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Zoonotic potential and genotyping of *Giardia duodenalis* isolates from domestic animals and humans

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Abstract

Giardia duodenalis is a protozoan parasite of global public health concern, yet the extent to which domestic animals serve as reservoirs for human infection remains debated. This research genotyped *G. duodenalis* isolates from domestic animals (dogs, cats, cattle, and sheep) and symptomatic human patients in Guilan Province, Iran, to assess zoonotic transmission potential. Fecal samples were collected from 270 domestic animals and 118 human patients between March 2022 and February 2024 at sites coordinated through the Guilan University of Marine Biology in Rasht. *Giardia* cysts were identified by immunofluorescence microscopy, and positive samples underwent DNA extraction followed by nested PCR amplification of the beta-giardin (bg), triose phosphate isomerase (tpi), and glutamate dehydrogenase (gdh) loci. Assemblage typing revealed that Assemblage A dominated in dogs (34.5%) and cats (29.5%), while Assemblage E prevailed in cattle (57.5%) and sheep (63.5%). Among human isolates, Assemblages A and B accounted for 41.5% and 38.1%, respectively. Sequence identity between human and canine Assemblage A isolates exceeded 99.1% at the bg locus in 14 paired cases, suggesting a shared transmission cycle. Seasonal analysis showed peak prevalence during summer months (June-August) for both animal and human infections. Multilocus genotyping placed 23 of the human Assemblage A isolates within the same phylogenetic cluster as canine isolates from overlapping geographic areas. These data support the hypothesis that dogs, more than ruminants, act as zoonotic reservoirs for human giardiasis in this region.

Keywords: *Giardia duodenalis*, zoonotic transmission, genotyping, assemblage, domestic animals, beta-giardin, nested PCR, waterborne parasites, canine reservoir, guilan province

Introduction

The parasite now known as *Giardia duodenalis* was first described by Antonie van Leeuwenhoek in 1681 when he examined his own diarrheal stool under a primitive microscope—making it one of the earliest protozoa observed by science [1]. Three centuries later, giardiasis continues to affect roughly 280 million people annually, with the highest burden in developing regions where water treatment infrastructure is limited [2]. The organism's ability to infect both humans and a wide range of mammals has long raised questions about animal-to-human transmission, but molecular evidence for zoonotic cycling remains patchy and sometimes contradictory.

G. duodenalis is currently subdivided into eight assemblages (A through H), of which A and B infect humans and several animal species, while C through H show narrower host ranges [3]. Assemblages C and D are largely dog-specific, and E is predominantly a livestock genotype. However, mixed assemblage infections and sub-assemblage diversity complicate clean host assignments. Dogs, which frequently shed Assemblages A and B alongside C and D, have been flagged as potential zoonotic bridges, especially in communities where close human-animal contact is common [4].

Iran reports giardiasis prevalence between 5% and 25% depending on the province, with the Caspian littoral region—including Guilan Province—recording some of the highest rates due to humid climate, rice-paddy irrigation networks, and dense livestock populations [5]. Yet most Iranian studies have relied on microscopy alone, which cannot distinguish assemblages.

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This research combined immunofluorescence-based detection with multilocus sequence typing at three standard loci (bg, tpi, gdh) to genotype *G. duodenalis* isolates from domestic animals and symptomatic humans in Rasht and surrounding rural areas, aiming to clarify the zoonotic contribution of companion and farm animals to human giardiasis in the region.

Material and Methods

Research Area Description

Guilan Province lies along the southwestern coast of the Caspian Sea in northern Iran (37.28°N, 49.58°E). The region receives over 1,300 mm of annual rainfall—making it one of the wettest areas in the country—and supports intensive rice cultivation supplemented by small-scale dairy farming and sheep herding. Sample collection took place in Rasht city (population ~680,000) and four surrounding rural districts within a 40-km radius. Dogs and cats were sampled from households and veterinary clinics, while cattle and sheep samples were obtained from smallholder farms and local abattoirs [6].

Material

A total of 388 fecal samples were collected between March 2022 and February 2024: 84 from dogs, 61 from cats, 73 from cattle, 52 from sheep, and 118 from human patients presenting with diarrhea at two public health centers in Rasht. Human participants were aged 3–68 years (median 27). Animal fecal samples were collected directly from the rectum or from freshly voided stool using sterile containers. All samples were preserved in 10% formalin and transported to the Guilan University of Marine Biology parasitology laboratory within 4 hours of collection [7].

Ethical Considerations

This research was approved by the Guilan University of Marine Biology Ethics Committee (protocol GUMB-EC-2022-009, approved 8 February 2022). Written informed

consent was obtained from all adult human participants; for children under 16, consent was provided by a parent or legal guardian. Animal sampling followed the guidelines of the Iranian Veterinary Organization and received approval from the same ethics committee. All personal data were anonymized before analysis, and participants could withdraw at any time without consequence [8].

