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A one health study on phylogenetics and risk of pathogenic intestinal parasites at a ranch in Inner Mongolia

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ABSTRACT

Cryptosporidium, *Giardia duodenalis*, and *Enterocytozoon bieneusi* are widespread zoonotic pathogens causing gastrointestinal diseases in humans and various animal species. Inner Mongolia, a major beef production region in China, there is a notable absence of comprehensive research on intestinal parasitism. Thus, timely and comprehensive diagnosis is essential to mitigate disease spread and minimize economic losses in the livestock industry. In this study, we collected fecal samples from cattle and humans, as well as soil and water samples, and all samples were tested for pathogenic intestinal protozoa at the Simmental cattle ranch in Wengniute, Chifeng City, Inner Mongolia. Among the 393 samples tested, 76/371 (20.5 %) cattle, 6/11 (54.5 %) ranch workers, 1/7 (14.3 %) water, and 2/4 (50 %) soil samples were positive. Factors affecting the infection rate of intestinal protozoa were examined. Results showed that the infection rate was higher in June than in January, higher in calves than in adults, and higher in diarrheal calves than in healthy calves. Additionally, the infection rate of intestinal protozoa was higher in pathogen-contaminated water source sheds than in uncontaminated sheds. Genetic and evolutionary analyses revealed that the prevalent *E. bieneusi* subtypes are predominantly J, I, and BEB4, while the *G. duodenalis* subtypes are assemblages B and E. The *Cryptosporidium* species identified were *C. bovis*, *C. andersoni*, *C. parvum*, *C. ryanae*, and *C. suis*, with *C. parvum* being a notable zoonotic pathogen. The pathogen sequences from humans, cattle, water, and soil showed 99–100 % similarity, suggesting possible transmission or contamination between animals and the environment. This study contributes to the One Health approach by addressing the gap in research on intestinal protozoa in Inner Mongolia. It provides important data for other ranches in the region to understand the prevalence of such pathogens and develop effective control measures. Using the concept of One Health to analyze the spatiotemporal distribution of intestinal protozoa in pastures is of great significance for maintaining public health.

1. Introduction

Cryptosporidium, *Enterocytozoon bieneusi* and *Giardia duodenalis*, are three prevalent zoonotic pathogens that can cause gastrointestinal diseases in humans and multiple animal species [1–3]. Notably, Inner Mongolia, a major beef production area in China, exhibits even higher infection rates of *Cryptosporidium* and other intestinal protozoa, ranging between 25.93% and 29.9% [4–6]. Despite Inner Mongolia contributing

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to one-tenth of total beef cattle output in China, data on the prevalence of intestinal protozoan infections in this region remain sparse.

Understanding the distinct host adaptation characteristics and variations in species and genotypes of intestinal parasites is crucial for identifying infection sources and elucidating transmission routes. *Cryptosporidium* plays a key role in this context [7]. Notably, *C. parvum*, *C. andersoni*, *C. ryanae*, and *C. bovis* are among the nearly 40 known species and with over 120 genotypes [8], and are the most prevalent in cattle infections. Similarly, *E. bieneusi*, the most common among the 17 pathogenic microsporidia in humans [9], has been found in cattle across more than 40 genotypes since its initial discovery in calves in Germany [10]. *Giardia* sp., another common intestinal parasite in humans, includes at least eight assemblages (A-H), with assemblages A and B posing a zoonotic transmission risk to humans, cattle, sheep, dogs, and cats, specifically, subtypes AI-AIV and BIII-BIV are associated with human infections [11,12]. All three pathogens are usually spread through the contamination of water sources by the feces of infected animals. The presence of these intestinal protozoa not only directly causes economic losses through cattle mortality and treatment costs but also indirectly affects the economy by reducing milk production, increasing production costs, and posing significant public health risks by contaminating water or food supplies.

Cryptosporidium, *E. bieneusi*, and *G. duodenalis* are recognized as the principal causes of bovine diarrhea in China, with national average infection rates documented at 11.9% for *Cryptosporidium* [13], 5.4-52% for *E. bieneusi* [14,15], and 2.2-3.6% for *G. duodenalis* [16,17]. Additionally, existing studies on intestinal protozoa in Inner Mongolia have been limited to cattle, neglecting comprehensive assessments of water contamination and feeder health. The "One Health" framework, emphasizes that human and animal health are interdependent and connected to the ecosystems in which they exist [18]. This research applied the framework to examine the spatial and temporal distribution of intestinal protozoa across cattle, humans, and water sources at a specific ranch. This finding aims to enhance the understanding of infection dynamics and support the development of strategies for the detection, prevention, and management of intestinal protozoan infections, thereby contributing to both animal and worker health on the ranch.

2. Materials and methods

2.1. Sample collection

The experimental ranch is located in Wengniute, Chifeng City, Inner Mongolia, where about 2,000 Simmental cattle are raised in a total of 8 cattle sheds. Each pair of sheds shares a well, while a separate well is designated for the human living areas to provide drinking water.

