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Assessment of potential zoonotic transmission of *Giardia duodenalis* from dogs and cats

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ABSTRACT

Giardia duodenalis is one of the major causes of diarrhea among humans, especially in young children. Statistical analysis revealed that the pooled prevalence of *G. duodenalis* in humans, dogs, and cats was 9.72% (10,921/112383), 15.60% (7510/48140), and 14.53% (1125/7740), respectively. Unquestionably, the canine-specific assemblages C and D and the feline-specific assemblage F were the dominant genotypes in dogs and cats, respectively. Additionally, the prevalence of zoonotic *G. duodenalis* assemblages (A and B) in dogs and cats was 23.07% (875/3792) and 41.42% (169/408), respectively, implying that the potential transmission of *G. duodenalis* from dogs and cats to human infection cannot be ignored. The highest frequency of potentially zoonotic assemblages was found among working dogs (3.55%, 25/705) and the 1–5 age group (22.92%, 11/48). In summary, dogs and cats have a significant role in the zoonotic transmission of *G. duodenalis* due to their close contact with humans and the higher frequency presence of zoonotic assemblages. Further studies are necessary to explore the presence of *G. duodenalis* among humans and animals and in environmental samples. Researchers should adopt a one-health approach to gain a deeper understanding of *G. duodenalis* in dogs and cats and potential transmission routes to humans.

1. Introduction

Giardia duodenalis, also known as *G. intestinalis* or *G. lamblia,* is a globally distributed parasite widely reported in humans and many animals worldwide [1], first discovered in 1681 by Leeuwenhoek [2]. *G. duodenalis* infection causes watery diarrhea in 280 million people globally [3], including 28.5 million human giardiasis cases in China annually [4,5]. The giardiasis symptoms include watery diarrhea, vomiting, abdominal pain, malabsorption, and other associated symptoms, particularly in young children [6]. For example, *G. duodenalis* is associated with approximately 15,000 to 17,000 enteritis cases in children in the United States of America each year [7].

G. duodenalis life cycle consists of two stages: rapidly multiplying trophozoites and environmentally hardy cysts [8]. Trophozoites are the vegetative form of *G. duodenalis* that replicates in the host's small intestine. On the contrary, cysts are the environmentally stable phase of the parasite's life cycle, which are released into the environment in feces and transmitted via the fecal-oral route. Cysts release in the feces contribute to zoonotic transmission of *G. duodenalis* from one host into the environment and ingestion by another host, ultimately leading to waterborne or foodborne outbreaks [2,9–11]. While several drugs have been approved for treating giardiasis in humans, treatment failure is common, and no vaccine is available [2,12–14].

G. duodenalis is a multispecies complex with eight identified assemblages (A to H), genetically diverse within the species [15]. Among them, assemblages A and B predominantly infect humans and various animals, displaying a high potential for zoonotic transmission [1]. Assemblage A is further divided into AI, AII, and AIII sub-assemblages, and host adaptation has been observed among these three sub-assemblages. For example, sub-assemblage AI predominantly infects animals, sub-assemblage AII infects humans, and sub-assemblage AIII is widely detected in wild ruminants [2,5,16]. The other *G. duodenalis*

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assemblages demonstrated host adaptation, with assemblages C and D primarily infecting dogs and cats, assemblage E primarily infecting ungulate animals (cattle, sheep, goats, and pigs), assemblage F mainly infects felines, assemblage G primarily infects rodents and assemblage H is primarily associated with seals [15]. The genotyping methods for *G. duodenalis* are similar to those for other intestinal protozoa. They typically include the conventional PCR, nested PCR, and quantitative PCR. Among these methods, nested PCR is the most commonly utilized genotyping tool in laboratory settings. Common genotyping loci for *Giardia* include the *SSU* rRNA, elongation factor 1 alpha (*ef-1*), β-giardin (*bg*), glutamate dehydrogenase (*gdh*), and triosephosphate isomerase (*tpi*) genes. However, the *SSU* rRNA and *ef-1* gene loci are the most commonly used for genotyping [2,5,16], including in whole-genome sequencing [17].

Dogs and cats significantly impact our daily lives, providing emotional support and companionship to humans as beloved pets. While *G. duodenalis* assemblages C and D, and F are primarily associated with dogs and cats, respectively, zoonotic assemblages A and B are also frequently detected in these animals [1]. However, although there are many reports of *G. duodenalis* infections in dogs and cats, there are few

Table 1
Molecular prevalence and assemblage distributions of *Giardia duodenalis* in human worldwide.

