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FULL PAPER

Parasitology

Prevalence and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* from large-scale cattle farms in Anhui Province, China

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ABSTRACT. To investigate the prevalence of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* from large-scale cattle farms in Anhui Province, 955 fecal samples were collected from 16 cattle farms from March to October 2018, which included six dairy farms (526), seven yellow cattle farms (323), and three water buffalo farms (106) in different regions of Anhui Province. PCR was conducted on all fecal samples using the 18S ribosomal RNA of *Cryptosporidium* spp. and internal transcribed spacer gene of *E. bieneusi* to detect these two pathogens, and the positive samples were sequenced and analyzed. The results showed that 23 (2.4%) and 40 (4.2%) out of the 955 samples were positive for *Cryptosporidium* spp. and *E. bieneusi*, respectively. There were 11 (2.1%), 10 (3.1%), and 2 (1.9%) positive samples of *Cryptosporidium* spp. and 16 (3.0%), 23 (7.1%), and 1 (0.9%) positive samples of *E. bieneusi* collected from dairy cattle, yellow cattle, and water buffalo, respectively, and no co-infection was identified in this study. All positive samples of *Cryptosporidium* spp. were *C. andersoni* with some variations. Ten *E. bieneusi* genotypes were obtained, including two known genotypes, J and CHN11, and eight new genotypes, named AHDC1 and AHYC1-7. The genotype CHN11 belonged to zoonotic Group 1, and the other nine genotypes belonged to Group 2, which is mainly documented in ruminants. These results indicated that *Cryptosporidium* spp. and *E. bieneusi* infections were present in large-scale cattle farms in Anhui Province. Therefore, attention should be paid to the development of containment strategies of these two pathogens in cattle.

KEY WORDS: Anhui Province, cattle, *Cryptosporidium* spp., *Enterocytozoon bieneusi*, prevalence

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The infections of intestinal pathogens are very common and prevalent in cattle, which may be associated with the inevitable mortality of cattle and the retard of cattle growth. Moreover, several intestinal pathogens are zoonotic and considered important to public health, such as *Cryptosporidium* spp. and *Enterocytozoon bieneusi* (*E. bieneusi*) [3, 40]. Currently, nearly 40 *Cryptosporidium* species have been identified, among which *C. hominis* and *C. parvum* are the major human-pathogenic species, and *C. hominis* only infects humans [6]. Cattle are mostly infected with *C. parvum*, *C. andersoni*, *C. bovis*, and *C. ryanae*, and the infection of *C. parvum* causes severe diarrhea in cattle [37]. Therefore, cattle are the dominant reservoirs of zoonotic infection of *Cryptosporidium* spp. [26].

E. bieneusi is another common cause of opportunistic infection in human and animals [23, 38]. The infection of *E. bieneusi* is responsible for persistent diarrhea in both human and cattle worldwide, notably in immune deficient patients [4, 10, 23]. Thus far, more than 500 *E. bieneusi* genotypes have been identified on the basis of internal transcribed spacer (ITS) gene sequence analysis, and comprehensive phylogenetic analysis showed that there are 11 genetic groups [17]. Groups 1 and 2 are the two largest groups, while Groups 3–11 have fewer genotypes. Most genotypes in Group 1 have a wide host range including human, therefore Group 1 is the main group responsible for zoonotic transmission. The genotypes in Group 2 have mainly been identified in ruminants, whereas the genotypes in Group 3–11 are more host-specific [17, 27].

Epidemiological studies revealed that *Cryptosporidium* spp. and *E. bieneusi* were commonly detected in cattle, especially in China [21, 37]. As a result, cattle are suspected to be a potential infection source of these two pathogens. In recent years, the

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cattle raising industry has rapidly expanded in Anhui Province, which increases the contamination chances of soil and water by oocysts/spores, and the opportunities for workers to be exposed to *Cryptosporidium* spp. and *E. bieneusi*, further causing zoonotic transmission of these two pathogens. However, few studies have investigated the prevalence of these two pathogens in cattle, and none has been conducted in water buffalo from Anhui Province, China. Therefore, this study was conducted to investigate a molecularly epidemiological prevalence of *Cryptosporidium* spp. and *E. bieneusi* in dairy cattle, yellow cattle, and water buffalo in Anhui Province to help prevent and control the cattle intestinal pathogens in this region.

MATERIALS AND METHODS

Fecal sample collection

The collection of samples were consistent with the previous study [18]. Briefly, from March to October 2018, a total of 955 fecal samples were collected from 16 cattle farms in Anhui Province, China; Among them, 526 dairy cattle samples were collected from six large-scale farms in five cities, 323 yellow cattle samples from seven large-scale farms in six cities, and 106 water buffalo samples from three large-scale farms in three cities. These cattle were divided into four age groups: <3 months (n=42), 3–12 months (n=121), 13–24 months (n=466), and >24 months (n=326). The samples were either directly collected from the rectum or ground immediately after defecation using a sterile disposable polyethylene glove. They were marked with the individual's age, fecal form classified as normal and abnormal (pasty, poorly formed, or liquid), and geographical origin classified as the northern and mid-region of Anhui Province. An icebox was used to store the samples until transport to the laboratory, where they were stored at 4°C, generally within 24 hr. The collection details are shown in Tables 1 and 2.

The detection of fecal samples

The genomic DNA of the 955 fecal samples was extracted using the Stool DNA Kit (Tiangen, Beijing, China). The infections of *Cryptosporidium* spp. and *E. bieneusi* were detected by the previous PCR methods using the 18S ribosomal RNA (rRNA) gene of *Cryptosporidium* spp. and the ITS gene of *E. bieneusi* (Table 3) [29, 36]. Briefly, the genomic DNA of each sample was amplified by the outer specific primers of these two pathogens; then, the products were used as the templates in secondary nested reactions. The secondary PCR products were further detected by electrophoresis.

