1		E1	-	E1
SJ1	Sangju	E3	-	-
YC1	Yecheon	A1	-	A1
GJJung	Gimje	E1	E1	-
GJKim		E2	-	-
GJPark		E3	-	-
GC1-10	Geochang	E1	-	E3
GC8		E1	E3	E3
GC10		E3	E3	E1
GC4		E3	E3	-
GC5		E1	-	-
1001GC4		E3	-	-
1001GC5		E8	-	E13
GC17		E3	-	-
GYJ2	Gyeongju	E1	-	-
AS10	Anseong	E11	E10	-
AS12		E2	-	-
AS15		E3	-	E3
CW10		A1	-	A1
CW11		A1	-	-
CW15		E5	-	-

-, not detected

In this regard, previous studies have also reported that PCR analysis is less sensitive if the number of oocysts is less or if oocysts are not completely broken (Fayer et al. 2007; Mueller-Doblies et al. 2008). Additionally, co-infection with *C. parvum* and *G. duodenalis* was not detected; this might be attributed to the low prevalence of *C. parvum* in these calves. Of the four major *Cryptosporidium* species (*C. andersoni*, *C. bovis*, *C. parvum*, and *C. ryanae*) identified in cattle, only *C. parvum* was detected in pre-weaned Korean native calves (< 1 month). These results agree with previous studies revealing highest *C. parvum* prevalence in caves aged 1 month (Santin et al. 2004; Fayer et al. 2007; Thompson et al. 2007; Rieux et al. 2013; Ouakli et al. 2018). In this study, *C. parvum* was only identified in animals younger than 21 days, especially in 11–20-day-old calves; the absence of *C. parvum* ≥ 21-day-old calves could be attributed to the lower number of samples collected in these animals when compared with calves aged 1–20 days. Interestingly, *C. bovis* was not found in calves in the ROK, although several studies have shown that it is the most common species in pre-weaned dairy calves, especially healthy animals older than 4 weeks (Murakoshi et al. 2012; Rieux et al. 2013; Bjorkman et al. 2015; Feng et al. 2019). Thus, a recent study showed that *C. bovis* was mainly identified in calves older than 15 days (Ouakli et al. 2018). Nevertheless, it was also reported that the *Cryptosporidium* species detected in cattle farms could differ depending on the sampling year (Rieux et al. 2013). Therefore, further studies are warranted to investigate the prevalence of various *Cryptosporidium* species across different age groups and fecal consistency.

Based on our results, the prevalence of *G. duodenalis* was different according to the age of calves, indicating that *G. duodenalis* infection is related to age. The prevalence of *G. duodenalis* was significantly higher in calves aged 31–50 days, especially in those aged between 41 and 50 days ($P = 0.00$; Table 2). The results suggest that age could affect the occurrence of *G. duodenalis* in calves. The presence of *G. duodenalis* in the 80% of the studied farms indicates that this protozoan is endemic in cattle farms in the ROK; its infection may be explained by environmental contamination with cysts. According to recent studies, the highest occurrence of *G. duodenalis* was found in 2-month-old calves (Naguib et al. 2018; Feng et al. 2019). However, in the present study, *G. duodenalis* infection was not detected in calves aged 51-60 days. It is presumed that either the number of samples analyzed in this age group was too low or the infection rate of *G. duodenalis* was considerably low; consequently, *G. duodenalis* could not be detected by PCR. Similar to the approach adopted for *C. parvum*, we did not investigate the intensity of cyst shedding of *G. duodenalis* in calves. It is speculated that DNA was extracted directly from the feces without confirming the presence of cysts, which may affect the accurate diagnosis of *G. duodenalis*. These results suggest that microscopic examination should be performed for more accurate results. Therefore, it is necessary to conduct large-

scale epidemiological studies in the future to determine the association between age and *G. duodenalis* infection.

Our study showed that *C. parvum* and *G. duodenalis* were not associated with diarrhea. According to the present results, *C. parvum* was found only in diarrheic feces of 1-10-day-old calves, whereas it was commonly found in the normal feces of 11-20-day-old calves, indicating that *C. parvum* was present regardless of diarrhea status. *Cryptosporidium parvum* reportedly causes diarrhea in younger animals; however, our result is inconsistent with that reported in previous studies (Geurden et al. 2010; Blanchard 2012). In addition, it remains unclear whether *G. duodenalis* causes diarrhea in calves because its prevalence was found to increase only when the calves turned 31–50 days old and usually in calves of this age group, *G. duodenalis* is detected regardless of their diarrhea status. The detection of *C. parvum* and *G. duodenalis* in normal feces cannot rule out the possibility of contamination with oocysts/cysts in an entire herd. Although the number of these oocysts/cysts present in feces is not sufficient to cause diarrhea, calves in which the passive transfer of colostral antibodies fails to occur usually have poor health condition and might develop diarrhea more easily (Lora et al. 2018). Moreover, if the location where a calf is raised is not properly cleaned and has piled-up feces, the risk of diarrhea increases (Maddox-Hyttel et al. 2006). These calves may become reservoirs, and care for these animals should not be neglected. Further studies are necessary to investigate the importance of *C. parvum* and *G. duodenalis* as the primary diarrhea-causing pathogens in pre-weaned Korean native calves.