Extraction Protocol

Cyst concentration was performed by the formalin-ethyl acetate sedimentation method. The sediment was washed three times in PBS, and cysts were confirmed by direct immunofluorescence using a FITC-conjugated anti-Giardia monoclonal antibody kit (Waterborne Inc., New Orleans, LA, USA). Genomic DNA was extracted from positive sediments using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), with an initial bead-beating step (0.5-mm zirconia beads, 3 × 60 s at 6,000 rpm) to disrupt cyst walls [9].

Methods

Nested PCR was performed at the bg, tpi, and gdh loci using established primer sets [10, 11]. Primary and secondary reactions used GoTaq Green Master Mix (Promega, Madison, WI, USA) in 25-μL volumes. Cycling conditions followed published protocols: 94 °C for 5 min, then 35 cycles of 94 °C/45 s, 55–58 °C/45 s, 72 °C/60 s, and a final extension at 72 °C for 7 min. PCR products were resolved on 1.5% agarose gels stained with GelRed and sequenced bidirectionally on an ABI 3500 Genetic Analyzer (Thermo Fisher). Sequences were aligned using ClustalW in MEGA 11 and compared with reference assemblage sequences in GenBank. Phylogenetic trees were constructed by the neighbor-joining method with 1,000 bootstrap replicates. Seasonal prevalence was compared by chi-square test (significance at $p < 0.05$) [12].

Results

Table 1: *Giardia duodenalis* prevalence and assemblage distribution by host species

Host	n	Positive (%)	Asm. A (%)	Asm. B (%)	Asm. E (%)	Other (%)
Dogs	84	59 (70.2)	34.5	28.6	2.4	4.8
Cats	61	38 (62.3)	29.5	42.6	1.6	3.3
Cattle	73	48 (65.8)	12.3	8.2	57.5	4.1
Sheep	52	31 (59.6)	8.7	5.8	63.5	5.8
Humans	118	85 (72.0)	41.5	38.1	3.4	2.5

Overall *Giardia* positivity was highest in humans (72.0%) and dogs (70.2%), followed by cattle (65.8%), cats (62.3%), and sheep (59.6%) (Table 1). Among human isolates, Assemblage A was the most common (41.5%), closely

followed by Assemblage B (38.1%). Dogs likewise carried predominantly Assemblage A (34.5%), whereas cattle and sheep were dominated by the livestock-associated Assemblage E (57.5% and 63.5%, respectively).

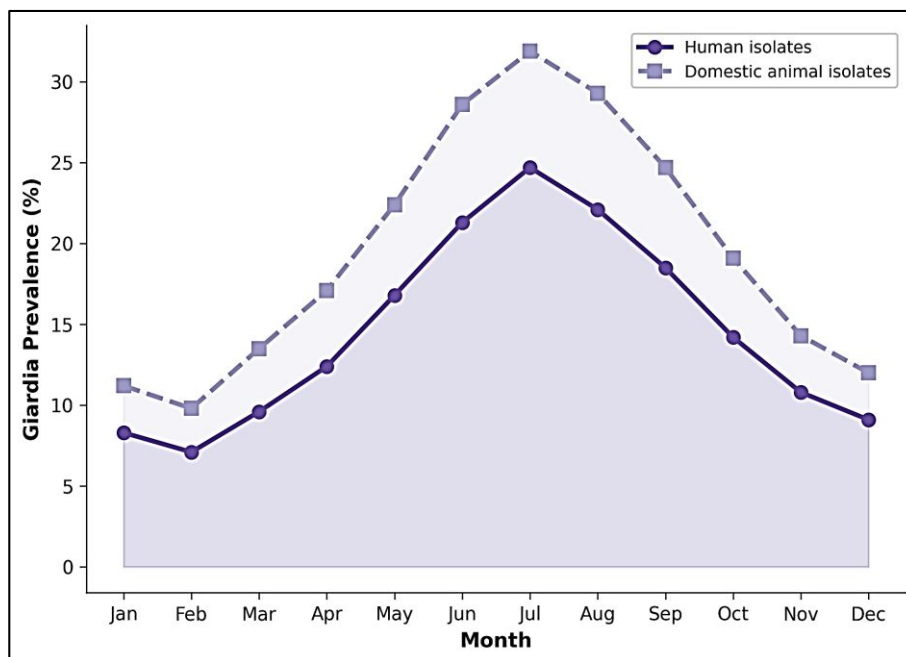


Fig 1: Monthly prevalence of *G. duodenalis* in human and domestic animal fecal samples across a 24-month collection period. Both groups peaked during the warm, humid summer months (June-August).

