Parasitology Research (2019) 118:3053–3060
https://doi.org/10.1007/s00436-019-06426-3

PROTOZOOLOGY - ORIGINAL PAPER

Infection patterns, clinical significance, and genetic characteristics of *Enterocytozoon bieneusi* and *Giardia duodenalis* in dairy cattle in Jiangsu, China

Rui Wang[1] · Na Li[2] · Wen Jiang[1] · Yaqiong Guo[2] · Xiaolan Wang[1] · Yue Jin[1] · Yaoyu Feng[1,2] · Lihua Xiao[2]

Received: 12 July 2019 / Accepted: 9 August 2019 / Published online: 16 August 2019

Abstract

The infection patterns and clinical significance of *Enterocytozoon bieneusi* and *Giardia duodenalis* in dairy cattle remain poorly investigated despite their common occurrence. Data on the genetic diversity are also needed to understand the transmission and human-infective potential of the two pathogens. In this study, fecal specimens from 1366 dairy cattle on a large farm were examined for the presence and genotype distribution of *E. bieneusi* and *G. duodenalis* by PCR and DNA sequencing. The overall infection rates of *E. bieneusi* and *G. duodenalis* were 13.0% and 20.6%, respectively. Pre-weaned calves had significantly higher infection rates of both pathogens than post-weaned and adult cattle ($P < 0.001$), with peak occurrence of the pathogens in animals of 7–12 weeks. In both pre- and post-weaned calves, animals with diarrhea were 2.1–3.0 times more likely to be infected with either pathogen than those without diarrhea ($P < 0.01$). The *E. bieneusi* identified belonged to five genotypes, including J ($n = 138$), I ($n = 21$), BEB4 ($n = 10$), Type IV ($n = 1$), and a novel genotype CHC17 ($n = 1$). Genotype J was the dominant one in all age groups, whereas genotype I was only identified in calves of 6–11 weeks. Genotyping of *G. duodenalis* at three genetic loci identified assemblage E ($n = 278$), assemblage A ($n = 2$), and concurrence of the two ($n = 1$). Altogether, 13, 7 and 10 subtypes of assemblage E were detected at the *bg*, *gdh*, and *tpi* loci, respectively, forming 65 multilocus genotypes. The formation of two major clusters of MLGs in eBURST analysis indicated that intra-assemblage genetic recombination of two dominant MLGs could have led to the high genetic heterogeneity within assemblage E on a single farm. Results of this study provide much needed data on the pathogenicity of *E. bieneusi* and *G. duodenalis* in pre- and post-weaned calves. The clinical significance of the two pathogens in dairy cattle warrants further investigations.

Keywords *Enterocytozoon bieneusi* · *Giardia duodenalis* · Dairy cattle · Transmission · Multilocus genotyping

Rui Wang and Na Li contributed equally to this work.

Handling Editor: Una Ryan

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00436-019-06426-3) contains supplementary material, which is available to authorized users.

✉ Yaoyu Feng
yyfeng@scau.edu.cn

✉ Lihua Xiao
lxiao1961@gmail.com

[1] State Key Laboratory of Bioreactor Engineering, School of Resources and Environmental Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

[2] Key Laboratory of Zoonosis of Ministry of Agriculture, College of Veterinary Medicine, South China Agricultural University, 483 Wushan Road, Guangzhou 510642, China

Introduction

Enterocytozoon bieneusi and *Giardia duodenalis* are two common intestinal protists in humans, farm animals, domestic pets, and wild mammals (Ryan et al. 2019; Wang et al. 2018). Humans are infected by the two pathogens through a similar fecal–oral transmission route, after exposures to infected persons (anthroponotic transmission) or animals (zoonotic transmission), or consumption of contaminated food or water (food-borne or water-borne transmission). Recently, concerns have been raised on the potential of cattle as a source for zoonotic transmission of the two pathogens (Abeywardena et al. 2015; Feng et al. 2019).

Enterocytozoon bieneusi is the most prevalent human-pathogenic species of microsporidia (Didier and Weiss

2011). It forms at least 11 genotype groups with different host preferences, with Group 1 containing mostly zoonotic genotypes and the remaining groups being largely host adapted (Li et al. 2019; Li and Xiao 2019). The detection of *E. bieneusi* in cattle was first reported in 2000, with genotypes I and J being identified initially (Rinder et al. 2000). Since then, more than 50 *E. bieneusi* genotypes have been detected in cattle, mostly belonging to Group 2 (Zhang et al. 2018). Among them, some genotypes in Groups 1 (D, Type IV, Peru 6, CHN4, and EbpA) and 2 (J, I, BEB4, BEB6, CHN3, CS-4, and EbpC) have been identified in humans, indicating the zoonotic potential of bovine *E. bieneusi* genotypes (Jiang et al. 2015; Yu et al. 2019).

