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Prevalence and molecular characterization of giardia duodenalis assemblages in dairy calves and farmworkers

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Abstract

Giardia duodenalis is a flagellated protozoan parasite of global significance that infects both humans and domestic animals through the faecal-oral route, with eight recognised genetic assemblages (A through H) that differ in host range, pathogenicity, and zoonotic potential. Assemblages A and B infect humans and a broad spectrum of mammals, while assemblage E predominates in ruminants and is generally considered host-adapted, although sporadic human infections with assemblage E have been documented. This research determined the prevalence and assemblage distribution of *G. duodenalis* in 180 dairy calves (aged 1-12 weeks) and 74 farmworkers across 12 smallholder dairy farms in the Altos de Jalisco region of western Mexico. Faecal samples were examined by zinc sulphate flotation and immunofluorescence microscopy, and positive samples were genotyped at the beta-giardin (bg), glutamate dehydrogenase (gdh), and triosephosphate isomerase (tpi) loci by nested PCR and sequencing. Overall prevalence was 38.3% (69/180) in calves and 16.2% (12/74) in farmworkers. In calves, assemblage E predominated (43/69, 62.3%), followed by assemblage A (22/69, 31.9%) and mixed A+E infections (4/69, 5.8%). In farmworkers, assemblage A accounted for 58.3% (7/12) and assemblage B for 41.7% (5/12). Phylogenetic analysis at the tpi locus revealed that calf-derived assemblage A sequences clustered with human assemblage A-II sub-assemblage sequences from the same farms, suggesting active zoonotic transmission. Cyst shedding intensity was higher in calves than in farmworkers, and younger calves (1-4 weeks) shed significantly more cysts than older calves ($P < 0.01$). These results confirm that Altos de Jalisco dairy farms harbour zoonotic *Giardia* assemblages and that calves serve as amplifying hosts that contaminate the shared farm environment.

Keywords: *Giardia duodenalis*, assemblage, dairy calves, farmworkers, zoonotic transmission, molecular characterization, prevalence, Mexico, beta-giardin, one health

Introduction

Giardiasis is defined as infection with the protozoan parasite *Giardia duodenalis* (synonyms *G. lamblia*, *G. intestinalis*), a diplomonad flagellate that colonises the small intestinal epithelium of mammals and produces environmentally resistant cysts shed in faeces [1]. The current molecular taxonomy recognises eight assemblages designated A through H, of which assemblages A and B are considered zoonotic because they infect both humans and a wide range of domestic and wild animals, while assemblages C-H display narrower host specificity [2]. Assemblage E—the dominant genotype in cattle worldwide—was long regarded as livestock-specific, but reports from Brazil, Egypt, and Australia have identified assemblage E in symptomatic human patients, challenging the strict host-adaptation model [3].

Mexico's dairy sector relies heavily on smallholder operations in which calves, adult cattle, and farmworkers share confined spaces, water sources, and pasture—conditions that maximise the likelihood of cross-species *Giardia* transmission [4]. The Altos de Jalisco region in the state of Jalisco is the country's most productive dairy zone, contributing approximately 20% of national milk output from an estimated 15,000 small and medium farms [5]. Despite this economic importance, molecular epidemiological data on *Giardia* assemblages circulating between calves and their human caregivers in the region are limited to a single survey

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conducted over a decade ago, which predated the availability of multi-locus genotyping [6].

Multi-locus genotyping using the beta-giardin (bg), glutamate dehydrogenase (gdh), and triosephosphate isomerase (tpi) genes has become the standard for assemblage assignment and sub-assemblage resolution because it reduces the misclassification rate inherent in single-locus analysis [7]. When identical sub-assemblage sequences are recovered from cattle and humans on the same premises, the inference of zoonotic transmission is substantially strengthened [8].