In January 2021, 200 samples were collected, including 49 calf fecal samples (32 from diarrheic calves and 17 from healthy calves, with calves defined as being less than 6 months old), 147 adult cattle fecal samples (all healthy), and 4 shed soil samples taken from the surface of the cattle sheds. In June 2021, 186 samples were collected, including 175 cattle fecal samples (8 from diarrheic cattle and 167 from healthy cattle) and 11 stool samples from workers (all healthy, including one driver and 10 breeders). 7 water samples were collected, including 6 animal drinking water sources and 1 human drinking water source, with each sample being approximately 10 liters.

All fecal samples were stored directly in -20°C freezer until DNA extraction. Water samples were taken to the laboratory on the day of collection and processed using EnviroChek-HV method to obtain oocyst enrichment fluids, which were also stored at -20°C until the DNA extraction.

2.2. DNA extraction and PCR amplification

For DNA extraction, each sample (approximately 500 mg) was extracted using the Fast DNA Spin Kit (MP Biomedicals) for soil for DNA extraction and stored at -20°C. Nested PCR, targeting the internal transcribed spacer (*ITS*) region gene for *E. bieneusi* and the β-giardin (*bg*) gene and the small subunit ribosomal RNA (*SSU rRNA*) gene for *G. duodenalis* and *Cryptosporidium* respectively, was employed for pathogen detection [19–21]. Following purification, all positive PCR products were directly sequenced [21]. The genotypes of *E. bieneusi* obtained were named as previously published if they matched the known genotypes in the GenBank database. Similarly, the species of *Cryptosporidium* and *G. duodenalis* were identified using BLAST (blast.ncbi.nlm.nih.gov/Blasst.cgi) based on the highest identity. The obtained nucleotide sequences have been deposited in GenBank database under the accession numbers OR485070-OR485112 (*Cryptosporidium*), OR491676-OR491694 (*E. bieneusi*), and OR526490-OR526518 (*G. duodenalis*).

2.3. Haplotype network analysis and phylogenetic reconstruction

To gain an intuitive understanding of the genetic structure of various pathogens present on this ranch, we analyzed the reported genotypes of *E. bieneusi*, along with the *SSU rRNA* gene sequences of *Cryptosporidium* (including *C. bovis*, *C. andersoni*, *C. parvum*, *C. ryanae*, *C. suis*, *C. canis* and *C. felis*) and *bg* gene sequences of *G. duodenalis* assemblages A, B, C, D, and E. The neighbor-joining (NJ) method was used to construct phylogenetic trees by MEGA11 (www.megasoftware.net). For constructing these trees, *C. felis* (MH115431.1) served as an outgroup to construct the *Cryptosporidium* tree, (DQ885585.1) from dogs for *E. bieneusi* tree, and assemblage C (LC437439.1) was used as an outgroup to construct the *G. duodenalis* tree. The Kimura two-parameter model was used as the nucleotide substitution model, with a gamma distribution was used as the rate among sites. The robustness of the phylogenetic trees was tested through 1000 bootstrap replicates.

Additionally, we selected *SSU rRNA* gene sequences of *C. andersoni* and *C. bovis* in cattle, *ITS* gene sequences of *E. bieneusi* and *bg* gene sequences of *G. duodenalis* assemblages B from Genbank, alongside sequences identified in this study, to create sequence collections. Multiple sequence alignments were performed using MEGA11, and haplotypes were identified using DnaSP27. Haplotype networks reconstructions were conducted using statistical parsimony network analysis with a 95% connection probability threshold, and these networks were analyzed using TCS Networks [22]. Sample frequencies were displayed in PopART [23].

2.4. Statistical analysis

To assess differences in infection rates across various time points, seasons, health statuses, and age groups, we employed the chi-square test to calculate the odds ratio (OR) and 95% confidence intervals (CI), along with corresponding *P*-values. Statistical significance was set at $P < 0.05$. GraphPad Prism version 8.02 (GraphPad Software Inc.) was used for analysis.

3. Results

3.1. Overall infection rates of intestinal protozoa in cattle

The study revealed an overall average infection rate of 20.5% (76/371) for intestinal protozoa among the sampled cattle population, encompassing infections with one or more of the following protozoans: *E. bieneusi*, *G. duodenalis* and *Cryptosporidium*.

Monthly variation in protozoan infections. **January**: The infection rate of intestinal protozoa was 16.3% (32/196). *E. bieneusi* infections were observed at a rate of 3.0% (6/196), involving genotypes J, I, and BEB4. *G. duodenalis* presented the lowest infection rate at 1.5% (3/196). *Cryptosporidium* was the most prevalent, with an infection rate of 13.3% (26/196), including *C. bovis* (9 cases), *C. andersoni* (12 cases), *C. parvum* (6 cases), and *C. suis* (1 case). **June**: The overall infection rate had

increased to 26.3% (44/175). *E. bieneusi* showed a higher infection rate of 6.8% (12/175) with the same genotypes as in January. Notably, *G. duodenalis* infections significantly increased to 12.0% (21/175). The infection rate of *Cryptosporidium* infection was 6.9% (12/175), including *C. bovis* (4 cases), *C. andersoni* (7 cases), and *C. ryanae* (1 case) (Table 1).