Table 1. The infection of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in cattle in Anhui Province, China

Species	Farms	No.of samples	*Cryptosporidium* spp.			*E. bieneusi*		
			Positive numbers	Infection rate (%)	Species	Positive numbers	Infection rate (%)	Genotypes
Dairy cattle	Bengbu1^{M}	113	1	0.9	*C. andersoni*	13	11.5	J (10), AHDC1 (3)
	Bengbu2^{N}	62	0	0.0		2	3.2	J (2)
	Suzhou^{N}	126	0	0.0		1	0.8	J (1)
	Huainan^{N}	50	0	0.0		0	0.0	–
	Fuyang^{N}	58	4	6.9		0	0.0	–
	Lu'an^{M}	117	6	5.1		0	0.0	–
	Subtotal	**526**	**11**	**2.1**		**16**	**3.0**	**J (13), AHDC1 (3)**
Yellow cattle	Bengbu2^{N}	45	0	0.0		6	13.3	J (6)
	Suzhou^{N}	97	0	0.0		15	15.5	J (6), AHYC1 (1), AHYC2 (2), AHYC3 (1), AHYC4 (1), AHYC5 (1), AHYC6 (2), AHYC7 (1)
	Huainan^{N}	50	0	0.0		1	2.0	J (1)
	Fuyang1^{N}	57	9	15.8		0	0.0	–
	Fuyang2^{N}	42	0	0.0		0	0.0	–
	Lu'an^{M}	22	1	4.6		1	4.6	AHYC3 (1)
	An'qing^{M}	10	0	0.0		0	0.0	–
	Subtotal	**323**	**10**	**3.1**		**23**	**7.1**	**J (13), AHYC1 (1), AHYC2 (2), AHYC3 (2), AHYC4 (1), AHYC5 (1), AHYC 6 (2), AHYC7 (1)**
Water buffalo	Wuhu^{M}	44	0	0.0		1	2.3	CHN11 (1)
	Lu'an^{M}	59	2	3.4		0	0.0	–
	An'qing^{M}	3	0	0.0		0	0.0	–
	Subtotal	**106**	**2**	**1.9**		**1**	**0.9**	**CHN11 (1)**
Total		**955**	**23**	**2.4**		**40**	**4.2**	**J (26), AHDC1 (3), AHYC1 (1), AHYC2 (2), AHYC3 (2), AHYC4 (1), AHYC5 (1), AHYC6 (2), AHYC7 (1), CHN11 (1)**

The superscript N represents that the sampling site is located in the north of Anhui Province; the superscript M represents that the sampling site is located in the middle of Anhui Province.

Table 2. Prevalence of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in cattle in different ages, feces forms and sampling areas

Factor		Sample size	*Cryptosporidium* spp.				*E. bieneusi*			
			No. of positives	Positive rate/%	*P* value	OR (95% CI)	No. of positives	Positive rate/%	*P* value	OR (95% CI)
Age (months)	Pre-weaned calves (<3)	42	5	11.9	0.000	Reference	1	2.4	0.482	Reference
	Post-weaned calves (3–12)	121	4	3.3			7	5.8		
	Juveniles (13–24)	466	12	2.6			22	4.7		
	Adults (>24)	326	2	0.6			10	3.1		
Feces form	Normal	857	17	2.1	0.014	0.3 (0.1–0.8)	35	4.1	0.634	0.8 (0.3–2.1)
	Abnormal	98	6	6.1			5	0.5		
Sampling area	Northern region	587	13	2.2	0.622	0.8 (0.4–1.9)	25	4.3	0.891	1.1 (0.5–2.0)
	Mid region	368	10	2.7			15	4.2		

OR, odds ratio; CI, confidence interval.

Table 3. Specific primers used for PCR detection in this study

Pathogens	Target genes	Specific primers		Primers (5′-3′)	PCR product size (bp)	Reference
Cryptosporidium spp.	18S rRNA	Outer	18SR	TTCTAGAGCTAATACATGCG	1,325	[36]
			18SF	CCCTAATCCTTCGAAACAGGA		
		Inner	18SNR	GGAAGGGTTGATTTATTAGATAAAG	830–840	
			18SNF	AAGGAGTAGGAAACAACCTCCA		
E. bieneusi	ITS	Outer	AL4037	GATGTCATAG GGATGAGAGCTT	410	[29]
			AL4039	AATAGGATCACTTGGCCGT		
		Inner	AL4038	AGGGATGAAGAGCTTCGGCTCTG	392	
			AL4040	AATATCCCTAATACAGGATCACT		

The analysis of sequences

The positive products were purified and confirmed by two-directional sequencing using the specific inner primers of each pathogen in Sangon Biotech (Shanghai, China). The obtained sequences were aligned using BioEdit 7.1 (http://www. mbio.ncsu.edu/BioEdit/bioedit.html) and compared with the sequences in GenBank database by BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). A part of the ITS region (243 bp) of *E. bieneusi* was used to name the genotypes according to the established nomenclature system [28]. The identified sequences of *E. bieneusi* that were identical to the sequences deposited in Genbank database were named as the first published name, and the other sequences that had a single nucleotide substitution/deletion/insertion were named as novel genotypes [28].