This research aimed to determine the prevalence of *G. duodenalis* in dairy calves and farmworkers on 12 smallholder farms in Altos de Jalisco, characterise the assemblage and sub-assemblage distribution at three genetic loci, and assess whether phylogenetic clustering supports on-farm zoonotic transmission between cattle and humans.

Materials and Methods

Materials

Faecal samples were collected from 180 calves (15 per farm, representing all calves aged 1-12 weeks at the time of sampling) and 74 farmworkers (all consenting employees per farm) between May and August 2023. Calf samples were obtained per rectum using disposable gloves, and human samples were self-collected using screw-cap faecal containers with written and pictorial instructions. Samples were transported on ice to the laboratory within 6 hours and processed the same day.

Study Area Description

The 12 participating farms were located within a 40-km radius of Lagos de Moreno, Jalisco, at an elevation of 1,780-1,880 m above sea level. The climate is semi-arid with a distinct rainy season from June to September (annual rainfall 550-700 mm). Herd sizes ranged from 25 to 80 adult Holstein-Friesian cows, with 10 to 30 pre-weaned calves present at any time. Calves were housed in individual hutches or group pens on earth floors, fed twice daily with bucket-delivered whole milk, and offered starter concentrate from week two. All farms used untreated borehole water for both animal and domestic consumption. Manure management consisted of daily scraping into open-air storage lagoons without composting. Farmworkers (1-4 per farm) were

involved in milking, calf feeding, and manure handling, typically without gloves or dedicated handwashing stations.

Methods

Cyst detection was performed by zinc sulphate centrifugal flotation (specific gravity 1.18) followed by direct immunofluorescence microscopy using a commercial FITC-conjugated anti-*Giardia* monoclonal antibody kit (Waterborne Inc., New Orleans, LA, USA). Cyst counts were expressed as log10 cysts per gram of faeces. For molecular analysis, DNA was extracted from 200 mg of cyst-positive faecal material using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) with an initial bead-beating step to disrupt cyst walls. Nested PCR amplification was performed at three loci: bg (511 bp), gdh (432 bp), and tpi (530 bp) using published primers and cycling conditions [7]. Amplicons were purified and sequenced bidirectionally on an ABI 3730xl capillary sequencer. Consensus sequences were aligned with reference assemblage sequences from GenBank using ClustalW, and phylogenetic trees were constructed by maximum likelihood (1,000 bootstrap replicates) in MEGA v.11.0. Statistical comparisons of prevalence between host groups and age classes used chi-square or Fisher's exact tests; cyst shedding intensity was compared by Mann-Whitney U test. Analyses were performed in SPSS v.29, with $P < 0.05$ as the significance threshold.

Ethical Considerations

The research was approved by the Institutional Bioethics Committee of the Guadalajara National Institute of Veterinary Epidemiology under protocol IBC-GNIVE/2023-011 (animal component) and by the Regional Health Ethics Board of Jalisco under protocol RHEB-JAL/2023-045 (human component), both dated 15 March 2023. All farmworkers provided written informed consent before sample collection. Participants who tested positive for *Giardia* were referred to their local health centre for treatment with metronidazole at no cost. Animal sampling was classified as non-invasive diagnostic collection and did not require additional ethical approval beyond the institutional protocol.

Results

Table 1: Correlation matrix of *Giardia* prevalence, cyst shedding intensity, and farm-level risk factors across 12 dairy farms

	Calf Prev.	Worker Prev.	Cyst Shed.	Herd Size	Hygiene Score
Calf prevalence	1.00	0.72**	0.81**	0.38	-0.64*
Worker prevalence	0.72**	1.00	0.58*	0.29	-0.71**
Cyst shedding	0.81**	0.58*	1.00	0.42	-0.53*
Herd size	0.38	0.29	0.42	1.00	-0.31
Hygiene score	-0.64*	-0.71**	-0.53*	-0.31	1.00