Similarly, molecular analyses have identified at least eight assemblages of A–H within *G. duodenalis*. Among them, zoonotic assemblages A and B infect humans and various animals, whereas host-adapted assemblages C–H each infects a specific group of animals (Feng and Xiao 2011). In cattle, assemblage E is the predominant *G. duodenalis* genotype found in most areas worldwide (Abdel-Moein and Saeed 2016; Hogan et al. 2014; Matsuura et al. 2017; Nguyen et al. 2016; Zhong et al. 2018), although zoonotic assemblages A and B were occasionally detected in some studies (Bartley et al. 2018; Lee et al. 2018; Wegayehu et al. 2016). Recently, a multilocus genotyping approach has been used in characterizations of *G. duodenalis* infections in cattle (Wang et al. 2014). This has led to the identification of numerous multilocus genotypes (MLGs) within assemblage E (Cui et al. 2018; Feng et al. 2019; Zhong et al. 2018). In some recent studies, assemblage E was found in a few human cases (Abdel-Moein and Saeed 2016; Fantinatti et al. 2016; Zahedi et al. 2017).

Despite the prevalence of *E. bieneusi* and *G. duodenalis* in dairy cattle, the clinical significance of two organisms in dairy cattle is not entirely clear. In a few studies, *G. duodenalis* infections in dairy cattle have been associated with the occurrence of diarrhea (Di Piazza et al. 2013; Toledo et al. 2017). This was supported by results of a case–control study in which a higher infection rate was seen in pre-weaned calves with diarrhea (Mahato et al. 2018). Healthy pre- and post-weaned calves, however, are also frequently infected with *G. duodenalis* (Feng et al. 2019; Kakandelwa et al. 2016). The association of *E. bieneusi* infection with the occurrence of diarrhea is even weaker, as most infected dairy cattle do not have diarrhea (Fayer et al. 2007; Tang et al. 2018).

In the present study, we examined the infection patterns, clinical significance, and genetic characteristics of *E. bieneusi* and *G. duodenalis* in dairy cattle on a large dairy farm in Jiangsu, China using molecular diagnostic tools.

Materials and methods

Ethics statement

This study was approved by the Ethics Committee of the East China University of Science and Technology. Fecal specimens from dairy cattle were collected with the permission of the farm manager. During the specimen collection, cattle were handled in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Specimen collection

From April 2016 to November 2017, a total of 1366 fecal specimens were collected from dairy cattle on one farm in Jiangsu Province, East China. The farm had ~ 5500 Holstein (> 99%) and Jersey (< 1%) cattle at the time of sampling. The dairy cattle were divided into four age groups: pre-weaned (< 3 months in age, $n = 615$) calves, post-weaned (3–12 months in age, $n = 508$) calves, adults (> 24 months in age, $n = 192$), and those without age information ($n = 51$). Diarrhea was observed in 240 sampled animals, with the majority in pre-weaned ($n = 158$) and post-weaned ($n = 74$) calves, while the remaining eight specimens were from cattle of unknown age. Fecal specimens were collected directly from the rectum of cattle into 50 mL centrifuge tubes by using disposable gloves, transported to the laboratory with ice packs, and stored in 2.5% potassium dichromate at 4 °C before DNA extraction. Each animal was sampled only once during the study period.

DNA extraction and PCR analysis

After being washed three times with distilled water by centrifugation at 2000×*g* for 10 min, 200 mg of fecal specimen was used in DNA extraction by using the FastDNA SPIN Kit for Soil (MP Biomedical, Santa Ana, CA, USA). The DNA was stored at − 80 °C before PCR analyses. For the detection and genotyping of *E. bieneusi*, a rRNA gene fragment covering the entire internal transcribed spacer (ITS) was amplified by nested PCR (Sulaiman et al. 2003). In contrast, three nested PCR assays targeting the β-giardin (*bg*), glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) genes were used in detecting and genotyping *G. duodenalis* (Caccio et al. 2008). Each specimen was analyzed at each genetic locus at least twice. DNA of genotype PtEb IX from dogs was used as the positive control in PCR analysis of *E. bieneusi*, while DNA of assemblage C from dogs was used as the positive control in PCR analysis of *G. duodenalis*, with reagent-grade water being used as the negative control. The secondary PCR products generated were examined by electrophoresis in 1.5% agarose gels stained with ethidium bromide.

Sequence analysis

All positive secondary PCR products were sequenced in both directions on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the BioSune Biotechnology Company (Shanghai, China). The obtained sequences were assembled using ChromasPro 2.1.6 (http://technelysium.com.au/ChromasPro.html), edited using BioEdit 7.1.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and aligned with each other and reference sequences in the GenBank database using ClustalX 1.81 (http://clustal.org) to determine the genotype identity of *E. bieneusi* and *G. duodenalis*. For *G. duodenalis*, a previous nomenclature system was used in naming *G. duodenalis* assemblage E subtypes at each locus (Wang et al. 2017). Genetic relationships among multilocus genotypes (MLGs) of assemblage E were accessed by using eBURST v3 (http://eburst.mlst.net).

Representative nucleotide sequences generated in this study were deposited in GenBank under the accession numbers MK890212 for *E. bieneusi* and MK890213–MK890222 for *G. duodenalis.*

Statistical analysis

The χ^2 test implemented in SPSS Statistics 21.0 (IBM Corp., New York, NY, USA) was used to compare differences in infection rates among age groups or clinical signs. Differences were considered significant at $P < 0.05$. Odds ratios with 95% confidence intervals (CIs) were calculated for the occurrence of each pathogen in animals with diarrhea.