Locations	Total No.	Positive No.	Infection rate %	No. genotyped	Assemblage distributions	Sub-assemblage A distributions
Albania	125	22	17.60%	22	A (10), B (12)	
Argentina	384	137	35.68%	84	A (13), B (69), D (1), mix (1)	AII (4), AIII (7)
Australia	440	96	21.82%	66	A (19), B (47)	AI (1), AII (9)
Bangladesh	2659	336	12.64%	305	A (38), B (250), mix (17)	AI (8), AII (22)
Belgium	373	15	4.02%	72	A (16), B (54), mix (2)	AII (2)
Brazil	6329	1122	17.73%	808	A (468), B (310), C (6), D (1), E (15), F (1), mix (7)	AI (120), AII (202), AIII (34)
Canada	818	110	13.45%	110	A (66), B (40), mix (4)	AI (20), AII (7)
China	51,924	210	0.40%	144	A (80), B (47), C (16), mix (1)	AI (16), AII (27)
Colombia	235	31	13.19%	24	A (20), B (4)	
Côte d'Ivoire Ivory Coast	9	2	22.22%			
Cuba	95	20	21.05%	20	A (9), B (11)	
Czech Republic		1		1	B (1)	
Egypt	2042	474	23.21%	381	A (129), B (179), C (1), E (27), mix (45)	AII (103)
Ethiopia	978	246	25.15%	104	A (37), B (52), mix (15)	AI (1), AII (27)
Europe		1658		1658	A (714), B (930), C (2), D (4), E (4), F (4)	AI (149), AII (466)
France		50		50	A (9), B (41)	AII (8)
Gabon	241	33	13.69%			
Germany	271	18	6.64%	17	A (14), B (3)	AI (5), AII (9)
Ghana	95	10	10.53%	5	A (3), B (2)	
Guinea Bissau	429	159	37.06%			
India	1496	346	23.13%	336	A (101), B (183), C (3), D (3), mix (46)	AI (6), AII (2)
Iran	1211	394	32.54%	279	A (200), B (69), mix (10)	AII (160)
Italy	2003	160	7.99%	152	A (81), B (57), mix (14)	AI (8), AII (57)
Jamaica	285	19	6.67%	19	A (19)	AI (3), AII (15)
Japan		26		24	A (14), B (10)	AI (12)
Kenya	172	30	17.44%	30	A (4), B (26)	
Korea		7		7	A (7)	
Malaysia	2027	356	17.56%	309	A (155), B (145), mix (9)	AII (30)
Mexico	395	116	29.37%	116	A (110), mix (6)	AI (72), AII (38)
Mongolia	419	14	3.34%			
Mozambique	4847	1488	30.70%	227	A (23), B (199), mix (5)	AI (2), AII (15)
Myanmar	172	19	11.05%	19	A (6), B (13)	
Nepal	6638	311	4.69%	35	A (7), B (26), mix (2)	
Netherlands	892	116	13.00%	116	A (43), B (73)	AI (7), AII (1)
New Zealands	66	6	9.09%	5	A (1), B (4)	
Nicaragua		119		119	A (25), B (94)	AII (16)
Norway		84		84	A (3), B (81)	AII (3)
Peru	2376	539	22.69%	205	A (80), B (103), mix (22)	AI (9), AII (65)
Poland	232	3	1.29%	3	A (2), B (1)	AII (2)
Portugal	190	32	16.84%	32	A (27), B (5)	AI (25), AII (2)
Qatar		54		54	A (9), B (30), mix (15)	AII (6)
Romania	7805	33	0.42%	30	A (27), B (3)	AII (27)
Saudi Arabia	1612	97	6.02%	40	A (23), B (15), mix (2)	AI (12), AII (11)
Slovakia	1262	53	4.20%	27	A (10), B (17)	AI (2), AII (8), BIII (5), BIV (3)
South Africa	968	92	9.50%			
Spain	1943	634	32.63%	259	A (79), B (176), mix (4)	AI (44), AII (31), AIII (1), AII/AIII (2)
Sweden		207		207	A (73), B (128), mix (6)	AII (64)
Tanzania	45	28	62.22%	28	A (6), B (22)	AII (3)
Thailand	989	154	15.57%	133	A (51), B (46), C (1), F (1), mix (34)	AII (21)
Tibet	1015	175	17.24%			
Turkey	4430	189	4.27%	100	A (52), B (46), mix (2)	AI (3), AII (21), AII/AIII (8)
Uganda	1136	132	11.62%	89	A (35), B (46), mix (8)	AI (1), AII (10)
United Arab Emirates	231	108	46.75%	82	A (37), B (37), mix (8)	AI (1), AII (6)
United Kingdom	79	28	35.44%	28	A (26), B (2)	AI (25), AII (1)
United States		2		2	B (2)	
Total	112,383	10,921	9.72%	7067	A (2981), B (3711), C (29), D (9), E (46), F (6), mix (285)	AI (552), AII (1501), AIII (42), AII/AIII (10), BIII (5), BIV (3)

comprehensive assessments of potential zoonotic transmission of *G. duodenalis* from these animals. Therefore, this review aims to assess the zoonotic potential transmission of *G. duodenalis* from dogs and cats to humans by analyzing the *G. duodenalis* prevalence, risk factors, and genotype distributions.

2. Search strategy and selection criteria

We searched PubMed, Web of Science, MEDLINE, ScienceDirect, and the China National Knowledge Infrastructure for all peer-reviewed publications written in English and Chinese documenting the prevalence of *G. duodenalis*. The search terms used included "*Giardia*" AND "human", OR "giardiasis" AND "human" for *G. duodenalis* populations in humans; "*Giardia*" AND "dog," OR "giardiasis" AND "dog" for *G. duodenalis* populations in dogs; and "*Giardia*" AND "cat," OR "giardiasis" AND "cat" for *G. duodenalis* populations in cats.

All the publications related to the molecular identification of *G. duodenalis* in humans, dogs, and cats published before December 31, 2022, were screened. First, their titles and abstracts were screened. Subsequently, the full texts were screened for the molecular prevalence records. Finally, the occurrence and genotype distribution of *G. duodenalis* in humans, dogs, and cats were recorded (Table S1).

3. Molecular epidemiology of *G. duodenalis* in human

3.1. Molecular prevalence of G. duodenalis

G. duodenalis infections in humans have been molecularly identified in at least 55 countries, with the molecular prevalence ranging from 0.03% (9/26886) in Xinjiang, China [18] to 82.05% (32/39) in Turkey [19]. The pooled molecular prevalence of *G. duodenalis* in humans is at 9.72% (10,921/112383), with infection rates ranging from 0.40% (210/51924) in China to 62.22% (28/45) in Tanzania. The prevalence of *G. duodenalis* in humans is highest in Tanzania (62.22%, 28/45), followed by the United Arab Emirates (46.75%, 108/231) and Guinea Bissau (37.06%, 159/429). Conversely, relatively low prevalence rates have been reported in China (0.40%, 210/51924), Romania (0.42%, 33/7805), and Portugal (1.29%, 3/232) (Table 1).