Statistical analysis of infection rates. **Age-related variability**: The analysis indicated significant differences in infection rates between calves and adult cattle (OR = 2.43, 95% CI: 1.09–5.47, $P < 0.05$) (Table 2). A particularly high risk of *E. bieneusi* infection was observed in calves (OR = 16.59,95% CI: 2.14-196.5, $P < 0.05$). Among the 8 positive diarrhea calves, *Cryptosporidium* spp. was found in 71.4% (5/7) of the cases, which was much more prevalent than *E. bieneusi* 28.6% (2/7) and *G. duodenalis* 17.3% (1/7).

3.2. Prevalence of pathogenic intestinal protozoa in ranch environment and workers

A statistical analysis comparing the prevalence of intestinal protozoa in cattle between January and June indicated a significantly higher infection rate in June (OR = 0.58, 95% CI: 0.35-0.96, $P < 0.05$) (Table 2). This finding led to suspicions of environmental contamination at the ranch, posing a potential threat to worker health. Consequently, soil, water sources and worker samples were collected and tested for intestinal protozoa in June.

One *C. parvum*, one *C. bovis* and one *E. bieneusi* were detected in the soil samples from 4 barn floor (Table 1). In cattle and soil, comparison of sequences showed 99%–100% similarity between *C. parvum, C. bovis* and *E. bieneusi*. Compared with *C. parvum* from cattle, *C. parvum* from soil has 4 base differences (2 A→ G and 2 T→ G) in the 445 bp conserved region. *E. bieneusi* has 1 base different (A→ G) in 242 bp. The sequences of *C. bovis* are exactly the same. Due to the current lack of relevant detection technology for *E. bieneusi* in water samples, we tested seven wells in the pasture for *Cryptosporidium* and *G. duodenalis*. Among them, contamination with the *C. ryanae* was detected in one well water sample (Table 1). The sequence of this pathogen sample is 100% similar to the sequence of *C. ryanae* found in cattle. In addition, in June, the positive rate of intestinal protozoa in cattle houses that were positive for intestinal protozoa in water sources was 34.7% (8/23), and the infection rate of negative cattle houses was 7.2% (11/152). Cattle houses with water supplies contaminated with intestinal protozoa and cattle houses without water sources differed statistically significantly throughout June (OR = 6.83, 95% CI: 2.2-18.8, $P < 0.05$).

Table 2
Prevalence and risk factors for intestinal protozoan infections in cattle.

Variable	Groups	No. tested	No. positive	OR (95% CI)	P-value
Host	People	11	6	4.66	$P <$
	Cattle	371	76	(1.32–13.74)	0.05
Time	January	196	32	0.5813	$P <$
	June	175	44	(0.35–0.96)	0.05
Age	Calf	49	13	2.43 (1.09–5.47)	$P <$
	Adult cattle	147	19		0.05

The infection rate of *Cryptosporidium* among ranch workers was 9.0% (1/11), and the test results showed that it was *C. bovis*. *G. duodenalis* was 45.5% (5/11), and no infection with *E. bieneusi* was found. The *C. bovis* found in humans has 100% similarity to the sequences found in soil and cattle. The *G. duodenalis* found in humans differs from that found in cattle by 2 bases (2 A→ G). There was a difference in the intestinal protozoa infection rate between drivers (1/1) and breeders among ranch workers (5/10).

The prevalence of intestinal pathogenic protozoa infection in cattle ranch workers was significantly higher than that in cattle (OR = 4.66, 95% CI: 1.32–13.74, $P < 0.05$) (Table 2). Notably, the infection rate of *G. duodenalis* was significantly higher in workers than in cattle (OR = 12.05, 95% CI: 3.75–45.47, $P < 0.05$), while there was no statistically significant difference in the infection rate of the other two intestinal protozoa (*E. bieneusi*: $\chi^2 = 0.59$, $P > 0.05$; *Cryptosporidium*: $\chi^2 = 0.02$, $P > 0.05$).

3.3. Phylogenetic relationship and haplotype analysis of E. bieneusi

A total of 19 *ITS* sequences of *E. bieneusi* were obtained from cattle and were trimmed to 243 bp after alignment with reference sequences. A phylogenetic tree and haplotype network were constructed using other genotypes from both human and cattle as references (Fig 1). The common host of Group 2 is cattle, and the sequences we found are all in Group 2 (Fig 1A).

Haplotype analysis of *E. bieneusi* revealed that all sequences could be classified into 33 distinct haplotypes, of which three haplotypes were present in the sequences we obtained. Specifically, the prevalence of

Table 1
Occurrence of *Cryptosporidium*., *E. bieneusi*, and *G. duodenalis* in workers, cattle, water and soil.

