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ORIGINAL PAPER

# Molecular Detection and Characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in Dairy Calves from Ningxia, China

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## Abstract

**Purpose** *Cryptosporidium* spp. and *Giardia duodenalis* are two important foodborne human and animal parasites that can be disseminated through both food and water, leading to diarrheal disease. Nevertheless, available information on the circumstances of *Cryptosporidium* and *Giardia duodenalis* from Ningxia is limited.

**Methods** A total of 208 stool samples of dairy calves derived from large-scale farms (> 1000 heads) of five cities randomly in Ningxia were gathered randomly, were amplified and analyzed by nested PCR based on the three target genes (18S rRNA, gp60 and tpi)and phylogenetic systematics.

**Results** The prevalence of cryptosporidiosis and giardiasis in dairy calves in Ningxia were 13.0% (27/208 samples, 95% CI 9.1–18.2%) and 1.9% (4/208, 95% CI 0.8–4.9%) respectively. Three *Cryptosporidium* species appeared in this study which are *Cryptosporidium parvum* (*C. parvum*), *Cryptosporidium andersoni* (*C. andersoni*) and *Cryptosporidium ryanae* (*C. ryanae*) based on the 18S rRNA gene sequence. IIdA15G1 and IIdA13G1 belonging to the subtypes of *Cryptosporidium* were detected by the *gp60* PCR. The genotypes of *Giardia duodenalis* were only assemblage E through the amplification of the triosephosphate-isomerase gene (*tpi* gene).

**Conclusion** There is a risk of transmission to humans in Ningxia because of zoonotic genotypes (*C. parvum, C. andersoni,* assemblage E) and subtypes (IId) of *Cryptosporidium* spp. and *G. duodenalis* in dairy calves, and it is necessary to pay attention to the disease to prevent a widespread epidemic of the disease with the purpose to protect human and livestock health.

**Keywords** *Cryptosporidium* · *Giardia duodenalis* · Zoonotic pathogen · Dairy calves · Ningxia

## Introduction

*Cryptosporidium* and *G. duodenalis* are two kinds of zoonotic parasites commonly found in a majority of countries which transmit by food and water, cause diarrhea in humans and animals, and can lead to fatality in severe cases [1, 2]. Dairy calves infected with *Cryptosporidium* and *G. duodenalis* can suffer from growth retardation due to diarrhea, thus causing huge economic losses to the cattle industry [3]. *C. parvum*, *C. andersoni*, *C. ryanae* and *Cryptosporidium bovis* (*C. bovis*) are the four commonest *Cryptosporidium* species in cattle [4]. The cattle of diverse age stages are infected by the different *Cryptosporidium* species [1]. For instance, *C. parvum* was detected predominantly in pre-weaned (< 2 months) calves, as compared to *C. ryanae* or *C. bovis* in post-weaned calves and *C. andersoni* in adults [2.4]. *G.duodenalis* has eight genotypes, especially, assemblage E is frequently observed in calves [3]. The above two *Cryptosporidium* (*C. parvum, C. andersoni*) and *G.duodenalis* (assemblage E) can infect humans, however,

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*C. ryanae* has not been found in humans currently [5–7]. The presence of *Cryptosporidium* and *G.duodenalis* varies in dairy calves in different regions due to geographical location, stocking density and other factors [8, 9]. *Cryptosporidium* and *G.duodenalis* extends a majority of hosts, including cattle, sheep, goat, pigs, cats, dogs, horses and other animals [10, 11]. The transmission risk of cryptosporidiosis and giardiasis in humans is increasing because of the frequent contact between hosts such as cats and dogs and them [11]. Cryptosporidium capable of infecting humans mainly includes *Cryptosporidium hominis* (*C. hominis)*, *C. andersoni*, *C. parvum*, *Cryptosporidium meleagridis* (*C. meleagridis*), *Cryptosporidium felis* (*C. felis*), *Cryptosporidium canis* (*C. canis*) and *Cryptosporidium suis* (*C. suis*) [12, 13]. Among them, *C. hominis* and *C. parvum* have the most serious impact on humans [5]. The zoonotic genotypes of *G. duodenalis* include assemblages A, B, C, D, E and F, A and B among them are highly prevalent in humans [14]. To date, there is still a lack of effective methods for the prevention and control of cryptosporidiosis and giardiasis [15, 16].

The climatic circumstance of Ningxia located in northwest China promotes the cultivation of farming including dairy farming which has become one of the important mainstays of the economy. The degree of intensification of dairy farming and poor sanitation in livestock breeding is associated with an increased infection risk of *Cryptosporidium* and *G. duodenalis*, and the probability of the two parasites transmitting to humans escalates during the rainy season and in areas of high population density [13]. Therefore, corresponding preventive measures should be taken against these two kinds of parasitosis to maintain public health. In recent years, various species of *Cryptosporidium* and *G. duodenalis* have been found on livestock and people throughout the whole country, thus we need to regularly check the prevalence of them in livestock and the surrounding environment, with special attention to the prevalence of human-animal [14].