Results

Infection patterns of *E. bieneusi* and *G. duodenalis* in dairy cattle

Of the 1366 fecal specimens collected from dairy cattle on the study farm, 177 (13.0%) were positive for *E. bieneusi* in ITS PCR. By age, the infection rate in pre-weaned calves (21.0% or 129/615; $P < 0.0001$) was significantly higher than rates in post-weaned calves (6.3% or 32/508) and adult cattle (2.6% or 5/192) (Table 1). Among pre-weaned calves (Fig. 1a), animals of 7–12 weeks of age had a much higher *E. bieneusi* infection rate than younger calves (27.4–53.5% vs. 0–9.8%); the overall infection rates were highly significant between the two age groups ($\chi^2 = 124.39$, $P < 0.0001$). In post-weaned calves, the *E. bieneusi* infection rate was the highest at 3 months of age (18.8% or 9/48). Afterward, the infection rate declined gradually with increased age (Fig. 1b).

Giardia duodenalis was detected in 281 (20.6%) of the 1366 specimens based on PCR positivity at any of the three genetic loci. The highest infection rate was 38.9% (239/615) in pre-weaned calves, followed by post-weaned calves (7.5% or 38/508) and adults (0.5% or 1/192) (Table 1). Pairwise differences in infection rates were all highly significant among the three age groups ($P < 0.001$). Similar to the *E. bieneusi* infection, increasing *G. duodenalis* infection rates were observed with increased age in pre-weaned calves, with infection rates at 6–12 weeks of age (43.9–86.3%) being significantly higher than 1–5 weeks (2.4–15.2%; $\chi^2 = 233.65$, $P < 0.0001$) (Fig. 1a). In post-weaned calves, the infection rate of *G. duodenalis* was the highest at 3 months of age (47.9% or 23/48). Afterward, *G. duodenalis* infection rates decreased rapidly (Fig. 1b).

In both pre- and post-weaned calves, infection rates of *E. bieneusi* or *G. duodenalis* in calves with diarrhea were significantly higher than those without diarrhea ($P < 0.01$) (Table 2). Pre-weaned calves with diarrhea were 2.5 (CI 1.7–3.8) or 2.1 (CI 1.4–3.0) times more likely to be infected with *E. bieneusi* or *G. duodenalis*, respectively. This was also the case in post-weaned calves, with animals experiencing diarrhea 2.9 (CI 1.3–6.5) or 3.0 (CI 1.5–6.3) times more likely to be *E. bieneusi* positive or *G. duodenalis* positive, respectively (Table 2).

Co-infection with *E. bieneusi* and *G. duodenalis* was detected in 98 animals including 93 pre-weaned and 5 post-weaned calves, with the infection rate in pre-weaned calves (15.0% or 93/615; $P < 0.0001$) being significantly higher than in post-weaned calves (1.0% or 5/508). Co-infection was not observed in adult cattle. Among pre-weaned calves, animals with diarrhea were 2.7 (1.7, 4.3) times more likely to have co-infection of both pathogens than those without diarrhea (Table 2).

E. bieneusi genotypes

Five genotypes were detected among 177 *E. bieneusi*-positive specimens, including J ($n = 138$), I ($n = 21$), BEB4 ($n = 10$), Type IV (n = 1), and a novel genotype named CHC17 ($n = 1$) (Table 1). Concurrence of two genotypes was detected in six animals, including J and I ($n = 5$) and J and BEB4 ($n = 1$). The ITS sequences generated from genotypes J, I, BEB4, and Type IV were identical to the GenBank reference sequence AF135837, AF135836, AY331008, and AF242478, respectively. Genotype CHC17 (MK890212) had two nucleotide substitutions compared with the BEB4 genotype. All the genotypes except Type IV belonged to Group 2.

Genotype J was the most common one identified in all three age groups. In contrast, genotype I was only detected in pre-weaned calves, genotypes Type IV and CHC17 were only found in adult animals, while BEB4 was detected in both pre- and post-weaned calves (Table 1). Within pre-weaned calves, genotype I was only detected in calves of 6–11 weeks (Fig. 2).

Table 1 Occurrence of *Enterocytozoon bieneusi* and *Giardia duodenalis* in dairy cattle in Jiangsu, China

Age (months)	Number of specimens	Number positive for *E. bieneusi* (%)	*E. bieneusi* genotypes (*n*)	Number positive for *G. duodenalis* (%)	*G. duodenalis* assemblages (*n*)
Pre-weaned (< 3)	615	129 (21.0)	J (97), I (20), BEB4 (7), J + I (5)	239 (38.9)	E (238), A (1)
Post-weaned (3–12)	508	32 (6.3)	J (28), BEB4 (3), J + BEB4 (1)	38 (7.5)	E (36), A (1), A + E (1)
Adults (> 24)	192	5 (2.6)	J (3), Type IV (1), CHC17 (1)	1 (0.5)	E (1)
Unknown	51	11 (21.6)	J (10), I (1)	3 (5.9)	E (3)
Total	1366	177 (13.0)	J (138), I (21), BEB4 (10), J + I (5), J + BEB4 (1), Type IV (1), CHC17 (1)	281 (20.6)	E (278), A (2), A + E (1)

G. duodenalis assemblages and subtypes

Of the 281 *G. duodenalis*-positive specimens, 258 were positive in *bg* PCR, 224 in *gdh* PCR, and 193 in *tpi* PCR. Among them, only assemblages E ($n = 278$) and A ($n = 2$) were identified, with the concurrence of A and E in one animal (Table 1).