To elucidate the genetic relationships of *Cryptosporidium* spp. and *E. bieneusi* isolated in this study, the accurate sequences confirmed through bidirectional sequencing (769 bp of *Cryptosporidium* spp. and 290 bp of *E. bieneusi*) were used to construct the neighbor-joining phylogenetic trees by MEGA6.0 (http://www.megasoftware.net/), and Kimura-2-parameter model with 1,000 bootstrap replicates was used to evaluate the relationship between the sequences identified in this study and the known sequences. In addition, the 18S rRNA sequence of *Isospora suis* (U97523) and ITS sequence of dog-specific *E. bieneusi* (DQ885585) were used as the outgroups for *Cryptosporidium* spp. and *E. bieneusi*, respectively.

Statistical analysis

Statistical analysis was performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). The differences of the infection among age, fecal form, and sampling area of cattle were analyzed by χ^2 test. The nonmetric variables were analyzed in the form of frequency tables, and $P<0.05$ was considered significant.

Nucleotide sequence accession numbers

Sequences obtained in this study were deposited in the GenBank database under accession numbers MW599393 and MW599394 for the 18S rRNA of *C. andersoni*, and MW621476-MW621485 for the ITS of *E. bieneusi*.

RESULTS

The infection of Cryptosporidium spp. in cattle

PCR results and the analysis of sequences showed that 23 (2.4%) fecal samples were positive for *Cryptosporidium* spp., among which 11 (2.1%), 10 (3.1%), and 2 (1.9%) were from dairy cattle, yellow cattle, and water buffalo, respectively. There was no significant difference in the infection of *Cryptosporidium* spp. among these cattle ($\chi^2=1.0$, $P=0.607$). However, the infection

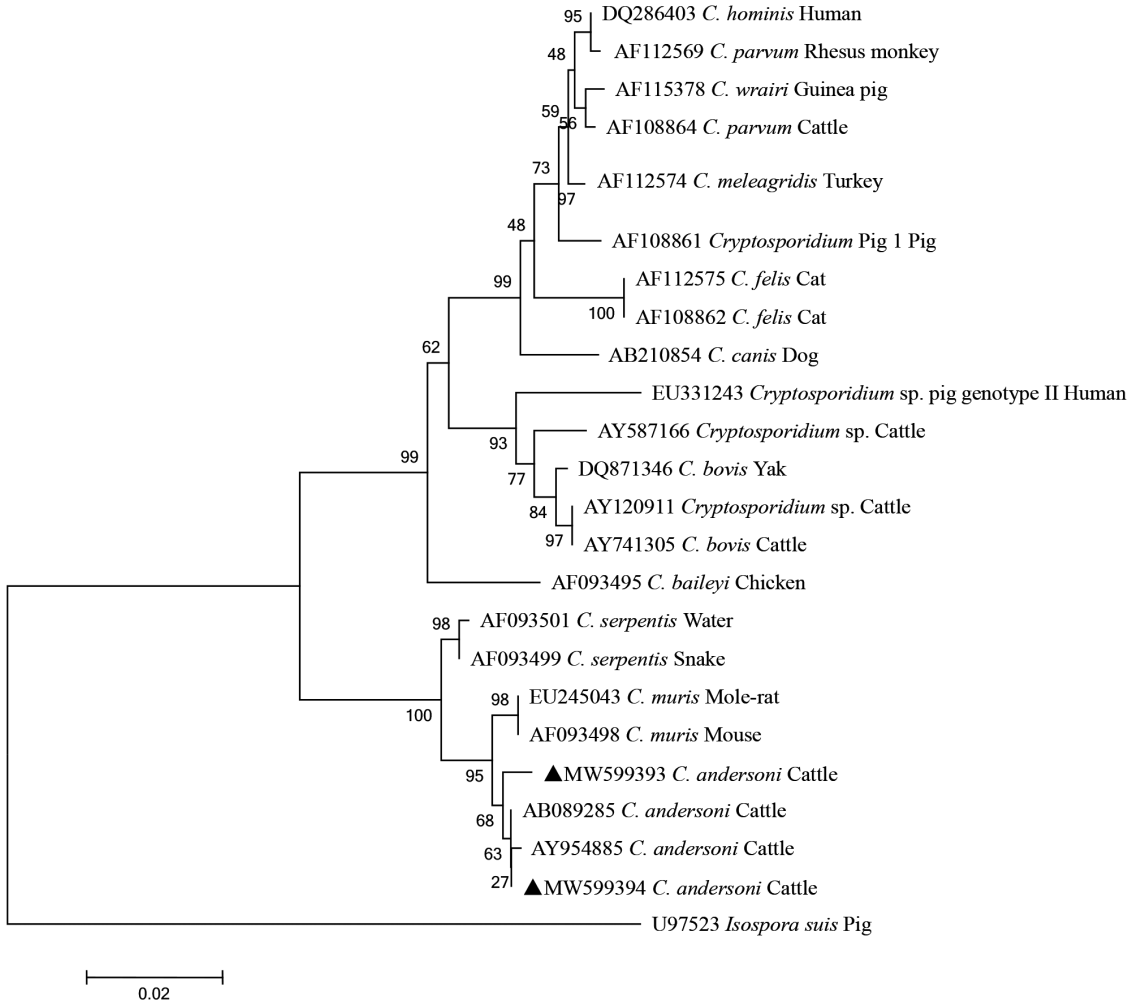

Fig. 1. Neighbor-joining tree showed the *Cryptosporidium* spp. 18S rRNA sequences obtained in this study (indicated by triangles), which were located on the *C. andersoni* branch.

rates of *Cryptosporidium* spp. in dairy cattle, yellow cattle, and water buffalo farms varied from 0–6.9%, 0–15.8%, and 0–3.4%, respectively, and the infection of *Cryptosporidium* spp. showed a significant difference between dairy cattle farms (χ^2=17.7, P=0.003) and yellow cattle farms (χ^2=38.6, P<0.001). The information of infection for each large-scale farm is shown in Table 1.

