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Prevalence of Intestinal Protozoan Parasites Among Food Handlers in Iran: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: Intestinal protozoa are significant causes of foodborne diseases in food handlers who may also act as carriers of no signs of infection. Food handling is an important occupation in Iran; although a national estimate of intestinal protozoan prevalence has been previously reported, it is now outdated and more recent data is required. Therefore, we aimed to determine the pooled prevalence of intestinal protozoan parasites and examine geographic variation among food handlers in Iran.
Methods: A systematic literature search was conducted using PubMed, Scopus, Embase, Web of Science, and Google Scholar up to 2025. Cross-sectional studies reporting the prevalence of intestinal protozoa among Iranian food handlers were included. Pooled prevalence with 95% confidence intervals (CIs) was calculated using a random-effects model.
Results: Twenty-four studies with 110,404 food handlers were included. The overall pooled prevalence of intestinal protozoan infection was 14.1% (95% CI: 9.2% – 21.0%). *Blastocystis hominis* was the most common parasite (11.2%), followed by *Entamoeba coli* (8.2%), *Giardia lamblia* (5.4%), and *Chilomastix mesnili* (5.4%).
Conclusion: Approximately one in seven food handlers in Iran harbors intestinal protozoan parasites, with profound geographic disparities. Targeted interventions, including periodic stool screening and hygiene training, are urgently needed, particularly in high-prevalence provinces.

Keywords: Intestinal protozoa, Food handlers, Iran, Systematic review, Meta-analysis, Prevalence

Introduction

Foodborne illnesses remain a public health concern particularly in low and middle-income countries due to lack of robust food safety surveillance systems (1). Among many possible enteric pathogens, intestinal protozoan parasites namely *Giardia lamblia*, *Entamoeba histolytica*, *Blastocystis hominis* and *Cryptosporidium* species are important causes of infection with transmission through food and water contamination (2,3). Food handlers have a vital role as vectors for disseminating parasites, since they are in direct contact with food through preparation, serving and transportation of food even if asymptomatic (4,5). Several factors including poor hand hygiene practices, untrimmed fingernails, use of protective barriers, food safety training deficiencies, can lead to transmission of intestinal protozoa by food handlers (6,7).

Globally, the prevalence of intestinal protozoan infections among food handlers has been estimated in many systematic reviews. A recent systematic review and meta-analysis of 138 studies conducted in 28 countries has suggested that the global pooled prevalence is 14.3% (95%CI: 11.8-17.0%) and *B. hominis* was the predominant protozoan (8). However, this prevalence rate has wide variations across geographic, climate and sanitary practices (9).

Food handling is a pervasive occupation in Iran, especially in large cities and tourism regions where there are a considerable amount of street food vendors and small cafes (10). Gastrointestinal parasitic infections can lead to a broad range of adverse health effects, starting with simple diarrhea caused self-limited until serious life-threatening cases (11). G. lamblia can impact on various hosts resulting from minor diarrhea self-limited until severe cases resulting from significant damage to the hosts system. For example, *G. lamblia* depletes microvillar surface area of the small intestine. Chronic giardiasis can result in malabsorption, poor nutrition and poor development of child (12). E. histolyticais known to be pathogenic and invading causes inflammatory colonic lesions (colitis) that could potentially be accompanied by bloody diarrhea, amoebic dysentery, and extra intestinal abscesses, particularly the liver, that if untreated can be fatal (13). Although its pathogenicity is questionable, B. hominis infection have been positively linked to irritable bowel syndrome, inflammatory bowel disease, and chronic urticarial (14). Cryptosporidium spp. are water-borne enteric parasites that produce profusely water diarrhea. In immunosuppressed hosts such as HIV/AIDS patients, and recipients of organ or bone marrow transplants they cause chronic and severe, wasting and liver disease (15).

In young children, cryptosporidiosis is a major contributor to long-term malnutrition and growth faltering (16). Other species such as Cyclospora cayetanensis and Cystoisospora belli cause prolonged, relapsing diarrheal illness, particularly in immunocompromised hosts (17). Although E. coli**,** Endolimax nana, and Iodamoeba butschlii are generally non-pathogenic, their presence indicates fecal contamination and poor hygiene (8).