In this study, we randomly collected limited fecal samples from dairy calves between September 2021 and May 2023 and performed PCR amplification, genotype and genotype subtype analyses as a means of demonstrating the limited infections of *Cryptosporidium* and *G.duodenalis* in dairy calves in Ningxia, as well as updating the limited presence of genotypes and subtypes of the two diseases present in the Ningxia region.

## Materials and Methods

### Ethical Statement

This study was approved by the Animal Ethics Committee of Ningxia University, NXU (No. NXU 2021-042). The use of these field samples was approved by the Animal Ethics Procedures and Guideline of China.

### Specimen Collection

Some of the large-scale farms in Ningxia were used as the research object, in which the number of large-scale dairy cattle required was more than 1000 heads, and each farm took a sampling method of randomly for the collection of fecal samples. 208 fresh stool samples collected from the rectums of dairy calves at random on the randomized large-scale cattle farm from Wuzhong city, Zhongwei city, Yinchuan city, Shizuishan city, Guyuan city belonging to Ningxia between September 2021 and May 2023, were recorded in sterile bags and taken to the given laboratory, after that they were conserved at -80 °C for testing. Among them, Wuzhong city sampled five farms, Zhongwei city sampled two farms, Yinchuan city sampled six farms, Shizuishan city sampled one farm and Guyuan city sampled three farms and the sampling quantity of each farm (Fig. 1). Of these 208 dairy calves, they could be categorized as pre- and post-weaning or with or without diarrhea, with 107 and 101 pre- and post-weaning dairy calves, respectively, and with or without diarrhea dairy calves collected in numbers of 125 and 83. These samples in this study have obtained the permission of the farmers.

### DNA Extraction

The fecal samples were extracted for PCR whose procedure was performed on the grounds of the manual of Stool DNA Kit belonging to Omega (Takara, Beijing, China).

### Nested PCR Amplification and Sequencing

The amplification of the 18S rRNA gene (~ 819–825 bp) confirmed the presence of *Cryptosporidium*. *C. parvum* was subtyped by the *gp60* gene (~ 820–864 bp). All three aforementioned genes were amplified using Nested PCR amplification as well as Sanger sequencing. The primers and conditions used for nested PCR of the three genes are revealed in the Table 1. The primers of gp60 gene and 18S rRNA gene refer to this two references respectively [2, 13], including the procedure of PCR to amplify these two genes.

The presence of *G. duodenalis* was confirmed using amplification of the tpi gene (530 bp) (Table 1) [5]. The nested PCR system, the gel recovery and the sequencing for these three genes are described below.

Each PCR mixture (total volume, 25 µL) contained 12.5 µL Taq PCR Premix (Takara, Beijing, China), 8.5 µL ddH2O, 1 µL (each) primer (2.5µmol/L) and 2 µL DNA of samples. After the PCR mixture was prepared, the first round of PCR reaction was carried out according to Table 1, and then the products obtained in the first round