Among the three assemblage A-positive specimens, sequence analysis of the *bg*, *gdh*, and *tpi* genes showed the presence of subtype A5 in one specimen, A1 + A5 in one specimen, and A1 + E in one specimen, respectively. The sequences produced were identical to the reference sequences of the *bg* (KR075938), *gdh* (KR075940), and *tpi* (JX845464) genes. Only one of the three assemblage A specimens was positive by PCR at all three genetic loci. Based on the A1 and A5 subtype identity at these loci, the specimen apparently had the sub-assemblage AI (Table S1).

A higher sequence diversity was found within assemblage E at all three genetic loci (Table S2). At the *bg* locus, 13 subtypes were identified, eight of which were reported previously: E3 ($n = 117$), E2 ($n = 53$), E1 ($n = 26$), E5 ($n = 9$), E6 ($n = 8$), E29 ($n = 7$), E8 ($n = 6$), and E11 ($n = 6$); the remaining five represented novel subtypes, including E28 ($n = 14$), E18 ($n = 4$), E30 ($n = 4$), E27 ($n = 1$), and E32 ($n = 1$) (MK890213-MK890217). At the *gdh* locus, seven subtypes were identified, including five known subtypes of E3 ($n = 91$), E1 ($n =$

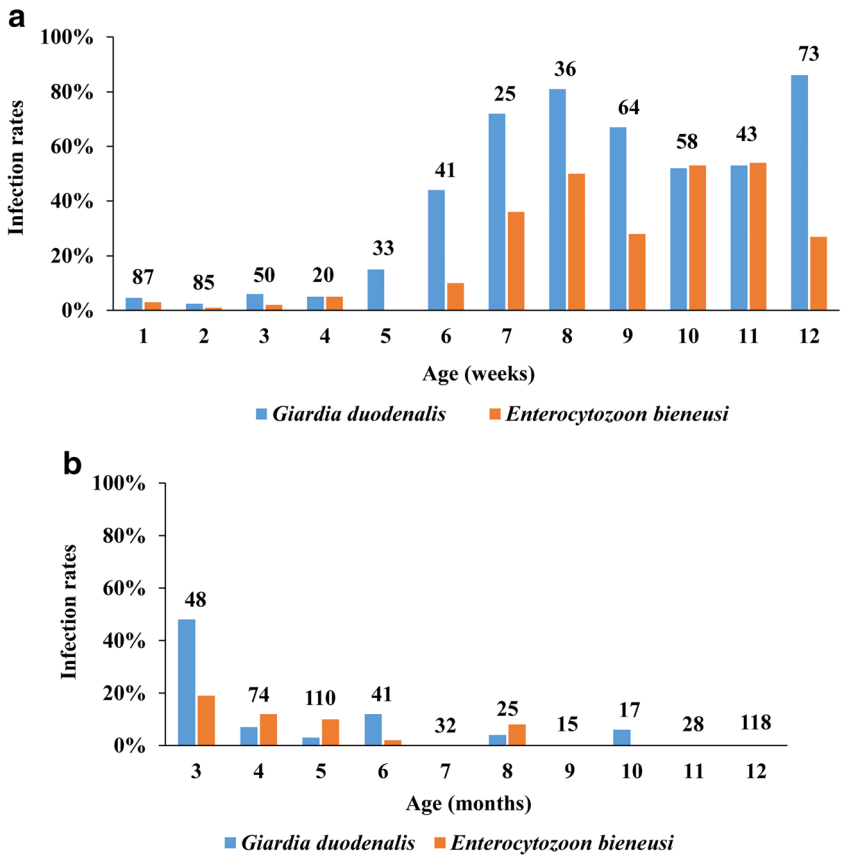

Fig. 1 Infection rates of *Enterocytozoon bieneusi* and *Giardia duodenalis* in pre-weaned (**a**) and post-weaned (**b**) dairy calves on a farm in Jiangsu, China by age

Table 2 Association between *Enterocytozoon bieneusi* or *Giardia duodenalis* infection and occurrence of diarrhea in dairy calves in Jiangsu, China

Age (months)	Animal group	Sample size	*E. bieneusi* infection				*G. duodenalis* infection				Co-infection			
			Number positive (%)	χ^2	OR (95% CI)	*P*	Number positive (%)	χ^2	OR (95% CI)	*P*	Number positive (%)	χ^2	OR (95% CI)	*P*
Pre-weaned (< 3)	Diarrhea	158	53 (33.5)				82 (51.9)				41 (25.9)			
	No diarrhea	457	76 (16.6)	20.264	2.5 (1.7, 3.8)	0.000**	157 (34.4)	15.21	2.1 (1.4, 3.0)	0.000**	52 (11.4)	19.421	2.7 (1.7, 4.3)	0.000**
Post-weaned (3–12)	Diarrhea	74	10 (13.5)				12 (16.2)				1 (1.4)			
	No diarrhea	434	22 (5.1)	6.274	2.9 (1.3, 6.5)	0.006**	26 (6.0)	9.551	3.0 (1.5, 6.3)	0.002**	4 (0.9)	NA	NA	NA