Analysis of the sequences obtained from the positive samples of Cryptosporidium spp. in cattle

Sequence analysis showed that all positive amplicons of *Cryptosporidium* spp. shared at least 99% identity with the 18S rRNA sequence of *C. andersoni* (AY954885), and can be classified into two sequences MW599393 and MW599394. The homology of these two sequences was 99.22%, and the phylogenetic tree further confirmed that the positive samples were *C. andersoni* (Fig. 1).

The infection of E. bieneusi in cattle

Of the 955 samples, 40 (4.2%) were positive for *E. bieneusi*, among which 16 (3.0%), 23 (7.1%), and 1 (0.9%) were from dairy cattle, yellow cattle, and water buffalo, respectively. The infection rates of *E. bieneusi* in dairy cattle, yellow cattle, and water buffalo were statistically significant (χ^2=11.4, P=0.003). Furthermore, the statistical significance of the infection of *E. bieneusi* was observed in different dairy cattle farms (χ^2=36.7, P<0.001) and yellow cattle farms (χ^2=23.4, P=0.001). Detailed information of the positive samples is shown in Table 1.

Genotype distribution of E. bieneusi in cattle

Analysis showed that, among the 40 positive samples, 10 genotypes of *E. bieneusi* were obtained, including two known genotypes, J and CHN11, and eight new genotypes, named AHDC1, AHYC1, AHYC2, AHYC3, AHYC4, AHYC5, AHYC6, and AHYC7. The homology among the 10 genotypes obtained in this study was from 95.58% to 99.07%. Genotype J was the predominant genotype. In dairy cattle, the detected genotypes were J (13/40) and AHDC1 (3/40). In yellow cattle, the detected genotypes were J (13/40), AHYC1 (1/40), AHYC2 (2/40), AHYC3 (2/40), AHYC4 (1/40), AHYC5 (1/40), AHYC6 (2/40), and

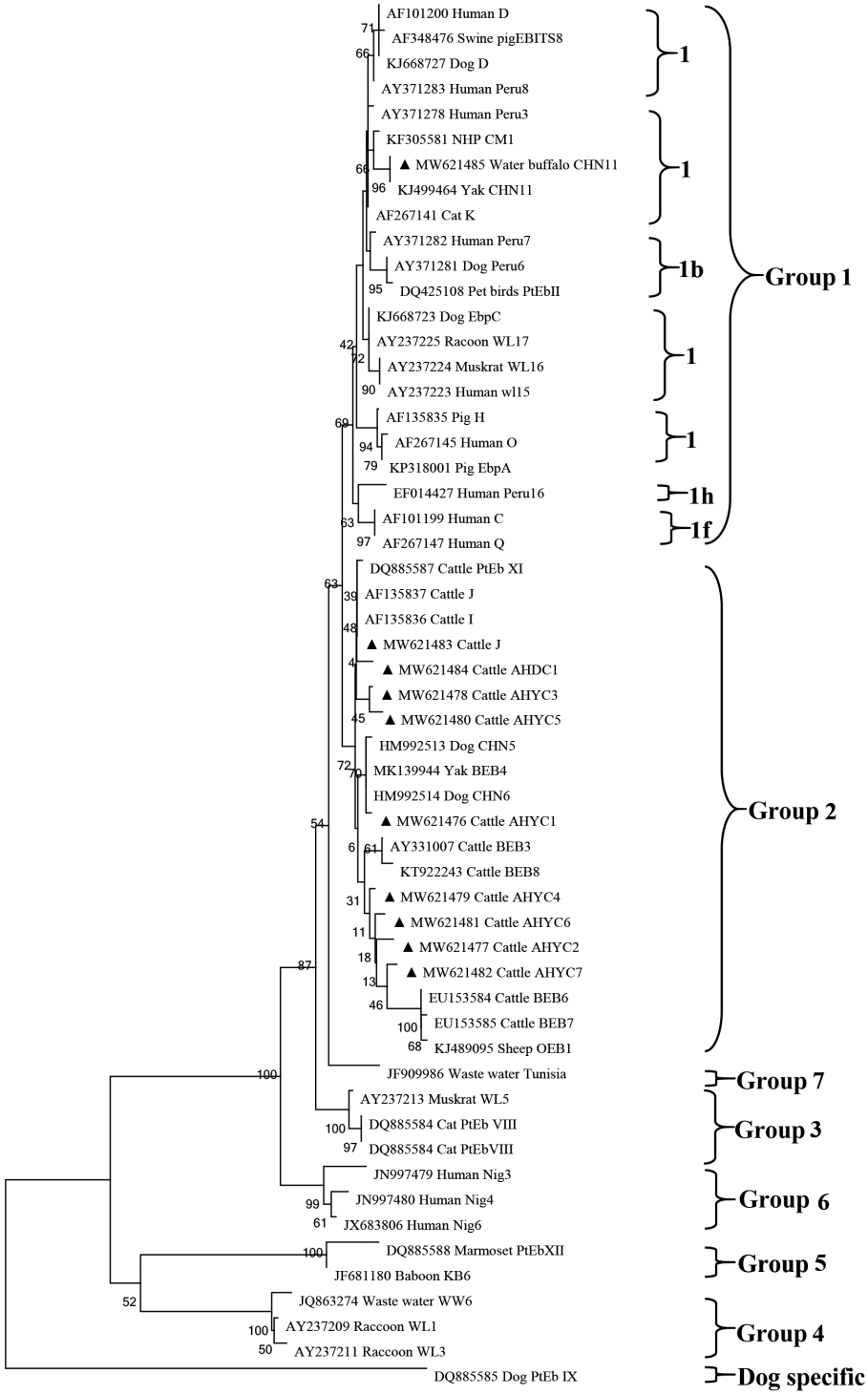

Fig. 2. Phylogenetic analysis of *Enterocytozoon bieneusi* internal transcribed spacer genotypes from different isolates. The *E. bieneusi* genotypes isolated in this study are indicated by triangles.