Group	Time	Age	Health status	No. specimens	No. positive for *Cryptosporidium* (%)	*Cryptosporidium* species (no.)	No. positive for *E. bieneusi* (%)	*E. bieneusi* genotype (no.)	No. positive For *G. duodenalis* (%)	Assemblage (no.)
Herd1	2021.1	Calf	Diarrhea	32	5 (15.6)	*C. bovis* (4), *C. parvum* (1)	2 (6.3)	J (2)	1 (3.1)	B (1)
			Non-diarrhea	17	3 (17.6)	*C. bovis* (2), *C. anderson* (1)	3 (17.6)	J (2), I (1)	1 (5.9)	E (1)
		Adult	Diarrhea	0	0	–	0	–	0	–
			Non-diarrhea	147	18 (12.2)	*C. bovis* (3), *C. andersoni* (11), *C. parvum* (3), *C. suis* (1)	1 (0.7)	BEB4 (1)	1 (0.7)	B (1)
Herd2	2021.6		Diarrhea	10	1 (10.0)	*C. bovis* (1)	2(20.0)	J (1), I (1)	1 (10.0)	B (1)
			Non-diarrhea	165	11 (6.7)	*C. bovis* (3), *C. andersoni* (7), *C. ryanae* (1)	10 (6.1)	J (5), I (1) BEB4 (4)	20 (12.0)	B (18), E (2)
Subtotal				371	38 (10.2)	*C. bovis* (13), *C. andersoni* (19), *C. parvum* (4), *C. suis* (1), *C. ryanae* (1)	18 (4.9)	J (10), I (3) BEB4 (5)	24 (6.5)	B (21), E (3)
Soil	2021.1			4	2 (50.0)	*C. bovis* (1), *C. parvum* (1)	1 (25.0)	BEB4 (1)	0 (0)	–
Workers	2021.6			11	1 (9.0)	*C. bovis* (1)	0 (0)	–	5 (45.5)	B (5)
Water	2021.6			7	1 (14.3)	*C. ryanae* (1)	–	–	–	–