** $P < 0.01$

NA not available, *OR* odds ratio, *CI* confidence interval

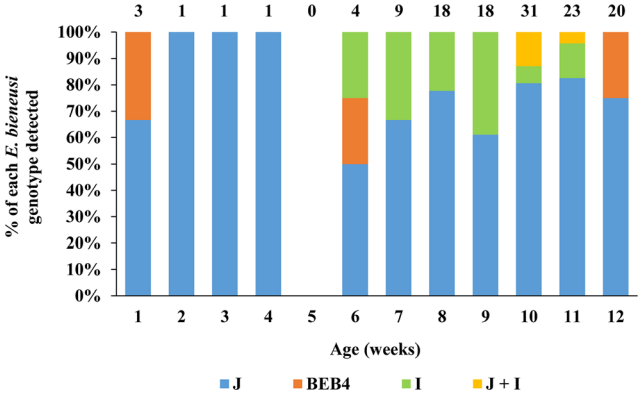

Fig. 2 Distribution of *Enterocytozoon bieneusi* genotypes in pre-weaned dairy calves on a farm in Jiangsu, China by age

75), E11 ($n = 7$), E2 ($n = 6$), and E15 ($n = 2$), and two novel subtypes of E32 ($n = 5$) and E33 ($n = 2$) (MK890218, MK890219). Double peaks were observed at nucleotide position 95 in *gdh* sequences from 34 specimens. At the *tpi* locus, 10 subtypes were detected including seven known subtypes and three novel ones. The former included E34 ($n = 112$), E17 ($n = 22$), E1 ($n = 11$), E53 ($n = 11$), E50 ($n = 6$), E54 ($n = 5$), and E52 ($n = 2$), whereas the latter included E55 ($n = 16$), E56 ($n = 4$), and E49 ($n = 3$) (MK890220–MK890222).

Multilocus genotypes of assemblage E

A total of 143 assemblage E-positive specimens were successfully subtyped at all three loci, forming 65 assemblage E MLGs (Table S1). A concurrent infection of A1 and E was excluded in the MLG analysis, as well as 31 additional specimens that showed the presence of double peaks at the *gdh* locus. All 143 specimens harboring 65 assemblage E MLGs were collected in April 2016. In pre-weaned calves, 59 assemblage E MLGs were identified with MLG-E1 ($n = 26$) and MLG-E2 ($n = 12$) as the most common MLGs. In post-weaned calves, 11 assemblage E MLGs were identified, including MLG-E1 in two specimens, and MLG-E6, MLG-E9, MLG-E17, MLG-E19, MLG-E25, MLG-E28, MLG-E43, MLG-E48, MLG-E60, and MLG-E63 each in one specimen.

The 65 assemblage E MLGs mostly belonged to two clusters in the eBURST analysis. Among them, two most common MLGs, MLG-E1 ($n = 28$) and MLG-E2 ($n = 12$), were identified as the primary and subgroup founders, respectively. They, however, were genetically related, differing only at one of the three genetic loci (Fig. 3).

Discussion

In this study, the overall infection rate of *E. bieneusi* was 13.0% in dairy cattle in Jiangsu Province, East China. This is within the range of reported *E. bieneusi* infection rates of

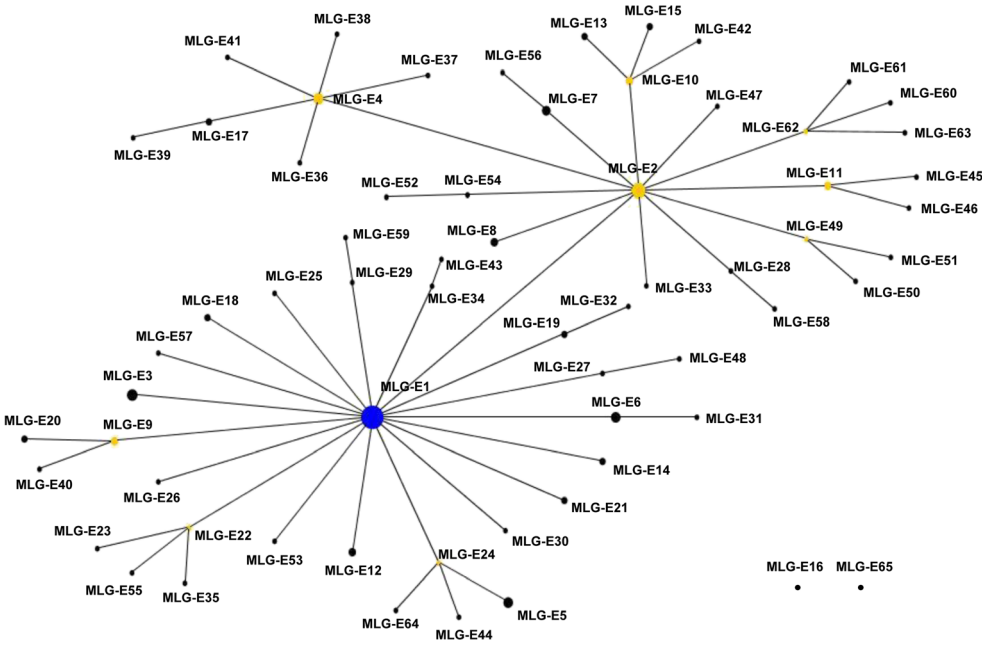

Fig. 3 Relationships among 65 multilocus genotypes (MLGs) of *Giardia duodenalis* assemblage E identified in dairy cattle by eBURST analysis of allelic data. Each dot represents a MLG; the size is proportional to the sample size of the MLG. Blue dot is the primary founder and yellow dots are subgroup founders. MLGs related by single-locus variants are linked by lines. The allelic profile of each MLG is indicated in Table S1