Given the mentioned health complications that these parasites would cause a high public and economic burden is generated and it is therefore important to screen these workers periodically, as well as to implement basic and effective hygiene interventions for such group of people. Hence, in the current study we aimed to perform a systematic review and meta-analysis to estimate the pooled prevalence and the factors associated with intestinal protozoan infections among food handlers in Iran.

Materials and Methods

Search strategy

This systematic review and meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1). To identify studies on the prevalence of intestinal protozoan parasites among food handlers in Iran, we systematically searched PubMed, Scopus, Embase, Web of Science, and Google Scholar. We also supplemented the electronic search by manually reviewing the reference lists of pertinent articles to find any additional studies that might have been missed.

The search strategy employed a combination of keywords using the Boolean operators "AND" and "OR". The following terms were used: "*Entamoeba histolytica*", "*E. histolytica*", "*Entamoeba dispar*", "*E. dispar*", "*Giardia* spp.", "*Giardia lamblia*", "*G. lamblia*", "*Giardia duodenalis*", "*G. duodenalis*", "*Giardia intestinalis*", "*G. intestinalis*", "*Entamoeba coli*", "*E. coli*", "*Iodamoeba butschlii*", "*I. butschlii*", "*Dientamoeba fragilis*", "*D. fragilis*", "*Blastocystis hominis*", "*B. hominis*", "*Chilomastix mesnili*", "*C. mesnili*", "*Endolimax nana*", "*E. nana*", "*Cryptosporidium* spp.", combined with "food workers", "food handlers", intestinal parasites", "intestinal protozoa", "epidemiology", "prevalence", "frequency", and "Iran".

Two reviewers (A.V.E and Z.R) independently examined the titles, abstracts, and full texts of all identified records. In cases of disagreement, consensus was reached via discussion. Following the removal of duplicates and unrelated studies, the bibliographies of the selected articles were further checked to identify any other potentially relevant studies.