In China, *G. duodenalis* infections in humans have been recorded in at least eight provinces, municipalities, or autonomous regions (Table S2), with a pooled molecular prevalence of 0.40% (210/51924). However, the prevalence varies across the different regions, ranging from 0.03% (9/26886) in Xinjiang [18] to 9.46% (7/74) in Shanghai [20]. Furthermore, analysis of *Giardia* occurrence in humans in China revealed the highest prevalence rates in Heilongjiang (10.17%, 42/413), followed by Anhui (4.12%, 40/972) and Shanghai (3.39%, 28/825). Conversely, relatively low prevalence rates were reported in Xinjiang (0.03%, 9/26886) and Jilin (0.04%, 3/8396) (Table S2).

3.2. Genotype distributions

Of the 10,921 positive samples for *G. duodenalis* in humans, 7067 were successfully genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multiple loci (Table 1). Statistical analysis revealed that the human-specific assemblages A and B were responsible for 42.18% (2981/7067) and 52.51% (3711/7067) of the genotyped samples, respectively. On the contrary, relatively low samples were infected with the felid-specific assemblage F (0.09%, 6/7067).

Of the 2105 human *G. duodenalis* assemblage A isolates identified, 26.34% (552/2095), 71.65% (1501/2095), and 2.00% (42/2095) were classified in sub-assemblages AI, AII and AIII, respectively. These results are consistent with previous studies [5], which revealed that sub-assemblage AI is predominantly found in animals and sub-assemblage AII in humans.

In China, out of the 210 positive samples for *G. duodenalis* in humans, only 114 samples were successfully genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multiple loci (Table 1). Statistical analysis revealed that the human-specific assemblages A and B were responsible for 55.56% (80/144) and 32.64% (47/144)genotyped samples, respectively, followed by the canine-specific assemblage C (11.11%, 16/144). Additionally, one isolate exhibited a mixed assemblage (A/B).

The data revealed that assemblages A and B are the major *G. duodenalis* genotypes infecting humans, consistent with previous studies [1]. Besides, humans can also be infected by canine-specific assemblages C and D and felid-specific assemblage F (including 38 assemblages C or D and 6 assemblages F).

4. Molecular epidemiology of *G. duodenalis* in dogs

4.1. Molecular prevalence of G. duodenalis

G. duodenalis infections in dogs have been documented in at least 38 countries, with the molecular prevalence ranging from 1.17% (8/682) in Qinghai, China [21], to 75.75% (25/33) in Italy [22]. The pooled molecular prevalence is at 15.60% (7510/48140), with infection rates ranging from 2.86% (2/70) in Singapore to 51.22% (63/123) in the Czech Republic. The highest *G. duodenalis* prevalence in dogs was reported in the Czech Republic (51.22%, 63/123), Argentina (44.44%, 16/36), and the Netherlands (31.38%, 107/341). Conversely, relatively low prevalence rates have been reported in Singapore (2.86%, 2/70), Iran (4.04%, 42/1040), and Ecuador (4.82%, 4/83) (Fig. 1).

In China, *G. duodenalis* infections in dogs have been recorded in at least 13 provinces, municipalities, or autonomous regions (Table S3), with a pooled molecular prevalence of 11.49% (1156/10062). The prevalence of *G. duodenalis* infections in dogs varied across the different regions, ranging from 1.17% (8/682) in Qinghai [21] to 63.50% (54/85) in Jilin [23]. The highest prevalence was reported in Shanghai (26.29%, 260/989), Yunnan (13.74%, 36/262), and Beijing (12.75%, 62/485). Conversely, a relatively low prevalence of *G. duodenalis* infections in dogs was reported in Qinghai (2.54%, 8/710), Fujian (3.17%, 10/315), and Xinjiang (3.64%, 22/604) (Fig. 1).

4.2. Risk factors for G. duodenalis infection in dogs

Several factors contribute to the variation in *G. duodenalis* infection rates in dogs (Table 3). For example, dogs in shelters exhibited the highest prevalence rate (28.02%, 1383/4936), followed by working dogs (15.18%, 107/705), stray dogs (15.24%, 199/1306), and pet dogs had the lowest infection rate (13.97%, 983/7039). In this study, working dogs encompassed shepherds, police, and hunting dogs. Interestingly, among the pet dogs, those in pet markets had a much higher infection rate (20.24%, 265/1309) compared to those in pet hospitals (14.16%, 142/1003), kennels (13.11%, 83/633), and families (12.04%, 493/4094). This difference may be attributed to the varying hygiene conditions, the level of care provided by breeders, and the immune status of the animals.

Furthermore, the prevalence of *G. duodenalis* in puppies less than one year old (16.19%, 1278/7893) was notably higher compared to other age groups. Interestingly, in the less than one year age group, the prevalence in the 0–3 month age range (23.80%, 173/727) was significantly higher than in the other age groups (Table 3).

However, there was no significant difference in the prevalence of *G. duodenalis* between female (17.04%, 847/4972) and male (15.63%, 888/5681) dogs ($P > 0.05$). Similarly, there was no significant difference in the prevalence of *G. duodenalis* between free-range dogs (16.73%, 41/245) and dogs kept in captivity (10.95%, 23/210) ($P > 0.05$). Additionally, the difference in prevalence based on the sterilization status of the dogs was insignificant ($P > 0.05$) (Table 3).