2.0–46.8% in dairy cattle in China (Qiu et al. 2019). By age, the *E. bieneusi* infection rate was significantly higher in pre-weaned calves than in post-weaned calves. This is similar to some previous studies in other provinces in China, which mostly reported a slightly higher rate in pre-weaned calves (Li et al. 2016b; Qi et al. 2017). In contrast, a lower *E. bieneusi* infection rate was observed in pre-weaned calves than in post-weaned calves in two other studies in China (Hu et al. 2017; Yu et al. 2019). Within pre-weaned calves, the *E. bieneusi* detection rate (21.0%) on the study farm is higher than rates reported in most other studies in China (Hu et al. 2017; Qi et al. 2017; Wang et al. 2016). In addition, *E. bieneusi* infection rates increased with age even in pre-weaned calves, with peak infection in calves of 7–12 weeks. This is largely in agreement with the age pattern described in two previous studies in the USA and China (Santin and Fayer 2009; Tang et al. 2018).

As in most previous studies in China and Egypt (Cui et al. 2018; Liu et al. 2015; Naguib et al. 2018), significant declining of *G. duodenalis* infection rates was also observed with increased age in this study, with the highest in pre-weaned calves (38.9%). Within pre-weaned calves, *G. duodenalis* infection rates were extremely low during the first 5 weeks and thereafter increased rapidly at 6–12 weeks of age, which is largely in concordance with observations in some earlier studies in the USA, New Zealand, and two recent surveys in Shanghai and Guangdong, China (Feng et al. 2019; Trout et al. 2004; Wang et al. 2017; Winkworth et al. 2008).

In both pre- and post-weaned calves in this study, the odds of *E. bieneusi* infection in animals with diarrhea were 2.5–2.9 times higher than in those without diarrhea, which is contrary to previous findings elsewhere that *E. bieneusi* infection was not associated with the occurrence of any clinical signs in infected dairy cattle (Qiu et al. 2019; Santin and Fayer 2011). Similarly, the chance for *G. duodenalis* infection was 2.1–3.0 times higher in calves with diarrhea. This observation is consistent with the results in some previous studies in Italy, Brazil, and Nepal (Di Piazza et al. 2013; Mahato et al. 2018; Toledo et al. 2017), further supporting the association between *G. duodenalis* infection and occurrence of diarrhea in dairy calves.

Similar to most previous studies in China (Hu et al. 2017; Li et al. 2016b; Qi et al. 2017), *E. bieneusi* genotype J was the dominant one in cattle of all ages in Jiangsu. Interestingly, genotype I, the second most common genotype in this study, was only detected in pre-weaned calves of 6–11 weeks. This is different from the age-related distribution of *E. bieneusi* genotypes in dairy cattle elsewhere in the world, where genotype I was commonly detected in all age groups (da Silva Fiuza et al. 2016; Santin and Fayer 2009; Zhang et al. 2018). One study in the USA found that genotype I was detected primarily in post-weaned calves (Santin and Fayer 2009).

Assemblage E was found to be the dominant *G. duodenalis* genotype in dairy cattle in Jiangsu. This is consistent with most previous studies in China and elsewhere (Bartley et al. 2018; Cui et al. 2018; Hu et al. 2017; Naguib et al. 2018). In addition, sub-assemblage AI was detected in three specimens in this study. Although both are more commonly seen in animals (Feng and Xiao 2011), *G. duodenalis* infection in dairy cattle may still present a potential risk for public health, as sub-assemblage AI and assemblage E have been found in humans in some recent studies (Abdel-Moein and Saeed 2016; Fantinatti et al. 2016; Zahedi et al. 2017).

A high genetic diversity was observed within assemblage E in this study, with 65 MLG-Es forming two major clusters on a single dairy farm. Previously, multiple MLGs of assemblages E had been reported in dairy cattle in China (Cui et al. 2018; Zhong et al. 2018). Those studies, however, were mostly conducted on multiple farms. The occurrence of multiple MLGs on a single farm suggests the likely occurrence of intra-assemblage genetic recombination, which could be responsible for the high genetic heterogeneity within assemblage E (Aguiar et al. 2016; Wang et al. 2017). In addition, the most common MLGs (MLG-E1 and MLG-E2) in the study were primary and subgroup founders in eBURST analysis, supporting the likely occurrence of genetic recombination of the two dominant MLGs. This needs substantiation by using population genetic analyses.