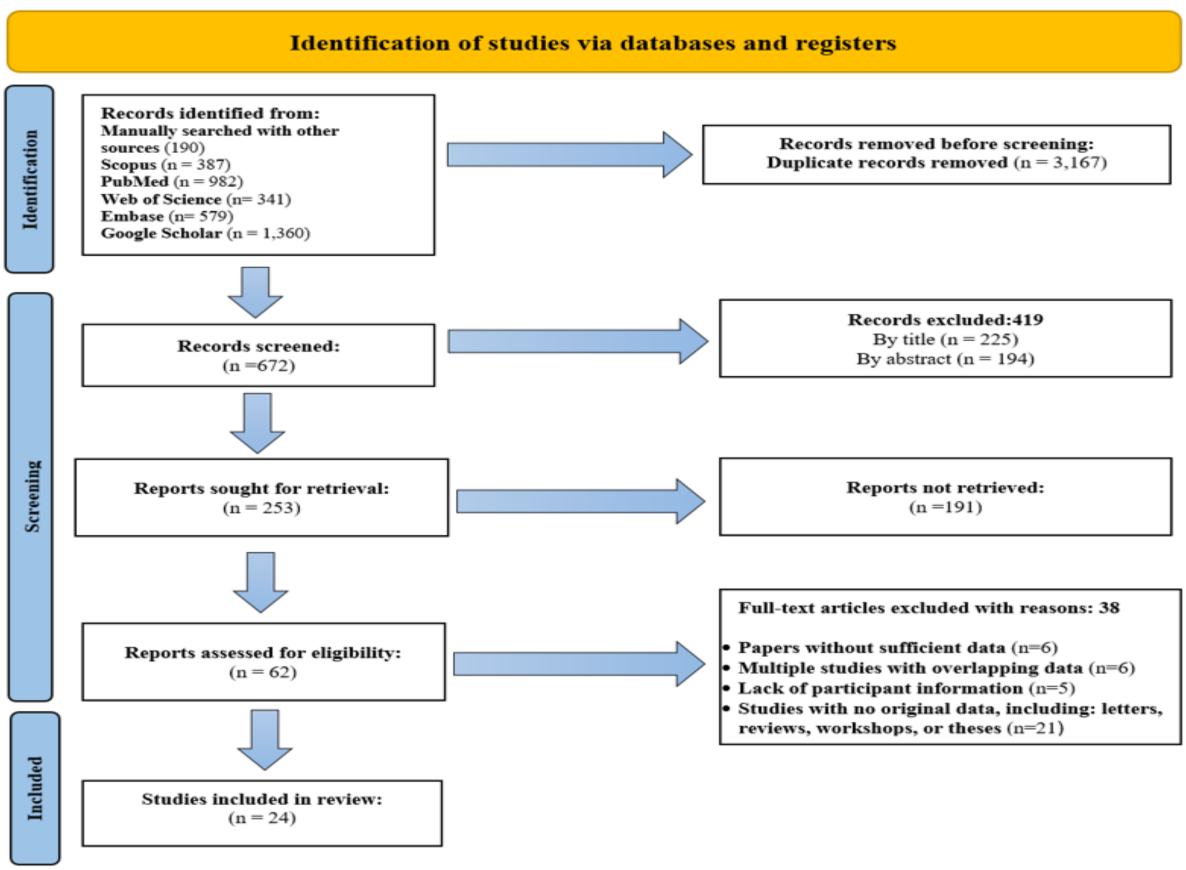

Figure 1: Flow diagram of the study design process

Inclusion and exclusion criteria

The following inclusion criteria were applied: (i) peer-reviewed original research articles; (ii) cross-sectional studies reporting the prevalence of intestinal protozoan parasites among food handlers in Iran; (iii) availability of both abstract and full text; and (iv) provision of numerator and denominator data sufficient to calculate prevalence.

We excluded studies that did not satisfy the predefined eligibility criteria. These included articles published in languages other than English or Persian, review papers without primary data, letters, conference abstracts, editorials, and studies with unclear or indeterminate findings. Furthermore, investigations that examined protozoan cysts, oocysts, or trophozoites in animal specimens, soil, or water samples were also omitted from the review.

Data synthesis and statistical analysis

The primary outcome was the pooled prevalence (overall, by parasite type, and by subgroup) of intestinal protozoan infections among Iranian food handlers. Given the expected high inter-study heterogeneity, a random-effects model with the DerSimonian-Laird estimator was employed to obtain pooled prevalence rates with 95% confidence intervals (CIs). Heterogeneity was quantified using the I^2 statistic and Cochran's Q test, with I^2 values of 0–25%, 25–50%, 50–75%, and >75% denoting low, moderate, high, and very high heterogeneity, respectively. A Q test P-value < 0.05 was considered significant. All analyses were performed using Comprehensive Meta-Analysis Software Version 3 (CMA V3).

Quality assessment

Two independent reviewers (ZR and FG) evaluated the methodological quality of all included studies using the Newcastle-Ottawa Scale (NOS) adapted for cross-sectional studies (18). Any differences in their assessments were settled through dialogue until a consensus was achieved. Each study could receive a maximum overall score of 9, allocated among three categories: participant selection (0–4 points), group comparability (0–2 points), and exposure/outcome evaluation (0–3 points). Based on the aggregate score, studies were categorized as poor (0–3 points), moderate (4–6 points), or high quality (7–9 points).

Results

Study characteristics

A total of 24 studies comprising 110,404 food handlers were included in the present meta-analysis. Among these participants, 24,113 individuals were infected with at least one intestinal protozoan parasite. The overall pooled prevalence was 14.1% (95% CI: 9.2% – 21.0%) (Figure 2 & Table 1). The included studies were conducted between 2000 and 2025 across various provinces of Iran, including Mazandaran, Isfahan, Fars, Lorestan, Hamadan, East Azerbaijan, Khuzestan, Golestan, Kerman, Qom, Tehran, Qazvin, Kurdistan, and Hormozgan.