Regarding the diarrhea status, dogs that exhibited diarrhea symptoms had a significantly higher prevalence of *G. duodenalis* (22.96%, 541/2356) than those without diarrhea symptoms (17.32%, 585/3377) ($P < 0.01$). Additionally, the prevalence of *G. duodenalis* in rural dogs

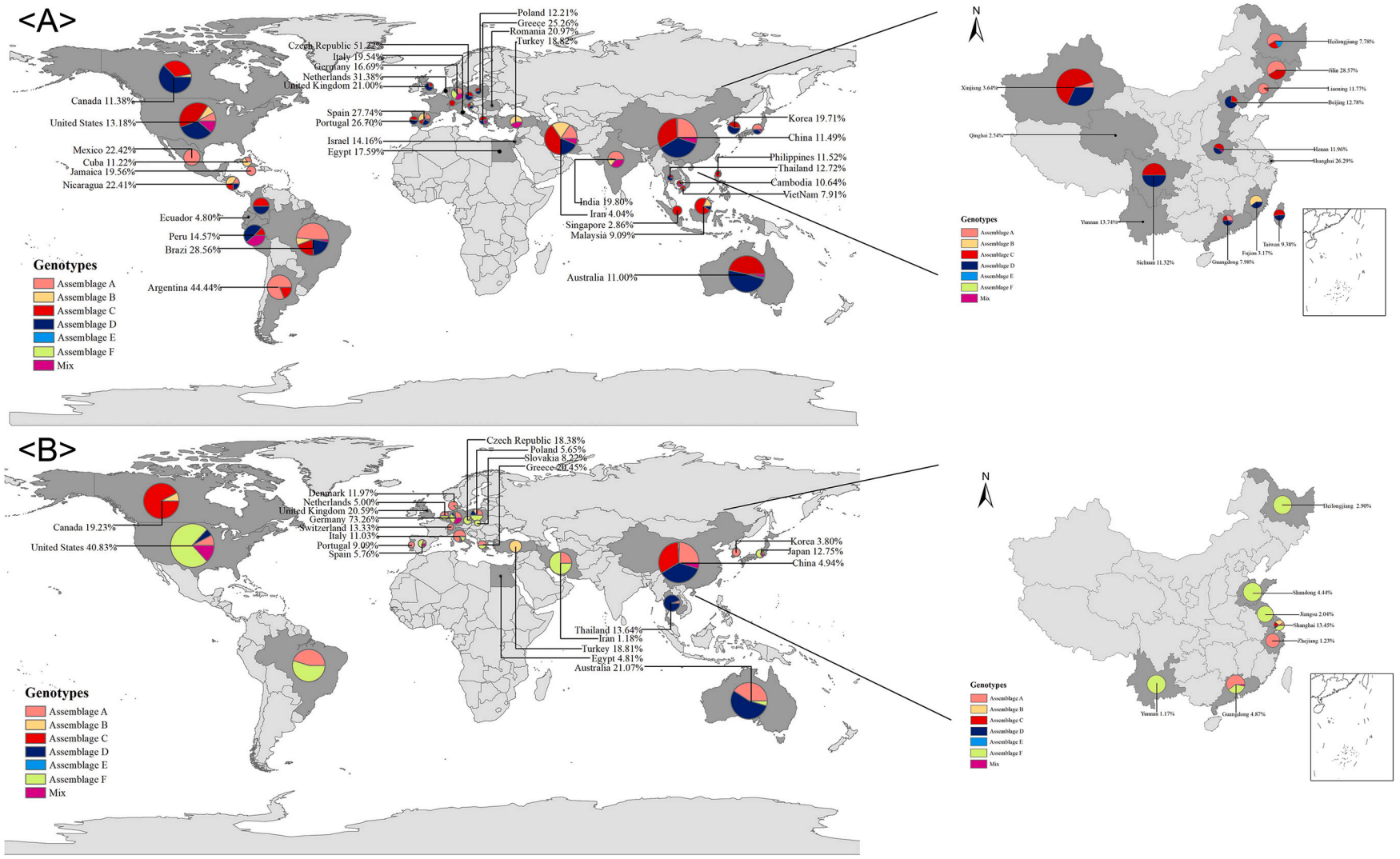

Fig. 1. Molecular prevalence and assemblages distributions of *Giardia duodenalis* in dogs (A) and cats (B) in worldwide and China.

(11.79%, 31/263) was significantly higher than in urban dogs (2.41%, 16/663) ($P < 0.01$). Furthermore, a significantly higher *G. duodenalis* prevalence was recorded in hybrid dogs than in purebred dogs. Dogs that had not been dewormed in the past month also recorded a significantly higher prevalence of *G. duodenalis* infections ($P < 0.01$) (Table 3).

Among the different seasons, the infection rate of *G. duodenalis* was the highest during summer, with a prevalence of 24.7% (92/373), followed by spring (22.94%, 120/523), winter (21.09%, 89/422), and autumn (16.67%, 47/282) (Table 3).

4.3. Genotype distributions

Among the positive samples for *G. duodenalis* identified in dogs worldwide, 3792 samples were successfully genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multi loci (Table 2), of which 66.59% (2525/3792) were canine-specific assemblages C and D. Specifically, 32.49% (1232/3792) of the successfully genotyped samples belonged to assemblage C and 34.10% (1293/3792) to assemblage D. Zoonotic assemblages A and B accounted for 23.07% (875/3792) of the positive samples, with assemblage A accounting for 16.85% (639/3792) and assemblage B for 6.22% (236/3792). There was a relatively low prevalence of the ruminant-specific assemblage E, which accounted for only 0.37% (14/3792) of the samples, and the felid-specific assemblage F (0.11%, 4/3792). Additionally, there was a considerable number (9.86%, 374/3792) of mixed infections, including assemblages C/D (63.37%, 237/374), A/B (14.17%, 53/374), and A/C (7.75%, 29/374) (Supplementary Fig. S1).

In this study, 259 canine *G. duodenalis* assemblage A isolates were identified, belonging to the three sub-assemblages: AI (66.80%, 173/259), AII (32.82%, 85/259), and AIII (0.39%, 1/259). Previous studies revealed that sub-assemblage AI is predominantly found in animals, while sub-assemblage AII is mainly found in humans [5]. This suggests that dogs infected with *G. duodenalis* may harbor the same genotype as humans, indicating the potential for zoonotic transmission.