In conclusion, *E. bieneusi* and *G. duodenalis* are common in dairy cattle in Jiangsu, China, with both more prevalent in pre-weaned calves than in post-weaned and adult cattle. The pathogenicity of *E. bieneusi* and *G. duodenalis* was also observed in both pre- and post-weaned calves, with the association between *E. bieneusi* infection and occurrence of diarrhea being described in dairy calves for the first time. Genetic recombination is suspected to be the cause of the high genetic heterogeneity within *G. duodenalis* assemblage E. Nevertheless, more advanced epidemiological studies are required to characterize the transmission and clinical significance of *E. bieneusi* and *G. duodenalis* on dairy farms.

Funding information This work was supported in part by the National Natural Science Foundation of China (31602042) and National Key R&D Program of China (2017YFD0501305, 2017YFC1601206).

Compliance with ethical standards This study was approved by the Ethics Committee of the East China University of Science and Technology. Fecal specimens from dairy cattle were collected with the permission of the farm manager. During the specimen collection, cattle were handled in accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Conflict of interest The authors declare no conflict of interest.

References

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

Abeywardena H, Jex AR, Gasser RB (2015) A perspective on *Cryptosporidium* and *Giardia*, with an emphasis on bovines and recent epidemiological findings. Adv Parasitol 88:243–301

Aguiar JM, Silva SO, Santos VA, Taniwaki SA, Oliveira TM, Ferreira HL, Keid LB, Gregori F, Soares RM (2016) Evidence of heterozygosity and recombinant alleles in single cysts of *Giardia duodenalis*. Rev Bras Parasitol Vet 25:187–195

Bartley PM, Roehe BK, Thomson S, Shaw HJ, Peto F, Innes EA, Katzer F (2018) Detection of potentially human infectious assemblages of *Giardia duodenalis* in fecal samples from beef and dairy cattle in Scotland. Parasitology:1–8

Caccio SM, Beck R, Lalle M, Marinculic A, Pozio E (2008) Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int J Parasitol 38:1523–1531

Cui Z, Wang L, Cao L, Sun M, Liang N, Wang H, Chang Y, Lin X, Yu L, Wang R, Zhang S, Ning C, Zhang L (2018) Genetic characteristics and geographic segregation of *Giardia duodenalis* in dairy cattle from Guangdong Province, southern China. Infect Genet Evol 66:95–100

da Silva Fiuza VR, Lopes CW, de Oliveira FC, Fayer R, Santin M (2016) New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. Vet Parasitol 216:46–51

Di Piazza F, Di Benedetto MA, Maida CM, Glorioso S, Adamo G, Mazzola T, Firenze A (2013) A study on occupational exposure of Sicilian farmers to *Giardia* and *Cryptosporidium*. J Prev Med Hyg 54:212–217

Didier ES, Weiss LM (2011) Microsporidiosis: not just in AIDS patients. Curr Opin Infect Dis 24:490–495

Fantinatti M, Bello AR, Fernandes O, Da-Cruz AM (2016) Identification of *Giardia lamblia* assemblage E in humans points to a new anthropozoonotic cycle. J Infect Dis 214:1256–1259

Fayer R, Santin M, Trout JM (2007) *Enterocytozoon bieneusi* in mature dairy cattle on farms in the eastern United States. Parasitol Res 102:15–20

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

Hogan JN, Miller WA, Cranfield MR, Ramer J, Hassell J, Noheri JB, Conrad PA, Gilardi KV (2014) *Giardia* in mountain gorillas (*Gorilla beringei beringei*), forest buffalo (*Syncerus caffer*), and domestic cattle in volcanoes National Park, Rwanda. J Wildl Dis 50:21–30

Hu S, Liu Z, Yan F, Zhang Z, Zhang G, Zhang L, Jian F, Zhang S, Ning C, Wang R (2017) Zoonotic and host-adapted genotypes of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy cattle in Hebei and Tianjin, China. Vet Parasitol 248:68–73

Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, Zhang S, Li W (2015) Zoonotic and potentially host-adapted *Enterocytozoon bieneusi* genotypes in sheep and cattle in Northeast China and an increasing concern about the zoonotic importance of previously considered ruminant-adapted genotypes. Appl Environ Microbiol 81:3326–3335

Kakandelwa C, Siwila J, Nalubamba KS, Muma JB, Phiri IG (2016) Prevalence of *Giardia* in dairy cattle in Lusaka and Chilanga districts, Zambia. Vet Parasitol 215:114–116

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 W, Xiao L (2019) Multilocus sequence typing and population genetic analysis of *Enterocytozoon bieneusi*: host specificity and its impacts on public health. Front Genet 10:307

Li J, Luo N, Wang C, Qi M, Cao J, Cui Z, Huang J, Wang R, Zhang L (2016) Occurrence, molecular characterization and predominant genotypes of *Enterocytozoon bieneusi* in dairy cattle in Henan and Ningxia, China. Parasit Vectors 9:142

Li N, Ayinmode AB, Zhang H, Feng Y, Xiao L (2019) Host-adapted *Cryptosporidium* and *Enterocytozoon bieneusi* genotypes in straw-colored fruit bats in Nigeria. Int J Parasitol: Par Wildl 8:19–24

Liu G, Su Y, Zhou M, Zhao J, Zhang T, Ahmad W, Lu H, Jiang N, Chen Q, Xiang M, Yin J (2015) Prevalence and molecular characterization of *Giardia duodenalis* isolates from dairy cattle in Northeast China. Exp Parasitol 154:20–24