AHYC7 (1/40). In water buffalo, only genotype CHN11 (1/40) was detected. The phylogenetic tree result showed that all genotypes identified in this study belonged to Group 2 except for genotype CHN11, which belonged to the zoonotic Group 1 (Fig. 2).

Risk factors for the infection of C. andersoni and E. bieneusi in cattle

For *C. andersoni*, there was a significant difference in the samples collected from differently aged individuals (χ^2=21.1, P<0.001), and pre-weaned calves had a much higher infection rate than other age groups. In addition, the positive rate was also significantly higher (χ^2=6.0, P=0.014) in the abnormal fecal group compared with that in the normal fecal group. Alternatively, for

E. bieneusi, no significant difference was identified among different groups of ages, fecal forms, and sampling areas. Among the positive samples of *C. andersoni* and *E. bieneusi*, there was no co-infection case. The detailed information is shown in Table 2.

DISCUSSION

Cryptosporidium spp. and *E. bieneusi* are intestinal opportunistic pathogens that infect a wide range of host species, including pets [22, 41], rodents [20], wildlife [1, 16], livestock [1, 21] and humans [33]. Numerous studies have indicated that these two pathogens were responsible for diarrhea, and had a high prevalence in cattle among livestock [11, 21]. However, in China, some studies mainly focus on the infection of yellow cattle and dairy cattle [8, 11, 34, 43], but few data are available on the molecular characterization of these two pathogens in water buffalo, especially in Anhui Province. Therefore, in this study, the infections of *Cryptosporidium* spp. and *E. bieneusi* in dairy cattle, yellow cattle, and water buffalo were detected and analyzed.

The average infection rate of *Cryptosporidium* spp. in this study was 2.1%, which was lower than that in Northern Thailand (7.6%), India (30.2%), Egypt (13.6%), and Northeast China (6.4%) [2, 11, 19, 24], but higher than that in Ningxia, China (1.6%) [9]. The average prevalence of *E. bieneusi* in cattle in Anhui Province was 4.2%, which was lower than that in Turkey (4.5%), Korea (15.0%), and Hainan Province (9.9%) [14, 38, 45]. In general, the infection rates of these two pathogens in Anhui Province were relatively low, which was probably due to that the feces after enrichment of oocysts/spores were used to extract the genomic DNA in other studies [19, 45]. However, the genomic DNAs were directly extracted from untreated fecal samples in this study. Furthermore, the Stool DNA Kits used in this study were different from those in the above reports, and the samples in this study were from large-scale farms with good breeding and management conditions, which may be also factors involved in the low infection rates.

Molecular studies have identified a wide range of *Cryptosporidium* species in cattle worldwide. The four major species in cattle are *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*, among which *C. parvum* is one of the most prevalent and pathogenic species in humans [6]. In this study, all of the positive samples of *Cryptosporidium* were identified as *C. andersoni*, consistent with the reports in Heilongjiang Province and Shanxi Province [19, 43]. However, our results differed from those in Henan and Ningxia Provinces [9, 32], in which more than one species of *Cryptosporidium* was detected, with *C. parvum* or *C. bovis* as the dominant species. This finding indicated that the distribution of *Cryptosporidium* species in cattle has regional characteristics, and the zoonotic transmission potential of *Cryptosporidium* spp. from cattle may be low in Anhui Province.

In this study, *C. andersoni* was found in all the age groups, which was consistent with the result in Czech Republic [12]. Pre-weaned calves in this study had a higher infection rate of *C. andersoni* than that in other age groups, while in other studies *C. andersoni* was commonly detected in older cattle [12, 43]. The result may due to the small number of samples examined (only 42 pre-weaned calves) in this study. In addition, four of the five positive samples of *C. andersoni* in pre-weaned calves were collected from Fengyang1 farm with a high prevalence of *C. andersoni*. The positive rate of *C. andersoni* was significantly higher in abnormal compared with normal feces in this study, which was inconsistent with the results in United Kingdom, in Estonia, and Sweden [13, 25, 29], those indicated that *C. andersoni* could not be associated with the presence of diarrhea. The difference of this result may be related to that in this study only *Cryptosporidium* spp. and *E. bieneusi* were detected, while other pathogens may be co-infected in the samples, this need to be further studied.

In the dairy cattle, yellow cattle, and water buffalo of this study, the infections of *E. bieneusi* varied significantly: yellow cattle had a much higher infection rate than dairy cattle and water buffalo, and the infection rate of *E. bieneusi* in yellow cattle in Anhui Province was higher than that in Tibet (2.5%) [35]. However, the infection rates of *E. bieneusi* in dairy cattle and water buffalo were lower than those previously reported in Northeast China (30.1%), Henan and Ningxia Provinces (24.3%), and Turkey (4.5%) [15, 38, 44]. These results indicated that the specie of cattle may influence the infection of *E. bieneusi*, while the infection can also be affected by the different degree of *E. bieneusi* contamination in soil of each farm. Therefore, further researches are needed to study the susceptibility of the species of cattle to *E. bieneusi*, and the prevention and control of *E. bieneusi* in yellow cattle should be strengthened in Anhui Province.