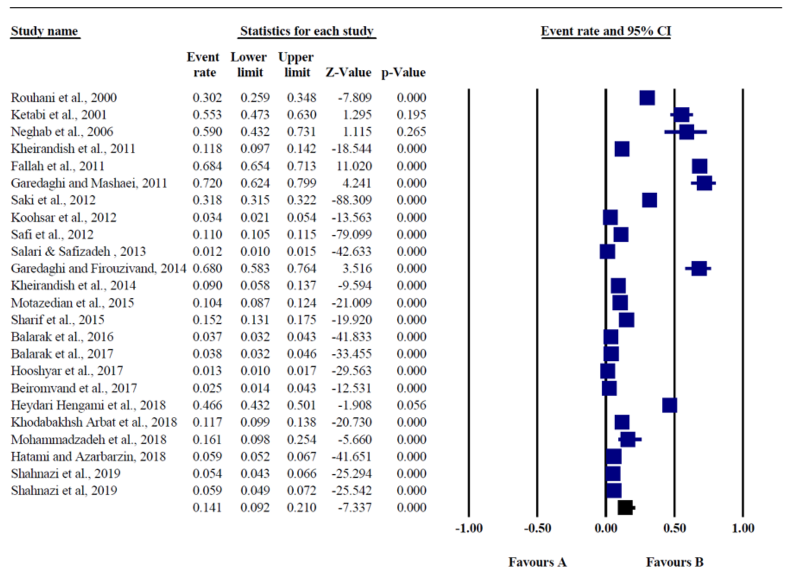

Figure 2: Forest plots for random-effects meta-analysis of intestinal protozoan parasites among food handlers in Iran.

Table 1: Main characteristics of the included studies reporting the prevalence of intestinal protozoan parasites among food handlers of Iran

Reference	*Author*	*Year*	*Province*	*District*	*Sample size*	*Infected*	*Type of intestinal protozoan parasites*
(19)	Rouhani et al.	2000	Mazandaran	Nowshahr and Chalous	411	124	*G. lamblia*
(20)	Ketabi et al.	2001	Isfahan	Isfahan	152	84	*G. lamblia*, *B. hominis*, *E. nana*, *E. coli*, *I. butschlii*
(21)	Neghab et al.	2006	Fars	Shiraz	39	23	*G. lamblia*, *B. hominis*, *E. coli*, *C. mesnili*
(22)	Kheirandish et al.	2011	Lorestan	Khorramabad	816	96	*G. lamblia*, *E. histolytica/dispar*, *E. coli*, *B. hominis*
(23)	Fallah et al.	2011	Hamadan	Hamadan	938	642	*G. lamblia*, *E. histolytica/dispar*
(24)	Garedaghi and Mashaei	2011	East Azerbaijan	Tabriz	100	72	*G. lamblia*, *E. histolytica/dispar*
(25)	Saki et al.	2012	Khuzestan	All district	62,007	19,741	*G. lamblia*, *B. hominis*, *I. butschlii*, *E. coli*, *E. histolytica/dispar*
(26)	Koohsar et al.	2012	Golestan	Gorgan	500	17	*G. lamblia*
(27)	Safi et al.	2012	Khuzestan	Ahvaz	14,614	1,604	*G. lamblia*, *E. coli*, *E. histolytica/dispar*
(28)	Salary & Safizadeh	2013	Kerman	Kerman	7,748	96	*G. lamblia*
(29)	Garedaghi and Firouzivand	2014	East Azerbaijan	Tabriz	100	68	*G. lamblia*, *E. histolytica/dispar*, *E. coli*