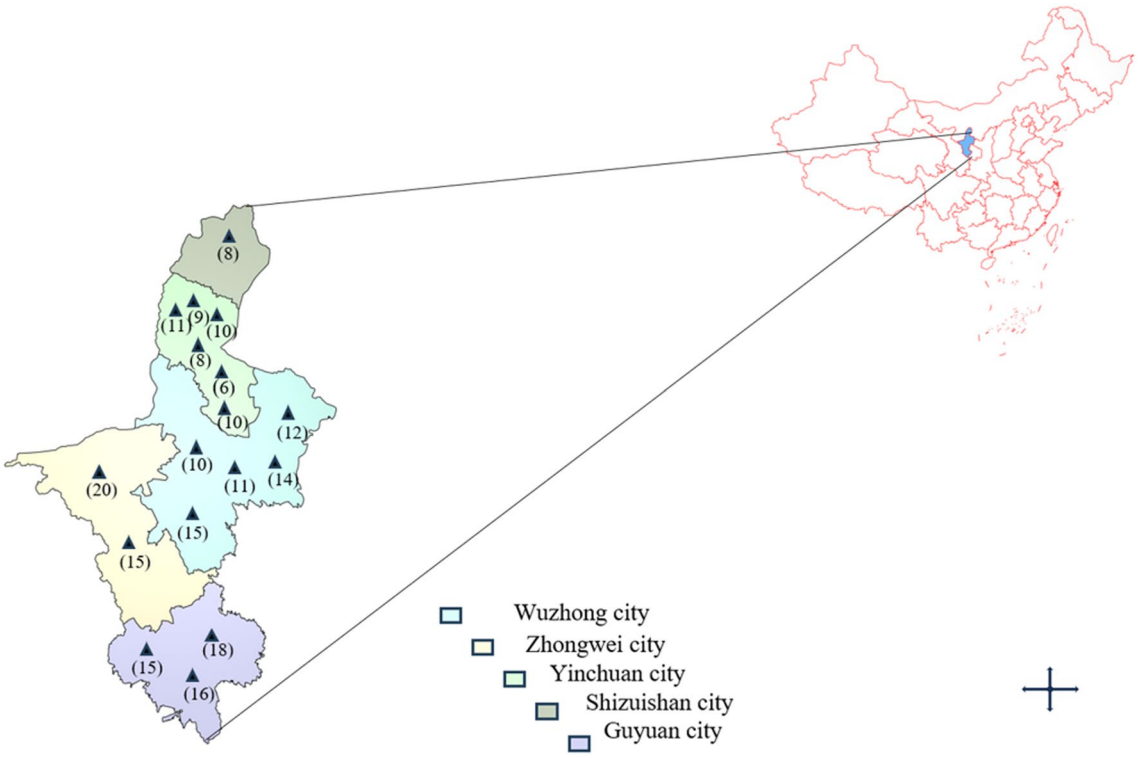


**Fig. 1** Locations of the seventeen farms in Ningxia from which faecal samples (numbers in parentheses) were collected by dairy calves and tested using PCR-based methods

**Table 1** Sequences of oligonucleotide primers and the condition for the PCR reaction

| Pathogen | Target gene | Primers | Oligonucleotide sequences (5′-3′) | Amplicon size | Condition for PCR |
|---|---|---|---|---|---|
| *Cryptosporidium* | 18S rRNA | F1 | TTCTAGAGCTAATACATGCG | | |
| | | R1 | CCCTAATCCTTCGAAACAGGA | | 94 °C/5 min—94 °C/45 s, 56 °C/45 s, 72 °C/1 min (35 cycles)—72 °C/7 min |
| | | F2 | GGAAGGGTTGATTTATTAGATAAAG | | |
| | | R2 | AAGGAGTAGGAAACAACCTCCA | ~830–840 bp | 94 °C/5 min—95 °C/45 s, 60 °C/45 s, 72 °C/1 min (35 cycles)—72 °C/7 min |
| *C. parvum* | *gp60* | F1 | ATAGTCTCCGCTGTATTC | | |
| | | R1 | GGAAGGAACGATGTATCT | | 95 °C/3 min—95 °C/45 s, 52 °C/45 s, 72 °C/1 min (40 cycles)—72 °C/10 min |
| | | F2 | TCCGCTGTATTCTCAGCC | | |
| | | R2 | GCAGAGGAACCAGCATC | ~820–850 bp | 95 °C/3 min—95 °C/45 s, 52 °C/45 s, 72 °C/1 min (40 cycles)—72 °C/10 min |
| *G. duodenalis* | *tpi* | F1 | AAATTATGCCTGCTCGTCG | | |
| | | R1 | CAAACCTTTTCCGCAAACC | | 94 °C/5 min—94 °C/45 s, 50 °C/45 s, 72 °C/1 min (35 cycles)—72 °C/10 min |
| | | F2 | CCCTTCATCGGTGGTAACTT | | |
| | | R2 | GTGGCCACCACTCCCGTGCC | 530 bp | 94 °C/5 min—94 °C/45 s, 55 °C/30 s, 72 °C/1 min (35 cycles)—72 °C/10 min |

were diluted 100-fold for the second round of PCR reaction. Two µL diluted PCR product is needed in the process of the secondary PCR step. For example, when amplifying 18S rRNA, the first round of reaction conditions were 94 °C pre-denaturation 45 min; 94 °C denaturation 45 s, 56 °C annealing 45 s, 72 °C extension 1 min, 35 cycles; 72 °C extension 7 min. 100-fold dilution of the first round of the product, 2 µl of the product added to the second round of the

system, the second round of the reaction conditions of the deformation temperature will be 94–95 °C, annealing temperature from 56 to 60 °C. After the amplification of nested PCR, 5 µL of products were inspected by 1.0% agarose gel electrophoresis. Clear and neat specific target bands could be amplified through electrophoretic detection, and there were no other non-specific bands. All target fragments were purified by the DNA Gel Extraction Kit of DiaSpin and were sent to Sangon (Shanghai, China) for Sanger sequencing.

### Subtyping of *Cryptosporidium* parvum Based the gp60 Gene

To date, the most commonly site which has polymorphism used subtyping for Cryptosporidium parvum is *gp60*. There are many different subtypes of Cryptosporidium parvum, such as IIa, IId, etc., including different the capability of transmission. The subtype is named by the number of trinucleotide repeat which are mainly TCA, TCG, or TCT and respectively represented by A, G, T [17]. Based on the number and type of trinucleotide repeat sequences, it is possible to classify subtypes of *C. parvum*. For example, IIdA19G1 indicates that it belongs to the IId subtype, with 19 consecutive TCA repeats and 1 TCG repeats.