The infection of *E. bieneusi* was detected in all age groups, among which post-weaned and pre-weaned calves had the highest and lowest infection rate, respectively, which was agreement with the age pattern described in the studies in Maryland, Henan, Hebei and Tianjin [8, 30, 39]. However, no difference was observed among the age groups of the infection of *E. bieneusi*. Furthermore, there was also no difference in the infection of *E. bieneusi* between normal and abnormal feces groups, which was consistent with previous findings in Brazil, Guangdong and Maryland [5, 7, 30], those indicated the infection of *E. bieneusi* was not associated with the occurrence of any clinical signs in infected cattle. However, a few studies indicated that *E. bieneusi* can cause diarrhea in pre-weaned cattle [10]. The differences of these results may be related to the age of cattle, and the immune system is not fully developed in pre-weaned calves.

The ITS sequencing has been generally used for the genotype of *E. bieneusi* isolated from many kinds of hosts [28]. In the 40 positive samples of *E. bieneusi* detected in this study, 10 genotypes were identified, including two known genotypes and eight novel genotypes. The dominant genotype in this report was genotype J, and this genotype was also detected in dairy cattle in Jiangsu Province [31], probably due to the geographical and climatic proximity of these two provinces. In other research, genotypes O and ALP3 were the dominant genotypes in Northeast China and Xinjiang [42, 44], respectively, whereas genotype I was the most prevalent genotype in Hebei Province, Shanxi Province, and Tibet [8, 34, 35]. These results indicated that different regions had their unique genotypes of *E. bieneusi*. The novel genotypes isolated in this study were mainly detected in yellow cattle, except for AHDC1, which was found in dairy cattle. These findings showed the genetic diversity of *E. bieneusi* in cattle, especially

in yellow cattle from Anhui Province. Furthermore, only one genotype identified in this study belonged to Group 1, and the others belonged to Group 2. Therefore, it seems that the zoonotic potential of *E. bieneusi* in cattle is low in Anhui Province [17]. However, most of the Group 2 genotypes were recently isolated in humans, which indicated the zoonotic potential [17]. Thus the genotypes in Group 2 detected in this study may be also responsible for zoonotic transmission, and this needs further investigation.

In conclusion, this study demonstrated that *Cryptosporidium* spp. and *E. bieneusi* are prevalent among dairy cattle, yellow cattle, and water buffalo in Anhui Province, China. *C. andersoni* was the only detected species of *Cryptosporidium* in this study, and its infection differed significantly among the ages and fecal forms of cattle. The 10 genotypes of *E. bieneusi* obtained in this study were clustered into Groups 1 and 2, which indicates that cattle may be responsible for the zoonotic transmission of *E. bieneusi*. These findings have important implications for the disease management of *Cryptosporidium* spp. and *E. bieneusi* in cattle in Anhui Province, China.

CONFLICT OF INTEREST. The authors declare no conflict of interest in relation with this paper.