Mahato MK, Singh DK, Rana HB, Acharya KP (2018) Prevalence and risk factors associated with *Giardia duodenalis* infection in dairy cattle of Chitwan, Nepal. J Parasit Dis 42:122–126

Matsuura Y, Matsubayashi M, Nukata S, Shibahara T, Ayukawa O, Kondo Y, Matsuo T, Uni S, Furuya M, Tani H, Tsuji N, Sasai K (2017) Report of fatal mixed infection with *Cryptosporidium parvum* and *Giardia intestinalis* in neonatal calves. Acta Parasitol 62:214–220

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

Nguyen ST, Fukuda Y, Nguyen DT, Tada C, Nakai Y (2016) Prevalence and first genotyping of *Giardia duodenalis* in beef calves in Vietnam. Trop Anim Health Prod 48:837–841

Qi M, Jing B, Jian F, Wang R, Zhang S, Wang H, Ning C, Zhang L (2017) Dominance of *Enterocytozoon bieneusi* genotype J in dairy calves in Xinjiang, Northwest China. Parasitol Int 66:960–963

Qiu L, Xia W, Li W, Ping J, Ding S, Liu H (2019) The prevalence of microsporidia in China: a systematic review and meta-analysis. Sci Rep 9:3174

Rinder H, Thomschke A, Dengjel B, Gothe R, Loscher T, Zahler M (2000) Close genotypic relationship between *Enterocytozoon bieneusi* from humans and pigs and first detection in cattle. J Parasitol 86:185–188

Ryan U, Hijjawi N, Feng Y, Xiao L (2019) *Giardia*: an under-reported foodborne parasite. Int J Parasitol 49:1–11

Santin M, Fayer R (2009) A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. Parasitol Res 105:141–144

Santin M, Fayer R (2011) Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. Res Vet Sci 90:363–371

Sulaiman IM, Fayer R, Lal AA, Trout JM, Schaefer FW 3rd, Xiao L (2003) Molecular characterization of microsporidia indicates that wild mammals harbor host-adapted *Enterocytozoon* spp. as well as human-pathogenic *Enterocytozoon bieneusi*. Appl Environ Microbiol 69:4495–4501

Tang C, Cai M, Wang L, Guo Y, Li N, Feng Y, Xiao L (2018) Genetic diversity within dominant *Enterocytozoon bieneusi* genotypes in pre-weaned calves. Parasit Vectors 11:170

Toledo RD, Martins FD, Ferreira FP, de Almeida JC, Ogawa L, Dos Santos HL, Dos Santos MM, Pinheiro FA, Navarro IT, Garcia JL, Freire RL (2017) *Cryptosporidium* spp. and *Giardia* spp. in feces and water and the associated exposure factors on dairy farms. PLoS One 12:e0175311

Trout JM, Santin M, Greiner E, Fayer R (2004) Prevalence of *Giardia duodenalis* genotypes in pre-weaned dairy calves. Vet Parasitol 124: 179–186

Wang H, Zhao G, Chen G, Jian F, Zhang S, Feng C, Wang R, Zhu J, Dong H, Hua J, Wang M, Zhang L (2014) Multilocus genotyping of *Giardia duodenalis* in dairy cattle in Henan, China. PLoS One 9: e100453

Wang XT, Wang RJ, Ren GJ, Yu ZQ, Zhang LX, Zhang SY, Lu H, Peng XQ, Zhao GH (2016) Multilocus genotyping of *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy and native beef (Qinchuan) calves in Shaanxi Province, northwestern China. Parasitol Res 115: 1355–1361

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

Wang SS, Wang RJ, Fan XC, Liu TL, Zhang LX, Zhao GH (2018) Prevalence and genotypes of *Enterocytozoon bieneusi* in China. Acta Trop 183:142–152

Wegayehu T, Karim MR, Erko B, Zhang L, Tilahun G (2016) Multilocus genotyping of *Giardia duodenalis* isolates from calves in Oromia special zone, Central Ethiopia. Infect Genet Evol 43:281–288

Winkworth CL, Matthaei CD, Townsend CR (2008) Prevalence of *Giardia* and *Cryptosporidium* spp. in calves from a region in New Zealand experiencing intensification of dairying. N Z Vet J 56:15–20

Yu F, Qi M, Zhao Z, Lv C, Wang Y, Wang R, Zhang L (2019) The potential role of synanthropic rodents and flies in the transmission of *Enterocytozoon bieneusi* on a dairy cattle farm in China. J Eukaryot Microbiol 66:435–441

Zahedi A, Field D, Ryan U (2017) Molecular typing of *Giardia duodenalis* in humans in Queensland—first report of assemblage E. Parasitology:1–8

Zhang Y, Koehler AV, Wang T, Haydon SR, Gasser RB (2018) *Enterocytozoon bieneusi* genotypes in cattle on farms located within a water catchment area. J Eukaryot Microbiol:1–7

Zhong Z, Dan J, Yan G, Tu R, Tian Y, Cao S, Shen L, Deng J, Yu S, Geng Y, Gu X, Wang Y, Liu H, Peng G (2018) Occurrence and genotyping of *Giardia duodenalis* and *Cryptosporidium* in pre-weaned dairy calves in Central Sichuan Province, China. Parasite 25:45