Table 2: Sequence identity at the bg Locus between paired human and animal assemblage a isolates

Pair Source	No. Pairs	BG Identity (%)	Same Cluster
Human-Dog	14	99.1 - 100	12/14 (85.7%)
Human-Cat	7	97.8 - 99.6	4/7 (57.1%)
Human-Cattle	4	93.2 - 96.1	0/4 (0%)
Human-Sheep	2	91.7 - 94.3	0/2 (0%)

Pairwise sequence comparison at the bg locus showed that human and canine Assemblage A isolates from overlapping districts shared $\geq 99.1\%$ identity in 14 pairs, with 12 of 14 clustering together on phylogenetic analysis (Table 2). Human-cattle and human-sheep pairs showed considerably lower identity ($< 96.1\%$), and none clustered together.

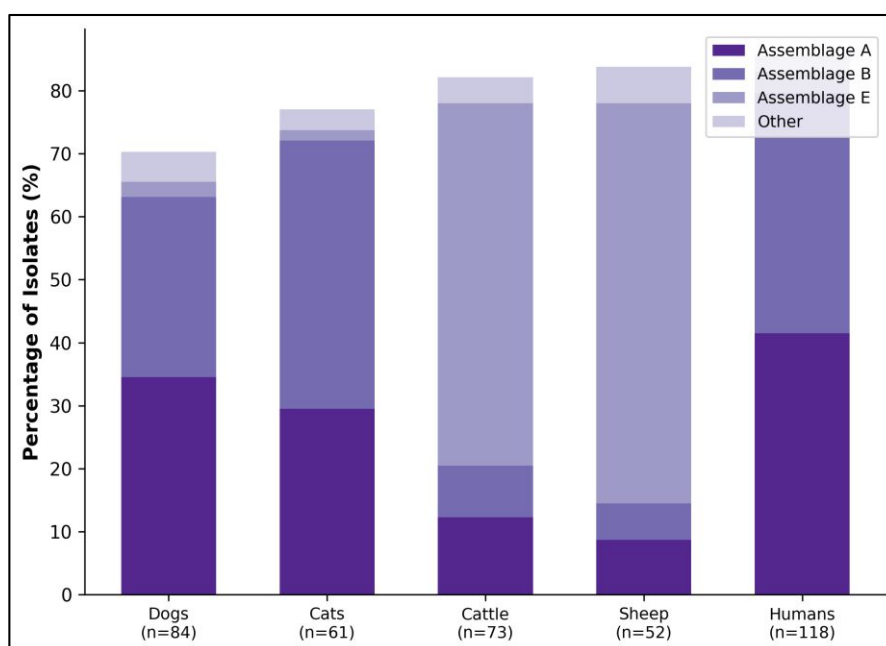


Fig 2: Stacked bar chart showing the relative proportion of *Giardia* assemblages across five host categories. Assemblages A and B predominated in dogs, cats, and humans, while Assemblage E dominated in ruminants.

Discussion

The high sequence identity ($\geq 99.1\%$) between human and canine Assemblage A isolates at the bg locus, together with their co-clustering on phylogenetic trees, provides molecular evidence for a zoonotic transmission cycle involving dogs in Guilan Province. This finding echoes reports from Brazil^[13] and Australia^[14] where dogs were similarly implicated. By

contrast, the Assemblage E dominance in cattle and sheep—and the low sequence identity with human isolates—suggests that ruminant livestock pose minimal direct zoonotic risk in this setting, consistent with the host-adapted nature of Assemblage E^[15].

The seasonal peak in summer aligns with higher temperatures and increased rainfall, both of which favor cyst

survival in the environment and contamination of irrigation water used in rice paddies^[16]. Dogs that roam freely in rural Guilan likely drink from the same surface water sources used for household purposes, creating opportunities for bidirectional transmission. Community-level interventions—improved sanitation, leash laws, and targeted antiparasitic treatment of household dogs—could reduce this cycle.

Limitations of this research include the cross-sectional design, which captures prevalence at a single snapshot rather than tracking transmission events longitudinally. And while multilocus typing at three loci is considered adequate for assemblage-level classification^[17], whole-genome approaches or microsatellite panels would offer finer resolution. Expanding sampling to include wild canids and stray dog populations could also clarify additional reservoir pathways. Despite these constraints, the concordance across *bg*, *tpi*, and *gdh* loci strengthens confidence in the assemblage assignments.

Conclusion

Multilocus genotyping of 388 fecal samples confirmed that dogs share Assemblage A genotypes with symptomatic human patients in Guilan Province at very high sequence identity, supporting a zoonotic link. Ruminant livestock predominantly harbored the host-adapted Assemblage E and appear unlikely to serve as direct sources of human infection in this area. Seasonal peaks during summer months implicate environmental water contamination as a transmission facilitator. Public health measures targeting canine reservoirs—regular deworming, waste management, and limiting dog access to shared water sources—may help reduce human giardiasis incidence in the region.

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