(30)	Kheirandish et al.	2014	Lorestan	Khorramabad	210	19	*G. lamblia*, *E. coli*, *B. hominis*, *E. histolytica/dispar*
(31)	Motazedian et al.	2015	Fars	Shiraz	1,021	106	*G. lamblia*, *B. hominis*, *I. butschlii*, *E. coli*, *C. mesnili*
(32)	Sharif et al.	2015	Mazandaran	Sari	1,041	158	*G. lamblia*, *B. hominis*, *E. histolytica/dispar*, *I. butschlii*, *E. coli*, *Cryptosporidium* spp.
(33)	Balarak et al.	2016	East Azerbaijan	Tabriz	4,612	172	*G. lamblia*, *E. coli*
(34)	Balarak et al.	2017	Qom	Qom	2,925	112	*G. lamblia*, *E. coli*
(35)	Hooshyar et al.	2017	Isfahan	Kashan	3,653	47	*G. lamblia*
(36)	Beiromvand et al.	2017	Khuzestan	Andimeshk	480	12	*G. lamblia*
(37)	Heydari-Hengami et al.	2018	Hormozgan	Bandar Abbas	800	373	*G. lamblia*, *B. hominis*, *D. fragilis*, *E. histolytica/dispar*, *I. butschlii*, *E. nana*, *E. coli*
(38)	Khodabakhsh Arbat et al.	2018	Isfahan	Kashan	1018	119	*G. lamblia*, *B. hominis*, *D. fragilis*, *E. histolytica/dispar*, *I. butschlii*, *E. nana*, *E. coli*, *C. mesnili*
(39)	Mohammadzadeh et al.	2018	East Azerbaijan	Tabriz	87	14	*E. nana*, *E. coli*, *B. hominis*, *C. mesnili*
(40)	Hatami and Azarbarzin	2018	Tehran	Tehran	4,072	241	*G. lamblia*, *E. histolytica/dispar*, *E. coli*
(41)	Shahnazi et al.	2019	Qazvin	Qazvin	1,530	82	*G. lamblia*, *B. hominis*, *E. nana*, *E. coli*
(42)	Shahnazi et al.	2019	Kurdistan	Saqqez	1530	91	*G. lamblia*, *B. hominis*, *E. nana*, *E. coli*, *I. butschlii*

Pooled prevalence of intestinal protozoan parasites

Based on the pooled prevalence estimates reported in Table 2, *B. hominis* was the most prevalent intestinal protozoan parasite, with a pooled prevalence of 11.20% (95% CI: 11.00-11.50; I^2 = 98%, $P < 0.001$). This was followed by *E. coli* at 8.20% (95% CI: 8.00-8.40; I^2 = 97%, $P < 0.001$). *G. lamblia* and *C. mesnili* showed identical pooled prevalence of 5.40%, with heterogeneity estimates of I^2 = 98%, $P < 0.001$ and I^2 = 91%, $P < 0.001$, respectively. The prevalence of *I. butschlii* was 4.90% (95% CI: 4.70-5.10; I^2 = 95%, $P < 0.001$), followed by *E. nana* at 4.40% (95% CI: 3.40--5.80; I^2 = 97%, $P < 0.001$). Lower prevalence rates were observed for *E. histolytica/dispar* (1.90%; I^2 = 99%, $P < 0.001$) and *T. hominis* (1.80%), while *Cryptosporidium* spp. showed a pooled prevalence of 0.50% and *D. fragilis* had the lowest prevalence at 0.30% (Table 2).

Table 2: Sub-group analysis and pooled prevalence of intestinal protozoan parasites based on type of intestinal protozoan parasites.

Type of intestinal protozoan parasites	*No studies*	*Sample size*	*Infected*	*Pooled prevalence (95% CI)*	*Heterogeneity*		
					I^2	τ^2	*P*-value
Blastocystis hominis	13	70,421	7,512	11.2 (11.0--11.5)	98	0.691	<0.001
Entamoeba histolytica / dispar	12	90,288	1,161	1.9 (1.8--2.0)	99	3.499	<0.001
Entamoeba coli	18	96,744	6,367	8.2 (8.0--8.4)	97	1.408	<0.001
Cryptosporidium spp.	1	1,041	5	0.5 (0.2--1.1)	-	-	-
Giardia lamblia	24	110,487	5,281	5.4 (5.3--5.5)	98	0.412	<0.001
Endolimax nana	6	5,117	54	4.4 (3.4--5.8)	97	4.402	<0.001
Trichomonas hominis	1	170	3	1.8 (0.6--5.3)	-	-	-
Chilomastix mesnili	5	1,469	31	5.4 (3.8--7.6)	91	2.814	<0.001
Iodamoeba butschlii	7	67,569	3,130	4.9 (4.7--5.1)	95	2.148	<0.001
Dientamoeba fragilis	1	1,081	3	0.3 (0.1--0.9)	-	-	-