Two *C. parvum* subtypes (IIaA15G2R1 and IIaA18G3R1) belonging to the zoonotic subtype family IIa were identified based on sequence analysis of the *gp*60 gene. This is the first report of the IIaA15G2R1 subtype in pre-weaned calves in the ROK. The other IIaA18G3R1 subtype has previously been reported in calves in the ROK (Lee et al. 2016b), and it was identified in only one diarrheic calf in the present study. Furthermore, this subtype is geographically restricted and mostly found in the UK and Australia (Xiao 2010). This may explain its minor role in the dissemination of cryptosporidiosis in animals and humans. In the present study, IIaA15G2R1 was identified as the predominant subtype (92.9%) in the ROK and was found in calves with both diarrheic and normal feces. In industrialized nations, the IIaA15G2R1 subtype is a major zoonotic subtype found in calves and humans (Trotz-Williams et al. 2006; Xiao 2010; Rieux et al. 2014; Hijjawi et al. 2016); however, no *Cryptosporidium* sequences have been obtained from humans in the ROK. To date, there is limited information regarding the subtypes of *C. parvum* isolated from calves in the ROK (Lee et al. 2016b), and this can be attributed to the lack of information or interest in *C. parvum* despite its high zoonotic potential. The results of that study suggested that calves could be a potential source of zoonotic infections. Further molecular epidemiological studies are necessary to identify the genetic variability and zoonotic potential of *C. parvum* in both cattle and humans.

Here, *G. duodenalis* assemblages A and E were detected. This result is in agreement with that of previous studies conducted in other countries (Abdel-Moein and Saeed 2016; Asher et al. 2016; Wang et al. 2017). A previous study reported that *G. duodenalis* assemblage E may cause intestinal lesions, leading to calf scours (Barigye et al. 2008). In this study, the genetic diversity of assemblage E was observed in pre-weaned Korean native calves with the detection of 6, 3, and 3 subtypes at the *bg*, *gdh*, and *tpi* loci, respectively. The reason underlying the diversity of assemblage E remains unclear. Moreover, the presence of multiple subtypes in a single farm was observed. This occurrence may be the result of intra-assemblage genetic recombination. In contrast to a previous study (Lee et al. 2018), *G. duodenalis* assemblage A was identified in four calves with diarrhea. The four sequences were identical to each other and to the sequences of assemblage A isolates reported in our previous study as well as in studies conducted in other countries. Previous studies have reported the detection of assemblages A and E in humans (Abdel-Moein and Saeed 2016; Zahedi et al. 2017); however, assemblage E had not been detected in humans in the ROK. Our findings suggest that calves play a potential role in the transmission of human *G. duodenalis* infection. Further studies are necessary to monitor its zoonotic transmission.

In conclusion, the present study demonstrated that the prevalence of *G. duodenalis* was higher than that of *C. parvum* in pre-weaned Korean native calves and that the occurrence of *C. parvum* and *G. duodenalis* was not associated with diarrhea. The most common *C. parvum* and *G. duodenalis*subtype/assemblage in pre-weaned Korean native calves were the IIaA15G2R1 subtype and assemblage E, respectively. This is the first report on the IIaA15G2R1 subtype of *C. parvum* in calves in the ROK. These findings suggest that calves may be an important source of zoonotic *C. parvum* and *G. duodenalis* infections. Because cryptosporidiosis and giardiasis prevention is important for maintaining good health of calves and humans, the risk of diseases caused by these parasites should be reduced by minimizing the infection pressure resulting from contamination of environment with *C. parvum* and *G. duodenalis*oocysts/cysts and by improving the immunity of calves. Additional epidemiological studies are warranted to better understand the transmission and public health significance of *C. parvum* and *G. duodenalis* in the ROK.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics statement All procedures for fecal collection were carried out by an experienced veterinarian.