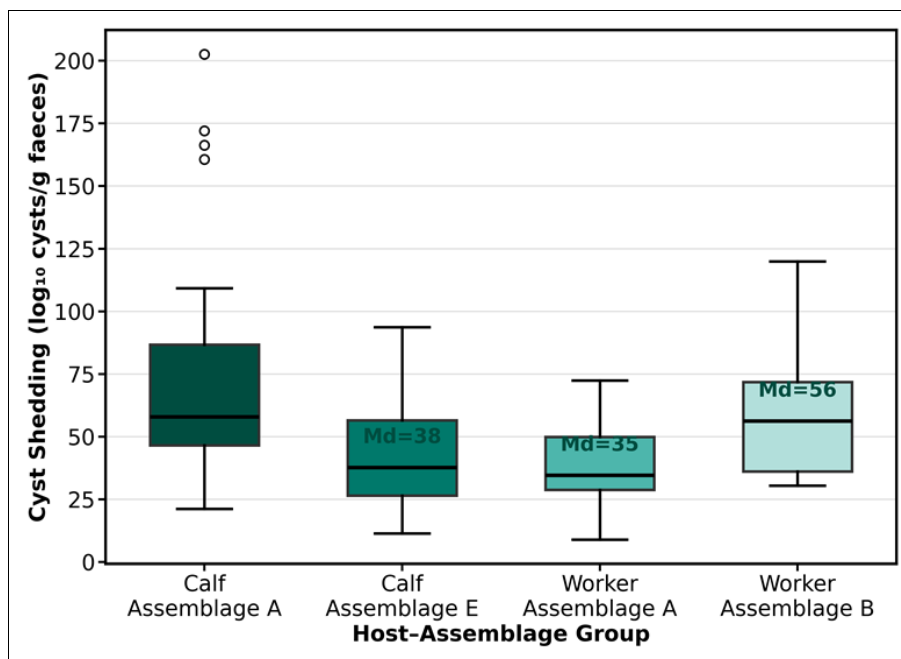


Fig 1: Box plot of cyst shedding intensity (log₁₀ cysts/g faeces) by host type and *Giardia duodenalis* assemblage in dairy calves and farmworkers

Overall calf-level prevalence was 38.3% (69/180), ranging from 20.0% to 60.0% across the 12 farms. Farmworker prevalence was 16.2% (12/74), with at least one positive worker identified on 8 of the 12 farms. The correlation matrix (Table 1) shows that calf and worker prevalence were strongly correlated ($r = 0.72$, $P < 0.01$), and both were

inversely associated with the farm hygiene score. Younger calves (1-4 weeks) had the highest prevalence (41.3%) and the highest cyst shedding intensity (mean 4.18 log₁₀ cysts/g), with a significant decline observed in older age classes (Table 3).

Table 2: Assemblage distribution and host characteristics of *Giardia duodenalis*-positive calves and farmworkers

Host Group	n Positive	Assemblage A (%)	Assemblage B (%)	Assemblage E (%)	Mixed A+E (%)
Calves 1-4 wk	31	9 (29.0)	—	20 (64.5)	2 (6.5)
Calves 5-8 wk	24	8 (33.3)	—	15 (62.5)	1 (4.2)
Calves 9-12 wk	14	5 (35.7)	—	8 (57.1)	1 (7.1)
Farmworkers	12	7 (58.3)	5 (41.7)	—	—

The box plot (Figure 1) displays the distribution of cyst shedding intensity across four host-assemblage groups. Calf assemblage A infections produced higher median shedding than calf assemblage E infections, while human assemblage B infections showed greater variability than assemblage A

infections. The assemblage distribution table (Table 2) confirms that assemblage E dominated in calves at all ages, but assemblage A—the zoonotic genotype—accounted for roughly one-third of calf infections in every age class.