### Phylogenetic Analyses

The sequencing was not successful for all positive samples, with 27 Cryptosporidium positives sent out and only 18 sequences obtained. Eighteen partial 18S rRNA sequences detected were deposited in GenBank under the accession nos. OQ923674-OQ923679, OQ926625-OQ926633 and OR363649-OR363651. The GenBank accession numbers for the ten *gp60* and four *tpi* sequences that have been uncovered are OR464713-OR464726. The obtained sequences were compared with 18S rRNA sequences of Cryptosporidium available in GenBank. Similar comparisons were also made with the obtained sequences of the *tpi* genes (OR464713-OR464716) and *gp60* genes (OR464717- OR464726). Then, phylogenetic trees were constructed using the neighbor-joining algorithm in the MEGA7.0 software, Specifically, firstly, sequence matching is performed, with parameters kept at default, and secondly, the neighbor-joining algorithm is utilized as a means of constructing an evolutionary tree.

### Statistical Analysis

The $\chi^2$ test was used to determine the correlation between the prevalence of *Cryptosporidium* infection and pre/post-weaning as well as sample size utilizing SPSS software on the basis of method of explanation. Differences were considered significant at $P < 0.05$. $P < 0.05$ suggests that the two are correlated.

## Results

### Prevalence of *Cryptosporidium* spp. and *G. duodenalis*

Positive samples were detected by PCR in 27 of 208 samples (7 from Wuzhong, 3 from Zhongwei, 3 from Yinchuan, 3 from Shizuishan and 11 from Guyuan). 12.98% (27/208) of the infection rate of *Cryptosporidium* in dairy calves is revealed in Ningxia (Table 2). Pre-weaned calves (19.6% of infection rate) demonstrated a significantly higher prevalence of infection with Cryptosporidium spp. compared to post-weaned calves (5.9%) ($\chi^2 = 8.62$, $P < 0.01$) (Table 2).

Four out of 208 samples (2 from Yinchuan, 1 from Shizuishan, 1 from Guyuan) were analyzed positively by PCR to detect *G.duodenalis*. The proportion of *G.duodenalis* infection in dairy calves in Ningxia was 1.92% (4/208) (Table 3). *G. duodenalis* infection was not associated with pre/post-weaned ($\chi^2 = 2.1$, $P > 0.05$).

Further analysis of the distribution of *Cryptosporidium* spp. and *G. duodenalis* in diarrheic and non-diarrheic calves of different age groups showed that *Cryptosporidium* spp. and *G. duodenalis* infections in all age groups of calves were not associated with diarrhea ($p > 0.05$). (Table 4).

### Mixed Infection of *Cryptosporidium* and *G. duodenalis*

Among the 208 fecal samples, there were 4 co-infections, meaning that they were infected with *Cryptosporidium* and *G.duodenalis* at the same time. These mixed infections occurred in Yinchuan, Shizuishan and Guyuan in Ningxia (Table 2, Table 3).

### Cryptosporidium Genotypes and Subtypes

The blast indicated Yinchuan-1 (OQ923675) has 99.6% similarity to *C. parvum* (OL378287.1), Yinchuan-2 (OQ926625) with 99.8%, Zhongwei-1 (OQ923676) with 99.5%, Zhongwei-2 (OQ926627) with 99.5%, Zhongwei-3 (OQ926628) with 99.1%, Wuzhong-2 (OQ923677) with 99.5%. Immediately following this, these evolutionary tree are bulit based on blast. By sequences analysis of 18S rRNA gene for them, Cryptosporidium spp., namely, C. parvum, C. andersoni, C. ryanae, were detected in Ningxia (Wuzhong (1 type), Zhongwei (1), Yinchuan (1), Guyuan (3)), which clustered with each of these three species when the 18S rRNA sequences data were analyzed by MEGA

**Table 2** Prevalence of *Cryptosporidium* in samples collected from dairy calves in five cities respectively in Ningxia