In China, 797 positive samples for *G. duodenalis* in dogs were genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multiple loci (Table S3). Among them, the canine-specific assemblages C and D accounted for 68.63% (547/797) of the identified *G. duodenalis*-positive samples in dogs. Independently, assemblage C accounted for 32.62% (260/797) of the positive samples, and assemblage D for 36.01% (287/797). Zoonotic assemblages A and B were responsible for 25.47% (203/797) of the samples, with assemblage A accounting for 24.47% (195/797) and assemblage B for 1.00% (8/797). The ruminant-specific assemblage E was identified in 0.63% (5/797) of the samples, and the feline-specific assemblage F in 0.38% (3/797). Additionally, there were mixed infections, which accounted for 4.89% (39/797) of the samples, including assemblages C/D (69.23%, 27/39), A/D (20.51%, 8/39) and A/C (10.26%, 4/39). Interestingly, only sub-assemblage AI was identified in dogs in Guangdong and Liaoning provinces in China [24].

Table 2
Molecular prevalence and assemblage distributions of *Giardia duodenalis* in dogs worldwide.

Locations	Total No.	Positive No.	Infection rate %	No. genotyped	Assemblage distributions	Sub-assemblage A distributions
Argentina	36	16	44.44%	16	A (13), C (3)	
Australia	2601	286	11.00%	62	C (29), D (30), E (1), mix (2)	
Brazil	1744	498	28.56%	179	A (87), B (13), C (36), D (38), mix (5)	AI (50), AII (23)
Cambodia	94	10	10.64%	10	B (2), C (4), mix (4)	
Canada	3406	388	11.38%	108	B (3), C (37), D (67), E (1)	
China	10,062	1156	11.49%	797	A (195), B (8), C (260), D (287), E (5), F (3), mix (39)	AI (51)
Columbia		4		4	C (2), D (2)	
Croatia	96	96		93	A (4), B (10), C (18), D (21), mix (40)	
Cuba	98	11	11.22%	9	A (5), B (4)	AI (4), AII (1)
Czech Republic	123	63	51.22%	54	C (21), D (32), mix (1)	
Ecuador	83	4	4.82%	0		
Egypt	108	19	17.59%	0		
Germany	1393	232	16.69%	184	A (46), B (3), C (38), D (51), F (1), mix (45)	
Greece	879	222	25.26%	99	A (5), C (45), D (28), mix (21)	AI (4), AII (1)
India	202	40	19.80%	17	A (8), B (3), mix (6)	AI (1), AII (4)
Iran	1040	42	4.04%	32	A (5), B (6), C (13), D (6), mix (2)	AII (1)
Israel	854	121	14.16%	0		
Italy	6744	1318	19.54%	558	A (64), B (8), C (248), D (207), mix (31)	AI (17)
Jamaica	225	44	19.56%	44	A (44)	AI (13), AII (31)
Japan	28	28		28	A (14), C (1), D (10), mix (3)	
Korea	842	166	19.71%	61	C (26), D (35)	
Malaysia	132	12	9.09%	11	B (2), C (8), D (1)	
Mexico	825	185	22.42%	48	A (48)	AI (30), AII (13)
Netherlands	341	107	31.38%	103	A (3), C (27), D (55), mix (18)	AI (2)
Nicaragua	58	13	22.41%	13	A (2), B (5), C (3), D (3)	
Peru	604	88	14.57%	67	C (9), D (32), mix (26)	
Philippines	165	19	11.52%	19	C (17), D (2)	
Poland	770	94	12.21%	58	A (7), B (1), C (23), D (25), E (1), mix (1),	
Portugal	206	55	26.70%	43	B (1), C (19), D (23),	
Romania	124	26	20.97%	0		
Singapore	70	2	2.86%	2	C (2)	
Spain	1370	380	27.74%	170	A (33), B (62), C (22), D (40), E (6), mix (7)	AII (11), AIII (1)
Switzerland	1	1		1	C (1)	
Thailand	1054	134	12.72%	56	A (2), C (16), D (36), mix (2)	
Turkey	473	89	18.82%	89	B (51), mix (38)	
United Kingdom	878	184	20.96%	41	A (1), C (10), D (29), mix (1)	AI (1)
United States	10,086	1329	13.18%	691	A (53), B (54), C (277), D (228), mix (79)	
Vietnam	354	28	7.91%	25	C (17), D (5), mix (3)	
Total	48,140	7510	15.60%	3792	A (639), B (236), C (1232), D (1293), E (14), F (4), mix (374)	AI (173), AII (85), AIII (1)

5. Molecular epidemiology of *G. duodenalis* in cats

5.1. Molecular prevalence of G. duodenalis

G. duodenalis infection in cats has been reported in 23 countries worldwide, with an overall pooled prevalence of 14.53% (1125/7740) (Table 4). The prevalence varies across regions, ranging from 1.18% (4/340) in Iran [25] to 40.83% (89/218) in the United States of America [26]. Germany has the highest prevalence of *G. duodenalis* in cats (73.26%, 63/86), followed by the United States of America (40.83%, 89/218), Australia (21.07%, 208/987), United Kingdom (20.59%, 224/1088), Greece (20.45%, 54/264), Canada (19.23%, 45/234), Turkey (18.81%, 38/202), and the Czech Republic (18.38%, 25/136) (Fig. 1). Generally, most countries have a *G. duodenalis* infection rate exceeding 15.00%. The United Kingdom (20.59%, 224/1088), Italy (11.03%, 87/789), and China (4.94%, 78/1579) have conducted more epidemiological surveys on *G. duodenalis* infection in cats, providing additional data on the prevalence of *G. duodenalis* infection in these countries.

In China, *G. duodenalis* infection in cats has been reported in seven provinces/municipalities (Table S3). The infection rates range from 1.17% (2/171) in Yunnan [27] to 13.45% (23/171) in Shanghai [28]. Thus, Shanghai has the highest prevalence of *G. duodenalis* infection in cats compared to other regions (Table S4).