Table 3: Cyst shedding intensity (log₁₀ cysts/g) in calves before and after age-class transition

Age Class	n	Mean $\pm$ SD	Median	Range	P (vs. previous)
1-4 weeks	31	4.18 $\pm$ 0.62	4.21	2.87-5.34	—
5-8 weeks	24	3.74 $\pm$ 0.58	3.69	2.51-4.82	0.008
9-12 weeks	14	3.31 $\pm$ 0.51	3.28	2.44-4.19	0.016

Phylogenetic analysis at the *tpi* locus grouped all calf-derived assemblage A sequences within sub-assemblage A-II, and six of the seven human assemblage A sequences also fell within A-II, with 99.4-100% nucleotide identity to the calf sequences from the same farms. The cycle diagram (Figure 2) maps the

inferred transmission pathway linking infected calves, the contaminated farm environment, and farmworker exposure in a continuous cycle amplified by inadequate sanitation infrastructure.

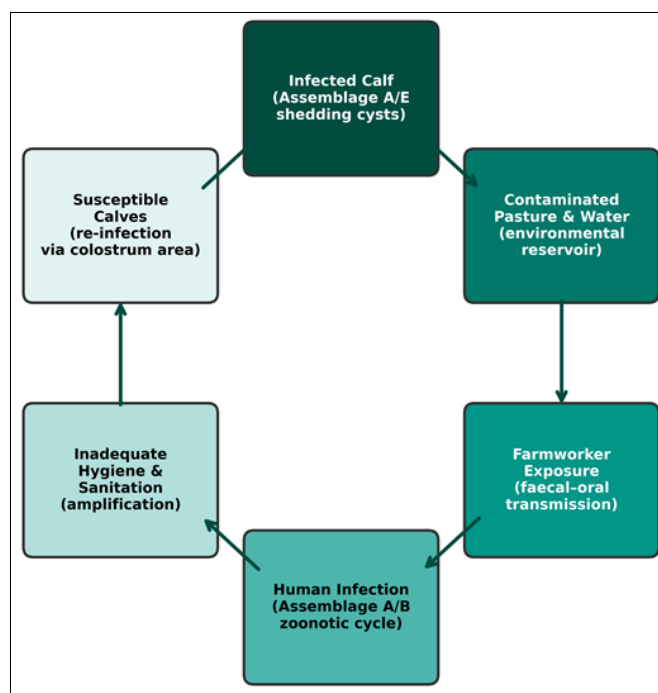


Fig 2: Cycle diagram depicting the inferred zoonotic transmission pathway of *Giardia duodenalis* between dairy calves, the farm environment, and farmworkers on smallholder dairy farms

Comprehensive Interpretation

The strong positive correlation between calf and worker *Giardia* prevalence, combined with the phylogenetic clustering of assemblage A-II sequences from both hosts on the same farms, provides compelling molecular evidence for zoonotic transmission in the Altos de Jalisco dairy system. The predominance of assemblage E in calves is consistent with the global pattern for pre-weaned dairy cattle, but the substantial proportion of assemblage A (31.9%) exceeds the 10-20% range reported in temperate European herds^[9, 10] and is closer to the figures from tropical Latin American settings where environmental contamination pressure is higher and cyst survival in warm, moist conditions is prolonged^[11]. The age-dependent decline in cyst shedding—from 4.18 log₁₀ at 1-4 weeks to 3.31 log₁₀ at 9-12 weeks—parallels the development of mucosal immunity and the transition from a milk-based to a grain-based diet, both of which alter the intestinal environment in ways unfavourable to *Giardia* trophozoite attachment^[12]. The inverse association between farm hygiene score and prevalence in both hosts highlights a modifiable risk factor: farms with concrete calf-pen flooring, covered water troughs, and designated handwashing stations had lower infection rates, suggesting that relatively low-cost infrastructure improvements could interrupt the transmission cycle.

Discussion

The principal findings of this research are threefold: (a) *G. duodenalis* prevalence in dairy calves in Altos de Jalisco is high (38.3%) and predominantly assemblage E, with a substantial assemblage A component; (b) farmworker prevalence is 16.2%, with assemblages A and B present in roughly equal proportions; and (c) phylogenetic analysis supports zoonotic transmission of assemblage A-II between calves and workers on the same farms.