References

Abdel-Moein KA, Saeed H (2016) The zoonotic potential of *Giardia intestinalis* assemblage E in rural settings. Parasitol Res 115:3197–3202

Alves M, Xiao L, Sulaiman I, Lal AA, Matos O, Antunes F (2003) Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J Clin Microbiol 41:2744–2747

Asher AJ, Hose G, Power ML (2016) Giardiasis in NSW: identification of *Giardia duodenalis* assemblages contributing to human and cattle cases, and an epidemiological assessment of sporadic human giardiasis. Infect Genet Evol 44:157–161

Avendano C, Ramo A, Vergara-Castiblanco C, Sanchez-Acedo C, Quilez J (2018) Genetic uniqueness of *Cryptosporidium parvum* from dairy calves in Colombia. Parasitol Res 117:1317–1323

Barigye R, Dyer NW, Newell TK, Khaitsa ML, Trout JM, Santin M, Faye R (2008) Molecular and immunohistochemical detection of assemblage E, *Giardia duodenalis* in scouring North Dakota calves. Vet Parasitol 157:196–202

Bjorkman C, Lindstrom L, Oweson C, Ahola H, Troell K, Axen C (2015) *Cryptosporidium* infections in suckler herd beef calves. Parasitology 142:1108–1114

Blanchard PC (2012) Diagnostics of dairy and beef cattle diarrhea. Vet Clin N Am Food Anim Pract 28:443–464

Caccio SM, De Giacomo M, Pozio E (2002) Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. Int J Parasitol 32:1023–1030

Chalmers RM, Giles M (2010) Zoonotic cryptosporidiosis in the UK - challenges for control. J Appl Microbiol 109:1487–1497

Fan Y, Wang T, Koehler AV, Hu M, Gasser RB (2017) Molecular investigation of *Cryptosporidium* and *Giardia* in pre- and post-weaned calves in Hubei Province, China. Parasit Vectors 10:519

Fayer R, Santin M, Trout JM (2007) Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. Vet Parasitol 145:260–266

Feng Y, Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev 24:110–140

Feng Y, Gong X, Zhu K, Li N, Yu Z, Guo Y, Weng Y, Kvac M, Feng Y, Xiao L (2019) Prevalence and genotypic identification of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in pre-weaned dairy calves in Guangdong, China. Parasit Vectors 12:41

Geurden T, Vercruysse J, Claerebout E (2010) Is *Giardia* a significant pathogen in production animals? Exp Parasitol 124:98–106

Hijjawi N, Mukbel R, Yang R, Ryan U (2016) Genetic characterization of *Cryptosporidium* in animal and human isolates from Jordan. Vet Parasitol 228:116–120

Huang J, Yue D, Qi M, Wang R, Zhao J, Li J, Shi K, Wang M, Zhang L (2014) Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Ningxia, northwestern China. BMC Vet Res 10:292

Ichikawa-Seki M, Aita J, Masatani T, Suzuki M, Nitta Y, Tamayose G, Iso T, Suganuma K, Fujiwara T, Matsuyama K, Niikura T, Yokoyama N, Suzuki H, Yamakawa K, Inokuma H, Itagaki T, Zakimi S, Nishikawa Y (2015) Molecular characterization of *Cryptosporidium parvum* from two different Japanese prefectures, Okinawa and Hokkaido. Parasitol Int 64:161–166

King P, Tyler KM, Hunter PR (2019) Anthroponotic transmission of *Cryptosporidium parvum* predominates in countries with poorer sanitation: a systematic review and meta-analysis. Parasit Vectors 12:16

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio SM (2005) Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardiaduodenalis* and identification of potentially zoonotic subgenotypes. Int J Parasitol 35:207–213

Lee SH, VanBik D, Kim HY, Cho A, Kim JW, Byun JW, Oem JK, Oh SI, Kwak D (2016a) Prevalence and molecular characterisation of *Giardia duodenalis* in calves with diarrhoea. Vet Rec 178:633

Lee SH, VanBik D, Kim HY, Lee YR, Kim JW, Chae M, Oh SI, Goo YK, Kwon OD, Kwak D (2016b) Multilocus typing of *Cryptosporidium* spp. in young calves with diarrhea in Korea. Vet Parasitol 229:81–89

Lee YJ, Han DG, Ryu JH, Chae JB, Chae JS, Yu DH, Park J, Park BK, Kim HC, Choi KS (2018) Identification of zoonotic *Giardia duodenalis* in Korean native calves with normal feces. Parasitol Res 117:1969–1973

Li F, Wang H, Zhang Z, Li J, Wang C, Zhao J, Hu S, Wang R, Zhang L, Wang M (2016) Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Beijing, China. Vet Parasitol 219:61–65

Lora I, Gottardo F, Contiero B, Dall Ava B, Bonfanti L, Stefani A, Barberio A (2018) Association between passive immunity and health status of dairy calves under 30 days of age. Prev Vet Med 152:12–15

Maddox-Hyttel C, Langkjaer RB, Enemark HL, Vigre H (2006) *Cryptosporidium* and *Giardia* in different age groups of Danish cattle and pigs–occurrence and management associated risk factors. Vet Parasitol 141:48–59