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REFERENCES

1. Abu Samra, N., Thompson, P. N., Jori, F., Zhang, H. and Xiao, L. 2012. *Enterocytozoon bieneusi* at the wildlife/livestock interface of the Kruger National Park, South Africa. *Vet. Parasitol.* **190**: 587–590. [Medline] [CrossRef]
2. Amer, S., Zidan, S., Adamu, H., Ye, J., Roellig, D., Xiao, L. and Feng, Y. 2013. Prevalence and characterization of *Cryptosporidium* spp. in dairy cattle in Nile River delta provinces, Egypt. *Exp. Parasitol.* **135**: 518–523. [Medline] [CrossRef]
3. Ayinmode, A. B., Zhang, H., Dada-Adegbola, H. O. and Xiao, L. 2014. *Cryptosporidium hominis* subtypes and *Enterocytozoon bieneusi* genotypes in HIV-infected persons in Ibadan, Nigeria. *Zoonoses Public Health* **61**: 297–303. [Medline] [CrossRef]
4. Bernard, E., Carles, M., Pradier, C., Boissy, C., Roger, P. M., Hebuterne, X., Mondain, V., Michiels, J. F., Le Fichoux, Y. and Dellamonica, P. 1995. [Persistent diarrhea in HIV infected patients: role of *Enterocytozoon bieneusi*]. *Presse Med.* **24**: 671–674. [Medline]
5. Feng, Y., Gong, X., Zhu, K., Li, N., Yu, Z., Guo, Y., Weng, Y., Kváč, M., Feng, Y. and 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. [Medline] [CrossRef]
6. Feng, Y., Ryan, U. M. and Xiao, L. 2018. Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol.* **34**: 997–1011. [Medline] [CrossRef]
7. da Silva Fiuza, V. R., Lopes, C. W., de Oliveira, F. C., Fayer, R. and Santin, M. 2016. New findings of *Enterocytozoon bieneusi* in beef and dairy cattle in Brazil. *Vet. Parasitol.* **216**: 46–51. [Medline] [CrossRef]
8. Hu, S., Liu, Z., Yan, F., Zhang, Z., Zhang, G., Zhang, L., Jian, F., Zhang, S., Ning, C. and 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. [Medline] [CrossRef]
9. Huang, J., Yue, D., Qi, M., Wang, R., Zhao, J., Li, J., Shi, K., Wang, M. and 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. [Medline] [CrossRef]
10. Hwang, S., Shin, S. U., Kim, S., Ryu, J. H. and Choi, K. S. 2020. Zoonotic potential of *Enterocytozoon bieneusi* in pre-weaned Korean native calves. *Parasit. Vectors* **13**: 300. [Medline] [CrossRef]
11. Inpankaew, T., Jiyipong, T., Sunanta, C., Kengradomkij, C., Pinyopanuwat, N. and Jittapalapong, S. 2017. Prevalence and molecular characterization of bovine *Cryptosporidium* from dairy cows in Northern Thailand. *Acta Parasitol.* **62**: 772–774. [Medline] [CrossRef]
12. Kvác, M., Kouba, M. and Vítovec, J. 2006. Age-related and housing-dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Vet. Parasitol.* **137**: 202–209. [Medline] [CrossRef]
13. Lassen, B., Viltrop, A., Raaperi, K. and Järvis, T. 2009. *Eimeria* and *Cryptosporidium* in Estonian dairy farms in regard to age, species, and diarrhoea. *Vet. Parasitol.* **166**: 212–219. [Medline] [CrossRef]
14. Lee, J. H. 2007. Prevalence and molecular characteristics of *Enterocytozoon bieneusi* in cattle in Korea. *Parasitol. Res.* **101**: 391–396. [Medline] [CrossRef]
15. Li, J., Luo, N., Wang, C., Qi, M., Cao, J., Cui, Z., Huang, J., Wang, R. and Zhang, L. 2016. Occurrence, molecular characterization and predominant genotypes of *Enterocytozoon bieneusi* in dairy cattle in Henan and Ningxia, China. *Parasit. Vectors* **9**: 142. [Medline] [CrossRef]
16. Li, J., Qi, M., Chang, Y., Wang, R., Li, T., Dong, H. and Zhang, L. 2015. Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou Zoo, China. *J. Eukaryot. Microbiol.* **62**: 833–839. [Medline] [CrossRef]
17. Li, W., Feng, Y. and Santin, M. 2019. Host specificity of *Enterocytozoon bieneusi* and public health implications. *Trends Parasitol.* **35**: 436–451. [Medline] [CrossRef]
18. Li, W. C., Huang, J. M., Fang, Z., Ren, Q., Tang, L., Kan, Z. Z., Liu, X. C. and Gu, Y. F. 2020. Prevalence of *Tetratrichomonas buttreyi* and *Pentatrichomonas hominis* in yellow cattle, dairy cattle, and water buffalo in China. *Parasitol. Res.* **119**: 637–647. [Medline] [CrossRef]
19. Liu, A., Wang, R., Li, Y., Zhang, L., Shu, J., Zhang, W., Feng, Y., Xiao, L. and Ling, H. 2009. Prevalence and distribution of *Cryptosporidium* spp. in dairy cattle in Heilongjiang Province, China. *Parasitol. Res.* **105**: 797–802. [Medline] [CrossRef]
20. Lv, C., Zhang, L., Wang, R., Jian, F., Zhang, S., Ning, C., Wang, H., Feng, C., Wang, X., Ren, X., Qi, M. and Xiao, L. 2009. *Cryptosporidium* spp. in wild, laboratory, and pet rodents in china: prevalence and molecular characterization. *Appl. Environ. Microbiol.* **75**: 7692–7699. [Medline] [CrossRef]
21. Ma, J., Li, P., Zhao, X., Xu, H., Wu, W., Wang, Y., Guo, Y., Wang, L., Feng, Y. and Xiao, L. 2015. Occurrence and molecular characterization of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in dairy cattle, beef cattle and water buffaloes in China. *Vet. Parasitol.* **207**: 220–227. [Medline]