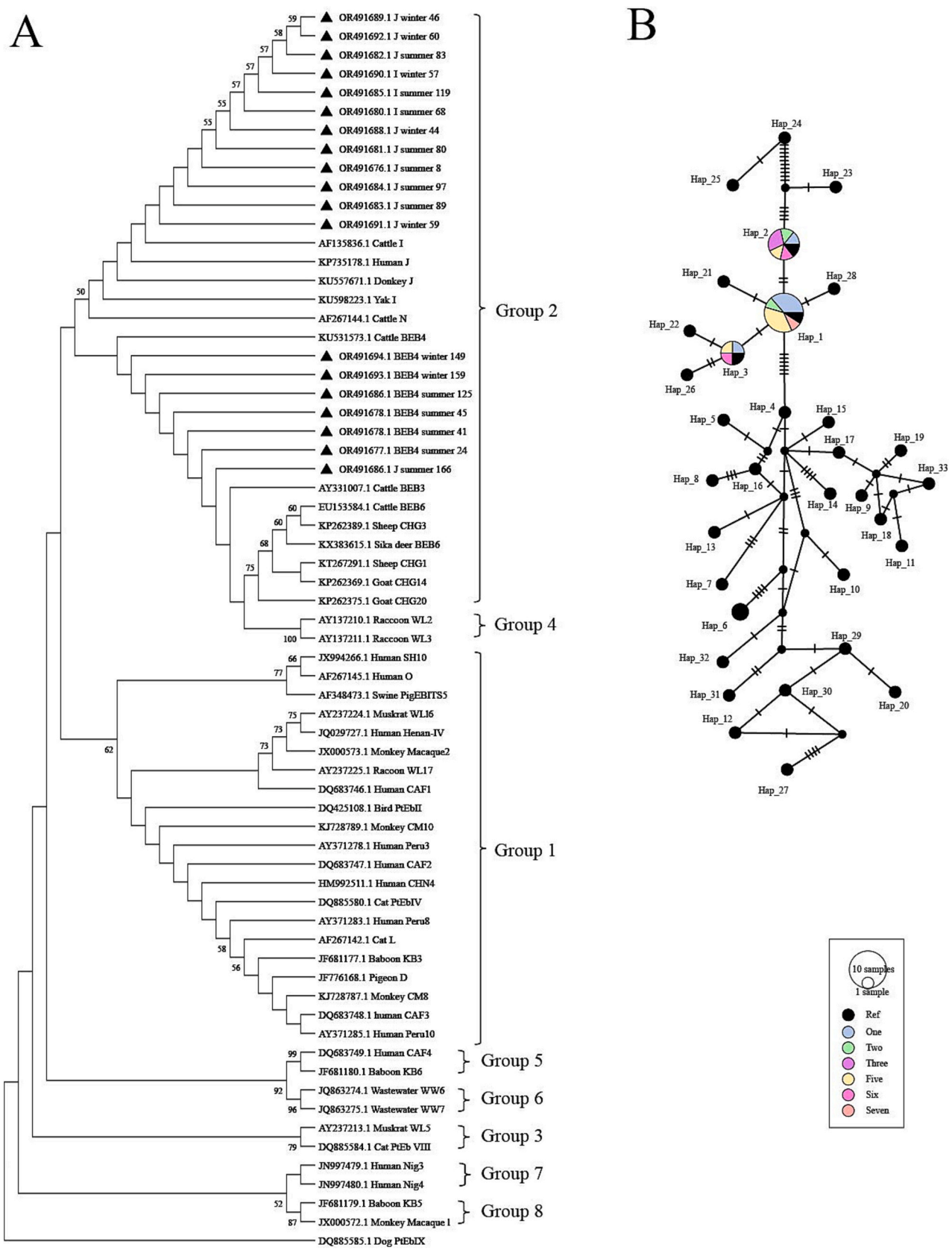

Fig. 1. Phylogenetic tree constructed using neighbor-joining (NJ) method and haplotype analysis network of *E. bieneusi* based on ~243 bp sequence of the internal transcribed spacer (*ITS*) region. (A) The phylogenetic tree was constructed using the Kimura two-parameter model with 1000 bootstrap replicates. (B) All the 19 sequences identified in this study and 23 previously published sequences were used to generate haplotype data. The TCS network algorithm was used to construct the network. The network was constructed using TCS Network algorithm. Various colors represent different groups and the sizes of the circle represent the numbers of haplotypes. One-Seven, cattle shed; Ref, reference published sequences.

Hap_1, Hap_2, and Hap_3 among cattle infected with *E. bieneusi* was 20.8%, 15.1%, and 5.7%, respectively. Hap_1 was the predominant haplotype, characterized by a central position relative to the other haplotypes. Notably, there was a one-base difference between Hap_1 and each of the other two haplotypes, Hap_2 and Hap_3 (Fig 1B).

3.4. Phylogenetic relationship and haplotype analysis of G. duodenalis

We obtained partial sequences of the *bg* gene from 29 isolates of *G. duodenalis* from both humans and cattle. These sequences were subsequently compared with reference sequences. Our study found that these sequences were divided into two groups: assemblage E and assemblage B (Fig 2A).

Assemblage B haplotype analysis showed a total of 12 haplotypes, with 9 haplotypes present in the sequences obtained. The percentage of Hap_1 among the nine haplotypes amounted to 60.5%, which was the dominant haplotype in *G. duodenalis* in this ranch. Two haplotypes, Hap_1 and Hap_9, were detected in human feces, whereas Hap_1-8 haplotypes were detected in cattle feces. Hap_1 may be the zoonotic haplotype, whereas Hap_9 may be more susceptible to human (Fig 2B).

3.5. Phylogenetic relationship and haplotype analysis of Cryptosporidium

Partial sequences of the *SSU rRNA* gene from 40 *Cryptosporidium* isolates obtained from humans, cattle, and water were trimmed and compared to reference sequences. All *Cryptosporidium* isolates identified in this study were categorized within their respective species branches (Fig 3A). For haplotyping analysis, reference sequences from previous studies were employed along with sequences of *C. bovis* and *C. andersoni* identified in our research.

C. bovis haplotype analysis revealed the presence of seven haplotypes, while our sequence results contained three haplotypes, Hap_1, Hap_6 and Hap_7. All three haplotypes were detected in cattle, whereas only Hap_1 was detected in human feces. In addition, the Hap_1 haplotype, which accounted for 86.0% of the total, may be the dominant haplotype on this cattle farm and is at risk of infecting humans (Fig 3B). *C. andersoni* haplotype analysis revealed the presence of 14 haplotypes, while our sequence results contained 11 haplotypes, of which Hap_3 was the shared haplotype. Furthermore, our experimental results showed that all 11 haplotypes were detected in cattle (Fig 3C).

4. Discussion

Previous studies have confirmed that *E. bieneusi* has a high prevalence of infection in cattle, such as in Henan, Ningxia [24], Beijing, and Tianjin [13]. In this ranch, the *E. bieneusi* infection rate (6.1%) was lower than reported infections in other provinces. Consistent with previous research, genotypes J and I are dominant genotypes in cattle in China [25]. In the haplotype network analysis, we have identified three distinct haplotypes. Among these haplotypes, Hap_2 is widely distributed across different cattle sheds within the ranch. The genetic differences between these haplotypes are minimal, suggesting a common source of infection within the ranch. The *E. bieneus*i detected in the soil differed from the cattle strain by just one base, indicating that cattle likely contaminated the surface soil.In this study, the infection rate of *G. duodenalis* was found to be 6.7 %, which is lower than rates reported in Heilongjiang [17], Henan [26], and Northeast China [27]. Assemblages A, B, and E have been detected, with assemblage E being predominant in previous studies. However, our findings indicate that assemblage B is more prevalent in cattle at this ranch compared to other assemblages. Notably, assemblage B, which is dominant among cattle here, was also identified in five human cases, exhibiting a high infection rate of 45.5 %. The *G. duodenalis* found in cattle differs from the human strain by only two bases (A → G), indicating that cattle likely transmitted *G. duodenalis* to humans. These results highlight the serious health risks *G. duodenalis* poses to both humans and animals on this ranch, underscoring the need for rigorous control measures.