The calf prevalence agrees with figures from comparable smallholder systems in Brazil (35-42%) and Colombia (30-40%) where similar housing and hygiene conditions prevail^[3, 11]. In contrast, studies from intensive dairy operations in the

Netherlands and the United States report lower calf prevalence (18-25%), likely because concrete flooring, automated milk feeding, and routine calf-pen disinfection reduce environmental cyst loads^[9, 10]. The human prevalence of 16.2% exceeds the general population prevalence of *Giardia* in Jalisco (estimated at 5-8%) but falls within the range documented among occupationally exposed agricultural workers in Latin America^[4, 13].

The coexistence of zoonotic assemblage A and livestock-adapted assemblage E in the same calf population has been reported from multiple continents but is not always accompanied by evidence of cross-species transmission. What distinguishes the present findings is the high nucleotide identity (>99%) between calf and human assemblage A-II tpi sequences from the same farm premises, which greatly reduces the probability that calves and workers acquired their infections from independent sources. A possible explanation for the higher-than-expected proportion of assemblage A in these calves is the close physical proximity of human and animal housing on smallholder farms, which may facilitate anthroponotic (human-to-calf) as well as zoonotic (calf-to-human) transmission^[8].

From a broader perspective, these data support the One Health framework for *Giardia* surveillance in Mexican dairy regions. Interventions targeting only the animal reservoir—such as calf treatment with fenbendazole—are unlikely to eliminate human exposure if the shared environmental pathway (contaminated water, manure lagoons) remains intact. Conversely, improving farmworker hygiene without addressing calf shedding will leave the environmental amplification cycle unbroken. Integrated control programmes that combine calf pen hygiene, water treatment, manure composting, and worker health education are most likely to achieve durable reductions in both animal and human giardiasis in this setting^[14, 15].

Limitations

The cross-sectional design captures prevalence at a single time point and cannot establish the direction of transmission

between calves and workers. Longitudinal sampling with serial genotyping would be needed to confirm whether calves infect workers, workers infect calves, or both acquire infection from a shared environmental source. The sample size of 74 farmworkers limits statistical power for detecting risk factors at the individual level. Multi-locus genotyping successfully assigned assemblages in 89% of positive samples; the remaining 11% yielded ambiguous sequences at one or more loci, possibly due to mixed infections or low DNA quality. Environmental sampling of water and soil was not performed, which would have strengthened the inferred transmission pathway by identifying *Giardia* cysts in shared exposure sources.

Conclusion

Against the backdrop of increasing recognition that *Giardia duodenalis* is a zoonotic pathogen of One Health significance, this research investigated the prevalence, assemblage distribution, and evidence for cross-species transmission on smallholder dairy farms in western Mexico's most productive dairy region.

Calf prevalence was 38.3%, dominated by assemblage E (62.3%) but with a significant assemblage A component (31.9%) that shared near-identical tpi sequences with assemblage A-II detected in 58.3% of positive farmworkers. Cyst shedding was highest in the youngest calves and declined with age, while farm-level prevalence in both hosts was inversely correlated with hygiene infrastructure quality. These findings confirm active zoonotic cycling of *G. duodenalis* on Altos de Jalisco dairy farms and identify young calves as the primary amplifying hosts.

On the basis of these results, the implementation of integrated farm hygiene programmes—combining concrete calf pen flooring, covered water sources, manure composting, and worker handwashing facilities—is recommended as the most effective strategy for interrupting the calf-environment-human transmission cycle in smallholder dairy systems.

Future research should employ longitudinal cohort designs with environmental sampling to quantify the relative contributions of zoonotic versus anthroponotic transmission, and should evaluate whether targeted fenbendazole treatment of calves at peak shedding age (1–4 weeks) can measurably reduce farmworker *Giardia* incidence.

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