| Source | No. positive | No. examined | Percentage (%) | Species (no.) | $\chi^2$ ($\pi-\varsigma\alpha\lambda\upsilon\varepsilon$) |
|---|---|---|---|---|---|
| Wuzhong city | 7 | 62 | 11.3 | *C. parvum* (2) | $\chi^2=5.6$ |
| Pre-weaned (< 3 months) | 4 | 11 | 36.4 | *C. parvum* (2) | $P<0.05$ |
| Post-weaned (3–6) | 3 | 51 | 5.9 | 0 | |
| Zhongwei city | 3 | 35 | 8.6 | *C. parvum* (3) | – |
| Pre-weaned (< 3 months) | 0 | 25 | 0 | 0 | |
| Post-weaned (3–6) | 3 | 10 | 30 | *C. parvum* (3) | |
| Yinchuan city | 3 | 54 | 5.6 | *C. parvum* (2) | $\chi^2=1.0$ |
| Pre-weaned (< 3 months) | 3 | 30 | 10 | C. parvum (2) | $P>0.05$ |
| Post-weaned (3–6) | 0 | 24 | 0 | 0 | |
| Shizuishan city | 3 | 8 | 37.5 | *C. parvum* (3) | – |
| Pre-weaned (< 3 months) | 3 | 8 | 37.5 | *C. parvum* (3) | |
| Post-weaned (3–6) | 0 | 0 | 0 | 0 | |
| Guyuan city | 11 | 49 | 22.5 | *C. parvum* (1), *C. andersoni* (2), *C. ryanae* (5) | $\chi^2=5.1$ |
| Pre-weaned (< 3 months) | 11 | 33 | 33.4 | *C. parvum* (1), *C. andersoni* (2), *C. ryanae* (5) | $P<0.05$ |
| Post-weaned (3–6) | 0 | 16 | 0 | 0 | |
| Total | 27 | 208 | 13 | *C. parvum* (11), *C. andersoni* (2), *C. ryanae* (5) | $\chi^2=8.62$ |
| Pre-weaned (< 3 months) | 21 | 107 | 19.6 | *C. parvum* (8), *C. andersoni* (2), *C. ryanae* (5) | $P<0.01$ |
| Post-weaned (3–6) | 6 | 101 | 5.9 | C. parvum (3) | |

**Table 3** Prevalence of *G.duodenalis* in samples collected from dairy calves in five cities respectively in Ningxia

| Source | No. positive | No. examined | Percentage (%) | Species (no.) | $\chi^2$ ($\pi-\varsigma\alpha\lambda\upsilon\varepsilon$) |
|---|---|---|---|---|---|
| Wuzhong city | 0 | 62 | 0 | 0 | – |
| Pre-weaned (< 3 months) | 0 | 11 | 0 | 0 | |
| Post-weaned (3–6) | 0 | 51 | 0 | 0 | |
| Zhongwei city | 0 | 35 | 0 | 0 | – |
| Pre-weaned (< 3 months) | 0 | 25 | 0 | 0 | |
| Post-weaned (3–6) | 0 | 10 | 0 | 0 | |
| Yinchuan city | 2 | 54 | 3.7 | Assemblage E (2) | $\chi^2=0.3$ |
| Pre-weaned (< 3 months) | 2 | 30 | 6.7 | 0 | $P>0.05$ |
| Post-weaned (3–6) | 0 | 24 | 0 | 0 | |
| Shizuishan city | 1 | 8 | 12.5 | Assemblage E (1) | – |
| Pre-weaned (< 3 months) | 1 | 8 | 12.5 | Assemblage E (1) | |
| Post-weaned (3–6) | 0 | 0 | 0 | 0 | |
| Guyuan city | 1 | 49 | 2 | Assemblage E (1) | $\chi^2=0$ |
| Pre-weaned (< 3 months) | 1 | 33 | 3 | Assemblage E (1) | $P>0.05$ |
| Post-weaned (3–6) | 0 | 16 | 0 | 0 | |
| Total | 4 | 208 | 1.9 | Assemblage E (4) | $\chi^2=2.1$ |
| Pre-weaned (< 3 months) | 4 | 107 | 3.7 | Assemblage E (4) | $P>0.05$ |
| Post-weaned (3–6) | 0 | 101 | 0 | | |

7.0 (Fig. 2). The remaining 11 sequences which are identical to these sequences already in the tree do not appear in the evolutionary tree because of ease of drawing, but have been uploaded to NCBI. Among three Cryptosporidium spp., Cryptosporidium parvum is the predominant one.

By selecting closer relatives as their outgroups and analyzing the *gp60* gene sequence utilizing MEGA 7.0 and referring to the official nomenclature[18], IIdA15G1 and IIdA13G1 existed (Wuzhong (IIdA15G1), Zhongwei (IIdA15G1, IIdA13G1), Yinchuan (IIdA15G1), Shizuishan (IIdA15G1). IIdA15G1 is a kind of primary subtype (Fig. 3).

**Table 4** Prevalence of *Cryptosporidium* spp. and *Giardia duodenali* in diarrheic and non diarrheic calves of different ages in Ningxia

| Species | Sources | No. Positive/Examined | | Percentage (%) | | $\chi^2$ (*p*-value) |
|---|---|---|---|---|---|---|
| | | Diarrheic | Non-Diarrheic | Diarrheic | Non-Diarrheic | |
| *Cryptosporidium spp.* | Pre-weaned (< 3 months) | 15/60 | 6/47 | 25.0 | 12.8 | $\chi^2=0$ $P>0.05$ |
| | Post-weaned (3–6) | 6/65 | –/36 | 9.2 | 0 | $\chi^2=0$ $P>0.05$ |
| *G. duodenalis* | Pre-weaned (< 3 months) | 4/60 | –/47 | 6.7 | 0 | $\chi^2=0$ $P>0.05$ |
| | Post-weaned (3–6) | –/65 | –/36 | 0 | 0 | $\chi^2=0$ $P>0.05$ |