5.2. Risk factors for Giardia duodenalis infections in cats

Various factors contribute to the differences in *G. duodenalis* infection rates in cats (Table 5). For example, stray cats have the highest prevalence (18.27%, 99/542) ($P < 0.01$), followed by shelter cats (13.48%, 93/690). Pet cats had the lowest infection rate (12.47%, 119/954). Interestingly, among pet cats, there was no significant difference in *G. duodenalis* infections between cats in pet families, pet hospitals, and pet markets ($P > 0.05$). However, the *G. duodenalis* prevalence in cats was slightly higher in kittens less than a year old (8.75%, 47/537) compared to those over a year old (6.71%, 54/805), though the difference was insignificant ($P > 0.05$). In addition, there were no significant differences in *G. duodenalis* infections among cats based on gender, feeding methods, living environments, sterilization status, or deworming status ($P > 0.05$) (Table 5). However, cats with diarrhea had significantly higher *G. duodenalis* infections (20.05%, 243/1212) than those without diarrhea symptoms (5.35%, 34/635) ($P < 0.01$).

5.3. Genotype distributions

Of the 1125 positive *G. duodenalis* samples identified in cats, 408 were successfully genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multiple loci (Table 4). Statistical analysis revealed that the felid-specific assemblage F accounted for 42.89% (175/408) of the genotyped samples. Zoonotic assemblages A and B accounted for 34.07% (139/408) and 7.35% (30/408) samples, respectively. However, the canine-specific assemblages C and D accounted for the least number of genotyped samples, with assemblage C accounting for 1.96% (8/408) and assemblage D 5.88% (24/408). Among the *G. duodenalis*-infected samples, 32 isolates exhibited mixed genotypes, with A/F (56.25%, 18/32) as the predominant mixed genotype, followed by B/F (12.5%, 4/32), and some mixed genotypes (Supplementary Fig. S1).

In China, out of the 78 *G. duodenalis* positive samples identified in cats, only 56 samples were successfully genotyped using *SSU* rRNA, *bg*, *gdh*, *tpi*, or multiple loci (Table S3). Of the 56 samples, the felid-specific assemblage F accounted for 44.64% (25/56) of the genotyped samples, followed by zoonotic assemblages A (35.71%, 20/56) and B (10.71%, 6/56). Further analysis revealed only AI *G. duodenalis* assemblage A subtype was identified in cats. The canine-specific assemblages C and D were the least, with assemblage C accounting for 5.36% (3/56) and assemblage D for 1.79% (1/56). Additionally, one isolate exhibited a mixed assemblage (A/C).

Overall, assemblage F was the major *G. duodenalis* genotype infecting the cats, consistent with previous studies [1,5].

6. Assessment of potential zoonotic transmission

6.1. Waterborne or foodborne zoonotic transmission

Giardiasis is a significant global health concern and one of the most common causes of waterborne and foodborne diseases worldwide. It accounts for over 280 million human diarrhea cases annually [3]. Besides, several major *G. duodenalis* outbreaks have been reported globally, highlighting its impact on public health. For example, in 1955, >50,000 people were infected with *G. duodenalis* through contaminated water in the United States of America [10]. In 2004, another extensive outbreak occurred in Bergen, Norway, affecting over 1500 individuals. This outbreak was traced back to drinking *Giardia* cysts-contaminated water due to leakage from a septic tank [29]. Since then, >300 outbreaks of

Table 4
Prevalence and assemblage distributions of *Giardia duodenalis* in cats worldwide.

Locations	Total No.	Positive No.	Infection rate %	No. genotyped	Assemblage distributions	Sub-assemblage distribution
Australia	987	208	21.07%	22	A (9), D (12), F (1)	
Brazil	56	56		20	A (9), F (11)	AI (9)
Canada	234	45	19.23%	13	B (12), C (1)	
China	1579	78	4.94%	56	A (20), B (6), C (3), D (1), F (25), mix (1)	AI (6)
Czech Republic	136	25	18.38%	25	A (2), F (23)	AI (2)
Denmark	284	34	11.97%	10	A (9), F (1)	
Egypt	104	5	4.81%			
Germany	86	63	73.26%	52	A (14), B (2), C (1), D (3), F (16), mix (16)	
Greece	264	54	20.45%	13	A (7), F (6)	
Iran	340	4	1.18%	4	A (1), F (3)	AI (1)
Italy	789	87	11.03%	69	A (52), C (2), D (3), F (10), mix (2)	
Japan	345	44	12.75%	44	A (5), C (1), F (31), mix (7)	
Korea	158	6	3.80%			
Netherlands	60	3	5.00%	2	A (1), F (1)	
Poland	301	17	5.65%	14	A (3), B (2), D (2), F (7)	
Portugal	22	2	9.09%	2	A (2)	
Slovakia	73	6	8.22%	6	F (6)	
Spain	243	14	5.76%	5	A (1), F (3), mix (1)	
Switzerland	105	14	13.33%			
Thailand	66	9	13.64%	2	A (1), D (1)	AI (1)
Turkey	202	38	18.81%	8	B (8)	
United Kingdom	1088	224	20.59%			
United States	218	89	40.83%	41	A (3), D (2), F (31), mix (5)	
Total	7740	1125	14.53%	408	A (139), B (30), C (8), D (24), F (175), mix (32)	AI (19)

Table 5
Giardia duodenalis infection in cats under different factors.