In our study, the infection rate of *Cryptosporidium* in cattle was 10.2%, lower than the national average in China, which stands at 14.5%. Notably, *C. andersoni* and *C. bovis* are the dominant species, which aligns with the common epidemic situation in China (*C. andersoni* > *C. bovis* > *C. parvum* > *C. ryanae*) [28]. *C. bovis* and *C. andersoni* were predominantly found in calves and adult cattle, respectively, aligning with findings from previous research by Xiao *et al.* [25]. *C. bovis* and *C. parvum* were detected in surface soil of cattle sheds, while *C. ryanae* was identified in the water sources on cattle farms. These findings highlight the potential environmental contamination from these pathogens. Despite detecting multiple species of *Cryptosporidium* on the ranch, only *C. ryanae* was identified in water samples, suggesting potential limitations in our sampling or detection methods or varying abilities of different *Cryptosporidium* strains to contaminate the environment. Additionally, the sequence of *C. bovis* from an infected ranch worker shared 100% similarity with strains isolated from cattle, indicating zoonotic transmission potential. Given that *C. parvum*, detected in water sources, is also known to infect humans, there remains a significant risk of human infection [29–32]. This study underscores the need for effective control measures to mitigate environmental contamination and minimize the risk of zoonotic transmission. The failure to amplify the *GP60* gene has limited our ability to fully investigate the genetic diversity of *Cryptosporidium* and the potential transmission routes. In our subsequent research endeavors, we aim to overcome this limitation by enlarging the sample pool, refining the amplification conditions for the GP60 gene, and exploring alternative molecular markers. The implementation of these measures will contribute to a more thorough and comprehensive understanding of the genetic characteristics and epidemiological patterns of *Cryptosporidium*.

The haplotype network analysis for *C. bovis* revealed that the Hap_1 haplotype had the highest infection prevalence. Additionally, the detection of Hap_1 in ranch workers underscores its significant zoonotic potential, indicating a substantial risk of transmission between humans and animals in both directions. The haplotype network analysis for *C. andersoni* demonstrated a diverse genetic structure within the pasture, with 11 distinct haplotypes identified. This diversity indicates multiple sources of infection and suggests potential genetic exchange between different strains. Such findings highlight the complex epidemiological dynamics of *Cryptosporidium* in this environment and underline the importance of monitoring genetic variations to understand transmission pathways better.

In addition to assessing the infection rates of various pathogens, our study investigated factors potentially influencing their transmission risk. **Time:** The infection rate of bovine intestinal protozoa was notably higher in June than January. This increase is likely due to soil contamination with oocysts following heavy rainfall in June. **Age:** Calves exhibited significantly higher infection rates than adults, possibly due to their less developed immune systems. **Health:** Among diarrheic calves, the infection rate for *Cryptosporidium* was higher than for other intestinal protozoa. This may be attributed to diarrhea-induced immune suppression, increasing susceptibility to *Cryptosporidium*. **Host:** Infection rates were higher in farm workers than in cattle, likely due to workers prolonged exposure to risk factors such as direct contact with animals and contaminated environments. Although our study comprehensively examined the prevalence of common intestinal protozoa on this ranch, it faced several limitations. Our testing was restricted to *Cryptosporidium* in water sources, and ranch staff preserved only fecal samples from June. Future research will employ diversified methods to detect additional protozoan species in water sources and expand sample collection periods. Moreover, increasing the sample size will help to better represent the regional prevalence of enteric protozoa.

Our study analyzed the spatial and temporal distribution of intestinal protozoa in humans, cattle, soil, and water, adopting a holistic health perspective on this ranch. The findings provide valuable insights into the patterns and transmission of these infections.