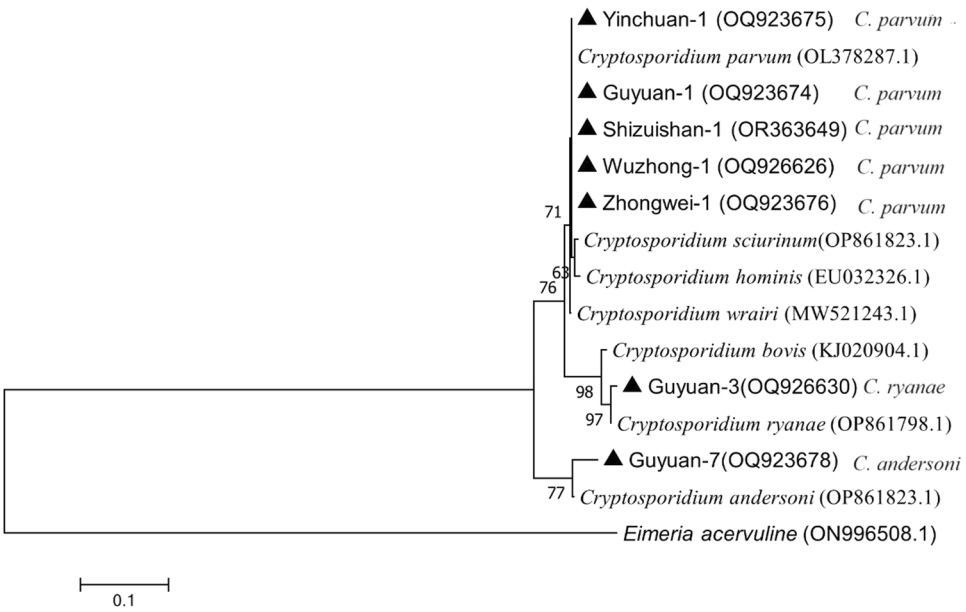


**Fig. 2** Neighbor-joining (NJ) phylogenetic analyses of *Cryptosporidium* spp. based on the 18S rRNA gene. The numbers indicate bootstrap values. Bootstrap values > 50% are shown. *Cryptosporidium* isolates identified in these samples are indicated by black triangles

### *G. duodenalis* Assemblages

Through applying blast to the Giardia positive samples, and then through selecting closer relatives as their outgroups and analyzing the *tpi* sequence data with MEGA 7.0, only one species clustered with them, hence only assemblage E was detected in Ningxia (Yinchuan (2 samples), Shizuishan (1), Guyuan (1)) (Fig. 4).

## Discussion

In this study, the limited number of samples and genotypic species as well as the selection of tpi with low sensitivity to detect *G. duodenalis* infection may have affected its results. The limited number of samples and genotypic species could have resulted in a poor representation of the results, for example, no genotypes of *C. bovis* was detected in this study. Considering only tpi for typing *G. duodenalis*, ignoring its lower sensitivity, may result in a lower infection rate compared to the actual situation. In the future, we can improve our strategy to minimize the limitations of the study results and better reflect the actual situation.

The mixed prevalence of *Cryptosporidium* and *G. duodenalis* detected at large-scale farms of five cities in Ningxia was 1.9% (4, n = 208, 95% CI 0.8–4.9%), which was higher than the co-infection rate of Tibetan sheep about rectum stool according to PCR in Qinghai (0.1%, n = 761), and was lower than that of calves about rectum stool according to PCR in Australia (11.9%, n = 177) [1, 19]. These differences may be highly related to the sampling time, sampling location, infected species, and the method of examining the positive percentages.

The prevalence of *cryptosporidium* was 13.0% (27/208). Among them, *C. parvum* was found to be the predominant species, at the same time, existing *C. andersoni* and *C. ryanae*. In 2015, the prevalence of *Cryptosporidium* in dairy cattle about rectum stool according to PCR measured