Factors			Positive no.	Total no.	Infection rate %	*P* value	χ^2 (95%CI)	Assemblage distributions
Source								
	Pet	Pet family	65	529	12.29%	Reference	Reference	F (2)
		Pet hospital	38	303	12.54%	0.915	0.011 (0.637–1.498)	
		Pet market	103	832	12.38%	0.960	0.003 (0.712–1.381)	A (1), B (2), C (2), D (1), F (5)
		Subtotal	119	954	12.47%	0.917	0.011 (0.712–1.358)	A (1), B (2), C (2), D (1), F (7)
	Shelter cats		93	690	13.48%	0.540	0.376 (0.641–1.263)	A (3), B (4), F (2)
	Stray cats		99	542	18.27%	0.007	7.378 (0.447–0.880)	F (10)
Age								
	≤1y		47	537	8.75%	Reference	Reference	A (4), B (2), C (2), D (1), F (3), mix (1)
	>1y		54	805	6.71%	0.164	1.934 (0.888–2.004)	A (1), B (4), F (3), mix (2)
Gender								
	Female		53	706	7.51%	Reference	Reference	A (2), mix (2)
	Male		43	578	7.44%	0.963	0.002 (0.665–1.534)	A (1), mix (1)
Feeding methods								
	Captivity		9	38	23.68%	Reference	Reference	
	Free-range		10	29	34.48%	0.331	0.944 (0.202–1.720)	
Diarrhea status								
	Yes		243	1212	20.05%	Reference	Reference	
	No		34	635	5.35%	0.000	70.585 (3.053–6.437)	
Living environment								
	Urban			13		Reference	Reference	
	Rural		5	48	10.42%	0.225	1.475 (1.014–1.229)	
Sterilization								
	Yes		1	34	2.94%	Reference	Reference	
	No		2	78	2.56%	0.910	0.013 (0.101–13.145)	
Breed								
	Purebred		20	187	10.70%			
	Hybrid							
Deworming								
	Yes		1	15	6.67%	Reference	Reference	
	No		5	19	26.32%	0.136	2.227 (0.021–1.938)	

giardiasis have been reported worldwide, with exposure to contaminated drinking or recreational water being the primary transmission route [30].

In addition to waterborne transmission, there are also documented cases of foodborne *G. duodenalis* outbreaks worldwide [10]. For example, *G. duodenalis* has been detected in fruits and vegetables in various countries, including Bangladesh, Brazil, Costa Rica, Egypt, Ethiopia, Ghana, India, Iran, Italy, Jordan, Norway, Saudi Arabia, Spain, and Sudan [31–39]. The average prevalence of *G. duodenalis* in these foods has been reported to be 4.8% [40].

These reports highlight the importance of addressing water and food safety measures to prevent *G. duodenalis* transmission and reduce the occurrence of giardiasis outbreaks.

6.2. Zoonotic potential of G. duodenalis in dogs and cats

G. duodenalis is commonly detected in domestic and wild animals, with a notable presence of zoonotic assemblages A and B. Notably, domestic animals such as sheep, goats, pigs, and calves, and wild animals including bison, wild raccoons, and wild canines harbor *G. duodenalis* [11,41–48]. Besides, a case-control study revealed that giardiasis was associated with contact with farm animals and pets, particularly pigs, dogs, and cats [49].

An assessment of the zoonotic potential of *G. duodenalis* in the different breeds of dogs revealed that the highest proportion of zoonotic potential assemblages is in working dogs (86.21%, 25/29). However, the *G. duodenalis* prevalence is lowest in working dogs (15.18%, 107/705) and highest in shelter dogs (28.02%, 1383/4936) (Table 3), implying that shelter dogs have a higher overall prevalence, but working dogs have a higher zoonotic potential for transmitting the infection.

Moreover, the prevalence of *G. duodenalis* in dogs decreases with increasing dog age (from 5.15% to 16.19%). However, the zoonotic potential increases with age (13.81% to 22.92%). Interestingly, no potential zoonotic assemblages were identified in dogs older than 5 years (Table 5), though the highest zoonotic potential was in the >5-year age group. Nonetheless, the dog gender and diarrhea status do not significantly influence their zoonotic potential. For cats, it is worth noting that the statistical results regarding zoonotic potential and age groups, particularly in shelter cats and cats over 1 year old, need to be verified with more samples, given the limited sample size (<20 cats) analyzed in this study (Table 6). Thus, further studies with a larger sample size are necessary to confirm these findings and provide more robust insights into the zoonotic potential of *G. duodenalis* in cats.

Given dogs and cats are common companion animals for humans, they have a higher chance of contact with humans, water, and food. Therefore, further studies are necessary to investigate *G. duodenalis* in human populations, livestock, pet animals, and environmental samples. It is crucial to adopt a multidisciplinary one-health approach involving zoologists, ecologists, veterinarians, and public health experts to gain a comprehensive understanding of *G. duodenalis* infections in dogs and cats and potential transmission routes. This collaborative effort will contribute to effective prevention and control strategies for *G. duodenalis* infections and mitigate the risk of zoonotic transmission.

7. Conclusion

G. duodenalis is an important zoonotic parasite transmitted between dogs, cats, and humans. The worldwide prevalence of *G. duodenalis* is significant in humans (9.72%), dogs (15.60%), and cats (14.53%). Human-specific assemblages A and B, canine-specific assemblages C and D, and felid-specific assemblages F are the dominant genotypes identified in humans, dogs, and cats, respectively. The zoonotic assemblages A and B cannot be ignored in dogs and cats, as they account for a considerable proportion of dogs and cats infections, respectively.

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Table 3
Giardia duodenalis infections in dogs under different factors.