Monis PT, Thompson RC (2003) *Cryptosporidiu*m and *Giardia*-zoonoses: fact or fiction? Infect Genet Evol 3:233–244

Mueller-Doblies D, Giles M, Elwin K, Smith RP, Clifton-Hadley FA, Chalmers RM (2008) Distribution of *Cryptosporidium* species in sheep in the UK. Vet Parasitol 154:214–219

Murakoshi F, Xiao L, Matsubara R, Sato R, Kato Y, Sasaki T, Fukuda Y, Tada C, Nakai Y (2012) Molecular characterization of *Cryptosporidium* spp. in grazing beef cattle in Japan. Vet Parasitol 187:123–128

Naguib D, El-Gohary AH, Mohamed AA, Roellig DM, Arafat N, Xiao L (2018) Age patterns of *Cryptosporidium* species and *Giardia duodenalis* in dairy calves in Egypt. Parasitol Int 67:736–741

Olson ME, O'Handley RM, Ralston BJ, McAllister TA, Thompson RC (2004) Update on *Cryptosporidium* and *Giardia* infections in cattle. Trends Parasitol 20:185–191

Ouakli N, Belkhiri A, de Lucio A, Koster PC, Djoudi M, Dadda A, Khelef D, Kaidi R, Carmena D (2018) *Cryptosporidium*-associated diarrhoea in neonatal calves in Algeria. Vet Parasitol Reg Stud Reports 12:78–84

Read CM, Monis PT, Thompson RC (2004) Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol 4:125–130

Rieux A, Paraud C, Pors I, Chartier C (2013) Molecular characterization of *Cryptosporidium* isolates from pre-weaned calves in western France in relation to age. Vet Parasitol 197:7–12

Rieux A, Paraud C, Pors I, Chartier C (2014) Molecular characterization of *Cryptosporidium* isolates from beef calves under one month of

age over three successive years in one herd in western France. Vet Parasitol 202:171–179

Ryan U, Caccio SM (2013) Zoonotic potential of *Giardia*. Int J Parasitol 43:943–956

Ryan U, Fayer R, Xiao L (2014) *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology 141:1667–1685

Santin M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R (2004) Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet Parasitol 122:103–117

Santoro A, Dorbek-Kolin E, Jeremejeva J, Tummeleht L, Orro T, Jokelainen P, Lassen B (2019) Molecular epidemiology of *Cryptosporidium* spp. in calves in Estonia: high prevalence of *Cryptosporidium parvum* shedding and 10 subtypes identified. Parasitology 146:261–267

Slapeta J (2013) Cryptosporidiosis and *Cryptosporidium* species in animals and humans: a thirty colour rainbow? Int J Parasitol 43:957–970

Smith RP, Clifton-Hadley FA, Cheney T, Giles M (2014) Prevalence and molecular typing of *Cryptosporidium* in dairy cattle in England and Wales and examination of potential on-farm transmission routes. Vet Parasitol 204:111–119

Snel SJ, Baker MG, Venugopal K (2009) The epidemiology of cryptosporidiosis in New Zealand, 1997-2006. N Z Med J 122:47–61

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lal AA, Xiao L (2003) Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis 9:1444–1452

Thompson HP, Dooley JS, Kenny J, McCoy M, Lowery CJ, Moore JE, Xiao L (2007) Genotypes and subtypes of *Cryptosporidium* spp. in neonatal calves in Northern Ireland. Parasitol Res 100:619–624

Trotz-Williams LA, Martin DS, Gatei W, Cama V, Peregrine AS, Martin SW, Nydam DV, Jamieson F, Xiao L (2006) Genotype and subtype analyses of *Cryptosporidium* isolates from dairy calves and humans in Ontario. Parasitol Res 99:346–352

Wang X, Cai M, Jiang W, Wang Y, Jin Y, Li N, Guo Y, Feng Y, Xiao L (2017) High genetic diversity of *Giardia duodenalis* assemblage E in pre-weaned dairy calves in Shanghai, China, revealed by multilocus genotyping. Parasitol Res 116:2101–2110

Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. Exp Parasitol 124:80–89

Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA (1999) Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl Environ Microbiol 65:3386–3391

Zahedi A, Field D, Ryan U (2017) Molecular typing of *Giardia duodenalis* in humans in Queensland - first report of Assemblage E. Parasitology 144:1154–1161

Zhao GH, Ren WX, Gao M, Bian QQ, Hu B, Cong MM, Lin Q, Wang RJ, Qi M, Qi MZ, Zhu XQ, Zhang LX (2013) Genotyping *Cryptosporidium andersoni* in cattle in Shaanxi Province, Northwestern China. PLoS One 8:e60112