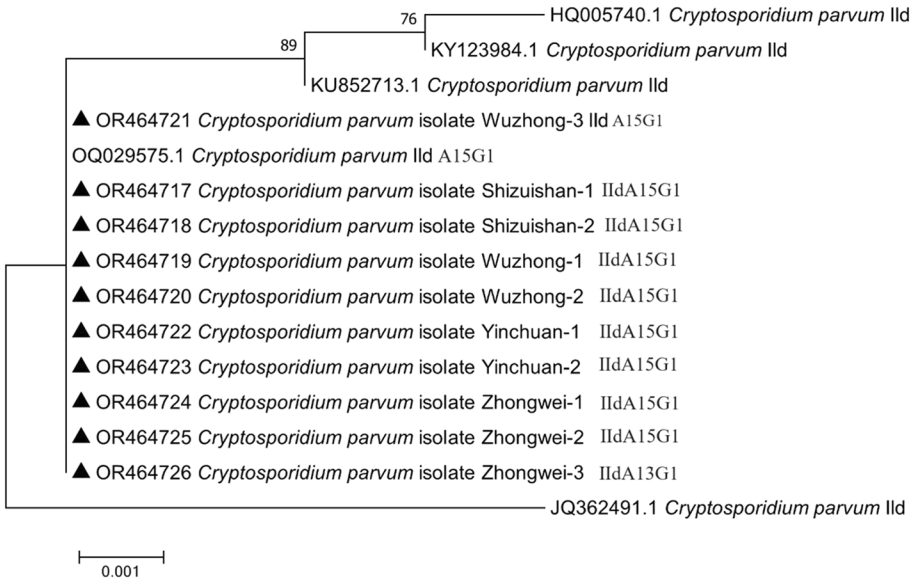


**Fig. 3** Neighbor-joining (NJ) phylogenetic analyses of *C. parvum* based on the *gp60* gene. The numbers indicate bootstrap values. Bootstrap values > 50% are shown. *C. parvum* isolates identified in these samples are indicated by black triangles

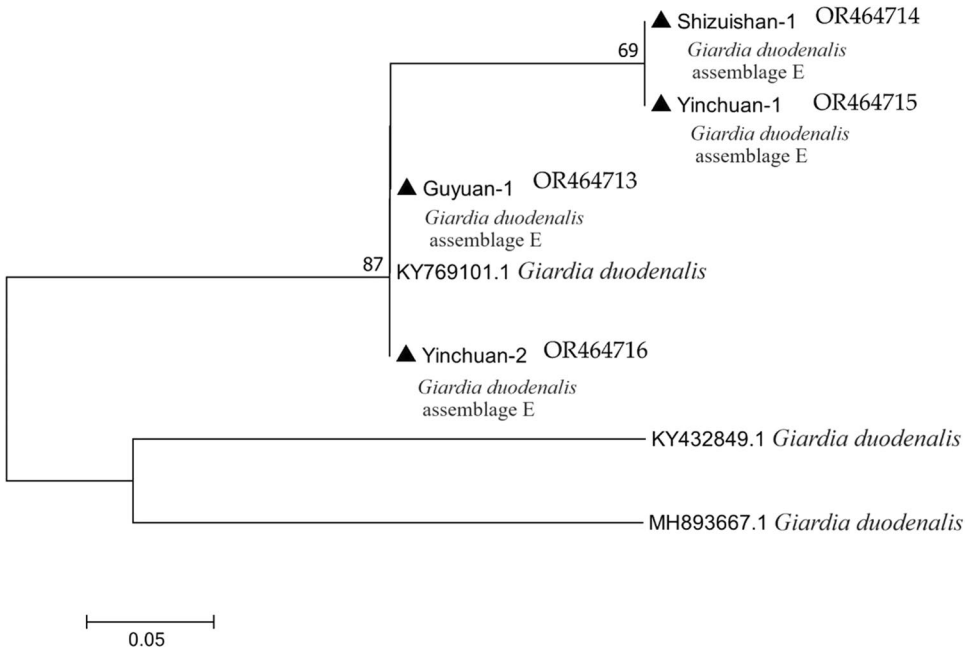


**Fig. 4** Neighbor-joining (NJ) phylogenetic analyses of *G.duodenalis* spp. based on the *tpi* gene. The numbers indicate bootstrap values. Bootstrap values > 50% are shown. *G.duodenalis* isolates identified in these samples are indicated by black triangles

in Ningxia was 5.5% (92/1688) lower than in this study [20]. The literature whose samples is dairy cattle, but the samples we have taken are dairy calve. The reasons for the different infection rates in the same area were according to sample age and sampling time. Recently, there are no pathological report of people infected with Cryptosporidium spp. in Ningxia. *C. parvum* was the dominant pathogen in pre-weaned calves in Austria, Germany and so on [21, 22]. In contrast, *C. bovis* was found to be the dominant in calves in Shaanxi Province, China [19], and in Korea, *C. ryanae* was the dominant in calves [23]. The infection rate of *Cryptosporidium* in pre-weaned dairy calves and diarrhea dairy calves was higher than that in post-weaned dairy calves and No diarrhea dairy calves in this paper. During this research, Pre-weaned calves are more likely to be infected with Cryptosporidium than post-weaned calves. The prevalence of *C. parvum* infection was greater in pre-weaned calves (7.5%) than in post-weaned calves (3.0%). *C. ryanae* and *C. andersoni* were observed only in pre-weaned calves (4.7%, 1.9%) as opposed to of *C. ryanae* which was found mainly in postweaning calves. Diseases caused by *Cryptosporidium* spp. and *G.duodenalis* are more frequent in warmer seasons such as summer. The rate of infection acquired varies from region to region due to the attention given to the disease and the climate and other factors that influence the spread of the disease in the region. Both diseases are more susceptible to calves, and

immunocompromised cattle are more susceptible to both diseases. All of the above comparisons show that such differences may depend on the time of sampling, the region and number of samples collected, age and health degree of samples and other factors. Infection rates have shown differences due to different detection methods, for example, the prevalence rates obtained by microscopic examination differ from those obtained by PCR, and methods for rapid detection with high accuracy are still being developed [24].