Factors			Positive No.	Total No.	Infection rate %	P-vlaue	χ^2 (95%CI)	Assemblage distributions	Sub-assemblage distribution
Source									
	Pet	Pet family	493	4094	12.04%	Reference	Reference	A (15), C (21), D (21), F (1), mix (7)	
		Pet hospital	142	1003	14.16%	0.069	3.306 (0.679–1.015)	A (6), C (15), D (45), mix (15)	
		Pet kennels	83	633	13.11%	0.444	0.587 (0.707–1.164)	C (7), D (7), mix (1)	
		Pet market	265	1309	20.24%	0.000	55.329 (0.458–0.636)	A (27), B (2), C (64), D (104), E (1), mix (16)	
		Subtotal	983	7039	13.97%	0.004	8.324 (0.751–0.947)	A (51), B (2), C (107), D (175), E (1), F (1), mix (35)	
	Working dogs		107	705	15.18%	0.020	5.404 (0.610–0.959)	A (25), C (2), D (2)	AI (25)
	Shelter dogs		1383	4936	28.02%	0.000	347.056 (0.314–0.394)	A (2), B (1), C (91), D (85), mix (23)	AI (1), AII (1)
	Stray dogs		199	1306	15.24%	0.003	9.049 (0.637–0.910)	A (9)	AI (9)
Age									
	≤1y	0-3 m	173	727	23.80%	Reference	Reference	C (3), D (3)	
		3-6 m	443	3462	12.80%	0.000	57.966 (1.746–2.593)	A (4), C (24), D (70), F (1), mix (11)	AI (2)
		6-12 m	662	3704	17.87%	0.000	13.945 (1.186–1.734)	A (22), C (30), D (32), mix (8)	AI (4)
		Subtotal	1278	7893	16.19%	0.000	27.858 (1.353–1.943)	A (29), C (57), D (105), mix (19)	AI (6)
	>1y		932	11,210	8.31%	0.000	194.824 (2.867–4.137)	A (11), C (23), D (28), mix (1)	AI (3)
	1-5y		761	7888	9.65%	0.000	137.859 (2.427–3.524)	A (11), C (14), D (22), mix (1)	AI (3)
	>5y		171	3322	5.15%	0.000	266.835 (4.573–7.241)	C (9), D (6)	
Gender									
	Female		847	4972	17.04%	Reference	Reference	A (20), C (28), D (39), mix (3)	AI (5)
	Male		888	5681	15.63%	0.050	3.835 (1.000–1.228)	A (15), C (47), D (43), F (1), mix (7)	AI (4)
Feeding methods									
	Captivity		23	210	10.95%	Reference	Reference		
	Free-range		41	245	16.73%	0.077	3.128 (0.354–1.058)		
Sterilization									
	Yes		61	333	18.32%	Reference	Reference		
	No		195	834	23.38%	0.059	3.562 (0.533–1.013)		
Diarrhea status									
	Yes		541	2356	22.96%	Reference	Reference	B (1), C (28), D (61), mix (16)	
	No		585	3377	17.32%	0.000	27.966 (1.248–1.622)	A (1), B (1), C (27), D (88), mix (22)	AI (1)
Living environment									
	Urban		16	663	2.41%	Reference	Reference		
	Rural		31	263	11.79%	0.000	34.324 (0.099–0.345)		
Breed									
	purebred dog		28	120	23.33%	Reference	Reference		
	hybrid dogs		66	162	40.74%	0.002	9.400 (0.261–0.749)		
Deworming									
	Yes		55	1058	5.20%	Reference	Reference	C (1), D (4)	
	No		64	560	11.43%	0.000	20.859 (0.292–0.619)	C (4), D (10), F (1)	
Season									
	Spring		120	523	22.94%	Reference	Reference	C (9), D (11)	
	Summer		92	373	24.66%	0.550	0.357 (0.666–1.242)	C (17), D (22)	
	Autumn		47	282	16.67%	0.036	4.392 (1.025–2.163)		
	Winter		89	422	21.09%	0.495	0.466 (0.817–1.519)	D (2)	,

Table 6
Assemblage distributions of *Giardia duodenalis* in dogs and cats under different factors.

Animals	Factors	Positive no.	Total no.	Infection rate %	No. of genotyped	Assemblage distribution	Zoonotic potential (no.)
Dogs	Source						
	Pet	983	7039	13.97%	372	A (51), B (2), C (107), D (175), E (1), F (1), mix (35)	14.24% (53)
	Working dogs	107	705	15.18%	29	A (25), C (2), D (2)	86.21% (25)
	Shelter	1383	4936	28.02%	203	A (3), B (1), C (91), D (85), mix (23)	1.97% (4)
	Stray dogs	199	1306	15.24%	9	A (9)	100% (9)
	Age						
	≤1y	1278	7893	16.19%	210	A (29), C (57), D (105), mix (19)	13.81% (29)
	>1y	932	11,210	8.31%	63	A (11), C (23), D (28), mix (1)	17.46% (11)
	1-5y	761	7888	9.65%	48	A (11), C (14), D (22), mix (1)	22.92% (11)
	>5y	171	3322	5.15%	15	C (9), D (6)	0 (0)
	Gender						
	Female	847	4972	17.04%	90	A (20), C (28), D (39), mix (3)	22.22% (20)
	Male	888	5681	15.63%	113	A (15), C (47), D (43), F (1), mix (7)	13.27% (15)
	Diarrhea status						
	Yes	541	2356	22.96%	106	B (1), C (28), D (61), mix (16)	0.94% (1)
	No	585	3377	17.32%	139	A (1), B (1), C (27), D (88), mix (22)	1.44% (2)
Cats	Source						
	Pet	119	954	12.47%	13	A (1), B (2), C (2), D (1), F (7)	23.08% (3)
	Shelter	93	690	13.48%	9	A (3), B (4), F (2)	77.78% (7)
	Stray cats	99	542	18.27%	10	F (10)	0 (0)
	Age						
	≤1y	47	537	8.75%	13	A (4), B (2), C (2), D (1), F (3), mix (1)	46.15% (6)
	>1y	54	805	6.71%	10	A (1), B (4), F (3), mix (2)	50.0% (5)
	Gender						
	Female	53	706	7.51%	4	A (2), mix (2)	50.0% (2)
	Male	43	578	7.44%	2	A (1), mix (1)	50.0% (1)

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Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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