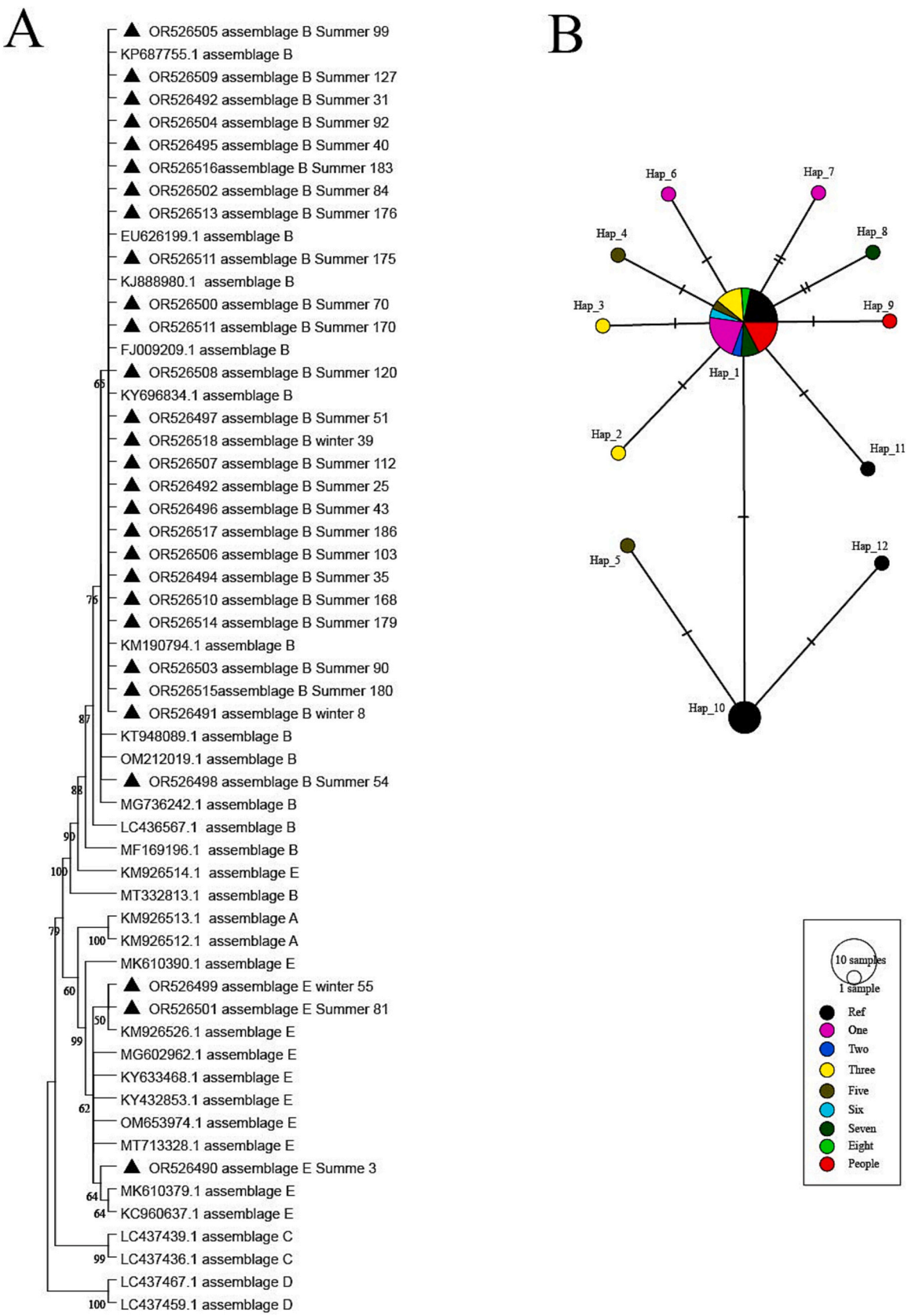

Fig. 2. Phylogenetic tree constructed using neighbor-joining (NJ) method and haplotype analysis network of *G. duodenalis* β-giardin (*bg*) gene. (A) The phylogenetic analysis tree was constructed using the Kimura two-parameter model with 1000 bootstrap replications. (B), All the 26 assemblag B sequences in this study and 12 published assemblag B sequences were used to generate haplotype data. The network was constructed using TCS Network algorithm. Various colors represent different groups and the sizes of the circle represent the numbers of haplotypes. One-Eight, cattle shed; Ref, reference published sequences.