The infection rate of *G. duodenalis* is 1.9% (4/208, 95% CI 0.8–4.9%) with Assemblage E genotype predominating, which was less than the infection rate of dairy calves about rectum stool according to PCR in Ningxia in 2014 occurred with assemblage E and B (2.1%) [2]. The occurrence of human infection with *G. duodenalis* in Ningxia has not been observed up to the present. No significant difference between pre-weaned and post-weaned calves infected with *G. duodenalis* in this paper. The dominant genotype of *G. duodenalis* was the same in different species at the same site. The infection prevalence of *G. duodenalis* in cattle about rectum stool according to PCR in other regions of China ranged from 2.2 to 74.2%, with genotypes A and E present, and the predominant parasites were all E, both of which were at risk of infecting humans [10, 25–27]. Depending on how the test is performed and the genes amplified can also lead to differences in infection rates [26]. It has also been found in some countries that dairy calves are a little more likely to be infected with *G. duodenalis* than other breeds of cattle [7]. In addition, the place of sampling, the breed of cattle (beef/dairy cattle), whether they have diarrhea and their age are all factors in the presence of *G. duodenalis*. In China, it is not only cattle that are infected with *G. duodenalis*, but also other animals such as sheep, Tibetan sheep, pigs, rodents and rabbits with varying prevalence rates, as well as the presence of zoonotic genotype B, which is not present in cattle [1, 2, 11]. The public health significance of *G. duodenalis* lies in its ability to infect humans. The prevalence of *G. duodenalis* in humans varies from country to country, ranging from 1.9% in China to 11.1% in Myanmar, which is probably due to geographic location, climatic conditions and the importance attached to *G. duodenalis* [11]. In addition, the gene used in the article to detect *G. duodenalis* was not selected for the more sensitive SSU, but for the less sensitive tpi, which would result in a possible lower-than-normal positivity rate for *G. duodenalis*, but tpi can be used to genotype *G. duodenalis* for achieving its genotypes.

IIdA15G1 and a new IIdA13G1 appeared in Ningxia this time. IIdA15G1 also appeared in dairy calves in Beijing [8]. IIdA15G1 was the dominant kind in the study, which was consistent with the results of the survey of dairy cattle in 2014 at Ningxia [2]. In contrast, the dominant species was IIdA15G1 in pre-weaned calves in Sichuan, but the dominant in dairy cattle in Beijing was IIdA19G1 [4, 25]. In addition to cattle, zoonotic IIdA19G1 has also been found in wildlife [14].This may be related to geographic location, age of sampling, and number of samples taken. Above is the typing of gp60 gene of *C. parvum.* The gp60 typing of *C. ryanae* was not performed because *C. ryanae* is not currently found in humans.

*C. parvum* (IId)*, C. andersoni* and assemblage E are pathogens that cause diarrhea and even death in children, and appear mostly asymptomatic but are associated with growth retardation and malnutrition [5–7]. Currently, no effective treatment for cryptosporidiosis and giardiasis has been identified, making disease prevention a top priority [28]. In particular, these two parasites can persist in water for extended periods for several months [29], necessitating regular testing of the water environment for *Cryptosporidium* and *G. duodenalis*. It was shown that calves infecting *G. duodenalis* and *Cryptosporidium* with prophylactic treatment are closely related to their gut microbiology, for example, the presence or changes in gut microorganisms can affect various biological pathways such as metabolism and immune responses in animals [30]. Improving the type as well as the number of their gut microbial species perhaps can prevent both diseases from occurring. Furthermore, the occurrence of both parasites in calves is closely related to farmland management [31], rational distribution of farms and effective hygiene management can reduce the prevalence of cryptosporidiosis and giardiasis, thereby promoting public health.

The infection rate, genotypes and the discovery of the new subtype of *C. parvum* IIdA13G1 in the results of this experiment can make their farms and the government pay more attention to the presence of *Cryptosporidium* and *Giardia duodenalis* and take a series of measures to prevent and control the occurrence of this disease. For example, measures such as strengthening the detection of *Cryptosporidium* and *Giardia duodenalis* in the environment and on farms, avoiding a continuous increase in the number of positive cows, and improving the farming environment on farms are some of the measures that can influence the development of the disease. All in all, animals are an important source of transmission of zoonotic parasites, and we should continued surveillance of animals is important.

## Conclusions

A new genetic subtype, namely IIdA13G1 appeared in dairy calves in Ningxia. *C. parvum* (IIdA15G1) emerged as the dominant species, and the favorable species of *G. duodenalis* was the zoonotic genotype E.

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**Data Availability** All data generated during this study are included in this published article.

## Declarations

**Conflict of Interest** The authors declare that they have no conflict of interest.

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