[CrossRef]
22. Morgan, U. M., Xiao, L., Monis, P., Fall, A., Irwin, P. J., Fayer, R., Denholm, K. M., Limor, J., Lal, A. and Thompson, R. C. A. 2000. *Cryptosporidium* spp. in domestic dogs: the "dog" genotype. *Appl. Environ. Microbiol.* **66**: 2220–2223. [Medline] [CrossRef]
23. Muadica, A. S., Messa, A. E. Jr., Dashti, A., Balasegaram, S., Santin, M., Manjate, F., Chirinda, P., Garrine, M., Vubil, D., Acácio, S., Köster, P. C., Bailo, B., Nhampossa, T., Calero-Bernal, R., Mwenda, J. M., Mandomando, I. and Carmena, D. 2020. First identification of genotypes of *Enterocytozoon bieneusi* (Microsporidia) among symptomatic and asymptomatic children in Mozambique. *PLoS Negl. Trop. Dis.* **14**: e0008419. [Medline] [CrossRef]
24. Paul, S., Chandra, D., Ray, D. D., Tewari, A. K., Rao, J. R., Banerjee, P. S., Baidya, S. and Raina, O. K. 2008. Prevalence and molecular characterization of bovine *Cryptosporidium* isolates in India. *Vet. Parasitol.* **153**: 143–146. [Medline] [CrossRef]
25. Robinson, G., Thomas, A. L., Daniel, R. G., Hadfield, S. J., Elwin, K. and Chalmers, R. M. 2006. Sample prevalence and molecular characterisation of Cryptosporidium andersoni within a dairy herd in the United Kingdom. *Vet. Parasitol.* **142**: 163–167. [Medline] [CrossRef]
26. Ryan, U., Fayer, R. and Xiao, L. 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitology* **141**: 1667–1685. [Medline] [CrossRef]
27. Santín, M. and Fayer, R. 2009. A longitudinal study of *Enterocytozoon bieneusi* in dairy cattle. *Parasitol. Res.* **105**: 141–144. [Medline] [CrossRef]
28. Santín, M. and Fayer, R. 2009. *Enterocytozoon bieneusi* genotype nomenclature based on the internal transcribed spacer sequence: a consensus. *J. Eukaryot. Microbiol.* **56**: 34–38. [Medline] [CrossRef]
29. Silverlås, C., Näslund, K., Björkman, C. and Mattsson, J. G. 2010. Molecular characterisation of *Cryptosporidium* isolates from Swedish dairy cattle in relation to age, diarrhoea and region. *Vet. Parasitol.* **169**: 289–295. [Medline] [CrossRef]
30. Sulaiman, I. M., Fayer, R., Lal, A. A., Trout, J. M., Schaefer, F. W. 3rd. and 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. [Medline] [CrossRef]
31. Wang, R., Li, N., Jiang, W., Guo, Y., Wang, X., Jin, Y., Feng, Y. and Xiao, L. 2019. Infection patterns, clinical significance, and genetic characteristics of *Enterocytozoon bieneusi* and *Giardia duodenalis* in dairy cattle in Jiangsu, China. *Parasitol. Res.* **118**: 3053–3060. [Medline] [CrossRef]
32. Wang, R., Wang, H., Sun, Y., Zhang, L., Jian, F., Qi, M., Ning, C. and Xiao, L. 2011. Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. *J. Clin. Microbiol.* **49**: 1077–1082. [Medline] [CrossRef]
33. Wang, R., Zhang, X., Zhu, H., Zhang, L., Feng, Y., Jian, F., Ning, C., Qi, M., Zhou, Y., Fu, K., Wang, Y., Sun, Y., Wang, Q. and Xiao, L. 2011. Genetic characterizations of *Cryptosporidium* spp. and *Giardia duodenalis* in humans in Henan, China. *Exp. Parasitol.* **127**: 42–45. [Medline] [CrossRef]
34. Wang, X. T., Wang, R. J., Ren, G. J., Yu, Z. Q., Zhang, L. X., Zhang, S. Y., Lu, H., Peng, X. Q. and Zhao, G. H. 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. [Medline] [CrossRef]
35. Wu, Y., Chen, Y., Chang, Y., Zhang, X., Li, D., Wang, L., Zheng, S., Wang, R., Zhang, S., Li, J. and Zhang, L. 2020. Genotyping and identification of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* from free-range Tibetan yellow cattle and cattle-yak in Tibet, China. *Acta Trop.* **212**: 105671. [Medline] [CrossRef]
36. Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A. A., Montali, R. J., Fayer, R. and Lal, A. A. 1999. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. *Appl. Environ. Microbiol.* **65**: 1578–1583. [Medline] [CrossRef]
37. Xiao, L. and Feng, Y. 2008. Zoonotic cryptosporidiosis. *FEMS Immunol. Med. Microbiol.* **52**: 309–323. [Medline] [CrossRef]
38. Yildirim, Y., Al, S., Duzlu, O., Onmaz, N. E., Onder, Z., Yetismis, G., Hizlisoy, H., Gonulalan, Z. and Yildirim, A. 2020. *Enterocytozoon bieneusi* in raw milk of cattle, sheep and water buffalo in Turkey: genotype distributions and zoonotic concerns. *Int. J. Food Microbiol.* **334**: 108828. [Medline] [CrossRef]
39. Yu, F., Qi, M., Zhao, Z., Lv, C., Wang, Y., Wang, R. and 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. [Medline] [CrossRef]
40. Yulfi, H., Fakhrur Rozi, M., Andriyani, Y. and Masyithah Darlan, D. 2021. Prevalence of *Cryptosporidium* spp. and *Blastocystis hominis* in faecal samples among diarrheic HIV patients in Medan, Indonesia. *Med Glas (Zenica)* **18**: 55–61. [Medline]
41. Zhang, H., Lin, Z., Jiang, Y., Qian, W., Lv, C., Zhang, L., Wang, S., Qi, M. and Xia, Z. 2020. Molecular identification of *Cryptosporidium* spp. in pet snakes in Beijing, China. *Parasitol. Res.* **119**: 3119–3123. [Medline] [CrossRef]
42. Zhang, Q., Wang, H., Zhao, A., Zhao, W., Wei, Z., Li, Z. and Qi, M. 2019. Molecular detection of *Enterocytozoon bieneusi* in alpacas (*Vicugna pacos*) in Xinjiang, China. *Parasite* **26**: 31. [Medline] [CrossRef]
43. Zhao, G. H., Ren, W. X., Gao, M., Bian, Q. Q., Hu, B., Cong, M. M., Lin, Q., Wang, R. J., Qi, M., Qi, M. Z., Zhu, X. Q. and Zhang, L. X. 2013. Genotyping *Cryptosporidium andersoni* in cattle in Shaanxi province, Northwestern China. *PLoS One* **8**: e60112. [Medline] [CrossRef]
44. Zhao, W., Zhang, W., Yang, F., Zhang, L., Wang, R., Cao, J., Shen, Y. and Liu, A. 2015. *Enterocytozoon bieneusi* in dairy cattle in the Northeast of China: genetic diversity of ITS gene and evaluation of zoonotic transmission potential. *J. Eukaryot. Microbiol.* **62**: 553–560. [Medline] [CrossRef]
45. Zheng, X. L., Zhou, H. H., Ren, G., Ma, T. M., Cao, Z. X., Wei, L. M., Liu, Q. W., Wang, F., Zhang, Y., Liu, H. L., Xing, M. P., Huang, L. L., Chao, Z. and Lu, G. 2020. Genotyping and zoonotic potential of *Enterocytozoon bieneusi* in cattle farmed in Hainan Province, the southernmost region of China. *Parasite* **27**: 65. [Medline] [CrossRef]