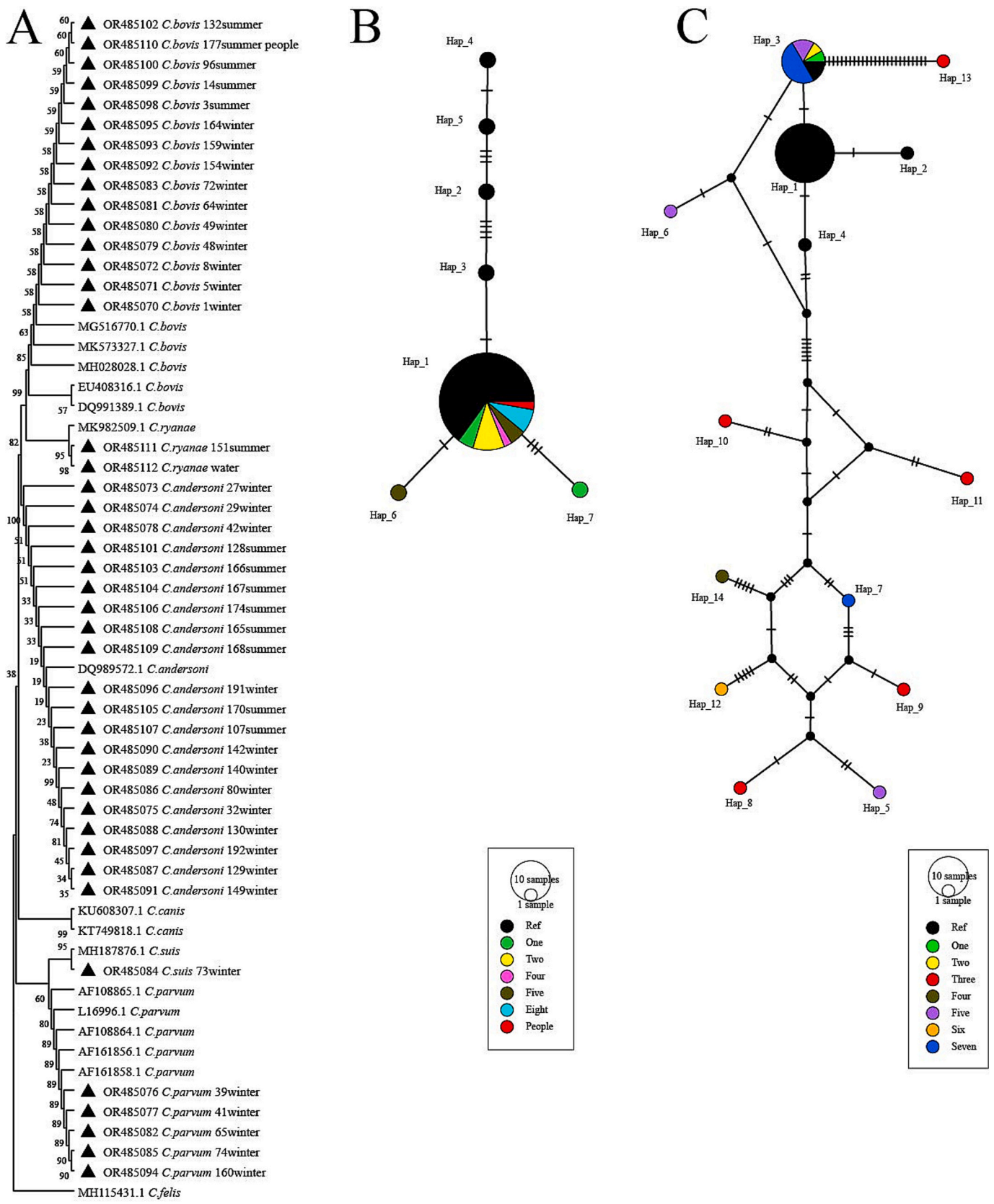

Fig. 3. Phylogenetic tree constructed using neighbor-joining (NJ) method and haplotype analysis network of *Cryptosporidium SSU rRNA* gene. (A) The phylogenetic analysis tree was constructed using the Kimura two-parameter model with 1000 bootstrap replications. (B) All the 14*C. bovis* sequences in this study and 28 published *C. bovis* sequences were used to generate haplotype data. The network was constructed using TCS Network algorithm. Various colors represent different groups and the sizes of the circle represent the numbers of haplotypes. One-Eight, cattle shed; People, workers; Ref, reference published sequences. (C) All the 19*C. andersoni* sequences in this study and 26 published *C. andersoni* sequences were used to generate haplotype data. The network was constructed using TCS Network algorithm. Various colors represent different groups and the sizes of the circle represent the numbers of haplotypes. One-Seven, cattle shed; Ref, reference published sequences.

5. Conclusions

Our study comprehensively analyzed the spatial and temporal distribution of intestinal protozoa across humans, cattle, soil, and water sources on this ranch, embracing a holistic health perspective. We identified a variety of protozoan pathogens, including *Cryptosporidium* species (*C. parvum*, *C. bovis*, *C. andersoni*, *C. ryanae* and *C. suis*), *E. bieneusi* (genotypes J, I, and BEB4), and *G. duodenalis* (Assemblages B and E) predominantly in cattle. Notably, *C. bovis* and *G. duodenalis* assemblage B were also found in humans, highlighting the zoonotic risk these pathogens pose. Additionally, the detection of *C. ryanae* in water sources emphasizes the environmental vectors facilitating the spread of these infections. The pathogen sequences from the four groups of samples (humans, cattle, water, and soil) showed up to 99-100% similarity, suggesting possible transmission or contamination of pathogens between animals and the environment. Meanwhile, the findings underscore the significant influence of seasonal changes and the age of cattle on the dynamics of protozoan transmission. In conclusion, understanding the epidemiology of these protozoan infections is crucial for both animal and public health. Our study advocates for the adoption of one health approach to effectively manage and mitigate the risks associated with intestinal protozoa, thereby safeguarding the health of cattle and humans and supporting the sustainability of livestock operations in Inner Mongolia.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Inner Mongolia University. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Ethics Committee of Inner Mongolia University.

Consent for publication

All participants consented to have their data published.

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CRediT authorship contribution statement

Ziran Mo: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Jingwei Quan:** Writing – original draft, Methodology. **Bin Xu:** Writing – original draft, Methodology. **Huixia Yu:** Methodology. **Junyan Li:** Methodology. **Xiaoping Luo:** Methodology. **Qimuge Wuyun:** Methodology. **Jian Li:** Writing – original draft, Validation, Methodology. **Wenbin Yang:** Writing – review & editing, Writing – original draft, Methodology. **Wei Hu:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

Data will be made available on request.

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Not applicable.

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