A province subgroup analysis was performed to assess the pooled prevalence of intestinal protozoan parasites among food handlers in Iran (Table 3). The results demonstrated substantial inter-provincial heterogeneity. The highest pooled prevalence estimates were observed in Hamadan (68.4%, 95% CI: 7.5–98.3), Hormozgan (46.6%, 95% CI: 3.2–95.9), and East Azerbaijan (30.8%, 95% CI: 7.8–70.1). Conversely, the lowest estimates were recorded in Kerman (1.2%, 95% CI: 0.0–251), Golestan (3.4%, 95% CI: 0.1–49.2), and Qom (3.8%, 95% CI: 0.1–51.5). Among provinces contributing more than one study, marked heterogeneity was evident, with I^2 values exceeding 98.1% ($P < 0.001$) in all such regions except Lorestan, which exhibited low heterogeneity ($I^2 = 18.8\%$, $\tau^2 = 0.090$, $P < 0.001$). These findings highlight pronounced geographical disparities in the prevalence of intestinal protozoan infections, which may be attributable to variations in environmental hygiene, socioeconomic conditions, and local food safety practices. The consistently high heterogeneity observed across multi-study provinces indicate considerable between-study variability, thereby warranting a cautious interpretation of the pooled estimates. Additionally, a map was created using QGIS 3 software (https://qgis.org/en/site/) to illustrate the distribution of prevalence of intestinal protozoan parasites among food handlers in Iran across different geographical regions of Iran, based on the included studies (Figure 3).

Publication bias

Publication bias was assessed using Egger's regression test. Egger's test revealed significant asymmetry in the funnel plot (intercept = 10.456, standard error = 3.965, 95% CI: 2.254 to 18.658; t = 2.637, df = 23, $P = 0.002$), suggesting potential publication bias (Figure 4). To further evaluate the robustness of the findings, a trim-and-fill analysis was performed; the adjusted pooled estimate remained unchanged (14.1%). The Classic Fail-safe N was calculated as 6,586, indicating that a very large number of missing studies would be needed to nullify the results. Overall, despite some asymmetry indicated by Egger's test, the pooled prevalence estimate appears to be robust and reliable.

Table 3: Subgroup analysis of intestinal protozoan parasites among food handlers in Iran, by province, based on included studies

Province	*NO. Studies*	*Pooled prevalence (95% CI)*	*Heterogeneity*		
			I^2	τ^2	*P*-value
Hamadan	1	68.4 (65.4-71.3)	-	-	-
Isfahan	3	11.4 (1.7-49.2)	99.5	1.96	<0.001
Hormozgan	1	46.6 (43.2-50.0)	-	-	-
East Azerbaijan	4	30.8 (7.8-70.1)	99.4	2.51	<0.001
Fars	2	28.6 (3.7-80.6)	98.16	1.76	<0.001
Qom	1	3.8 (3.2-4.5)	-	-	-
Golestan	1	3.4 (2.0-5.6)	-	-	-
Kerman	1	1.2 (1.0-1.5)	-	-	-
Khuzestan	3	10.5 (3.3-28.9)	98.9	2.04	<0.001
Kurdistan	1	5.9 (4.8-7.3)	-	-	-
Lorestan	2	10.3 (1.1-54.3)	18.80	0.090	<0.001
Qazvin	1	5.4 (4.3-6.7)	-	-	-
Tehran	1	5.9 (5.2-6.7)	-	-	-

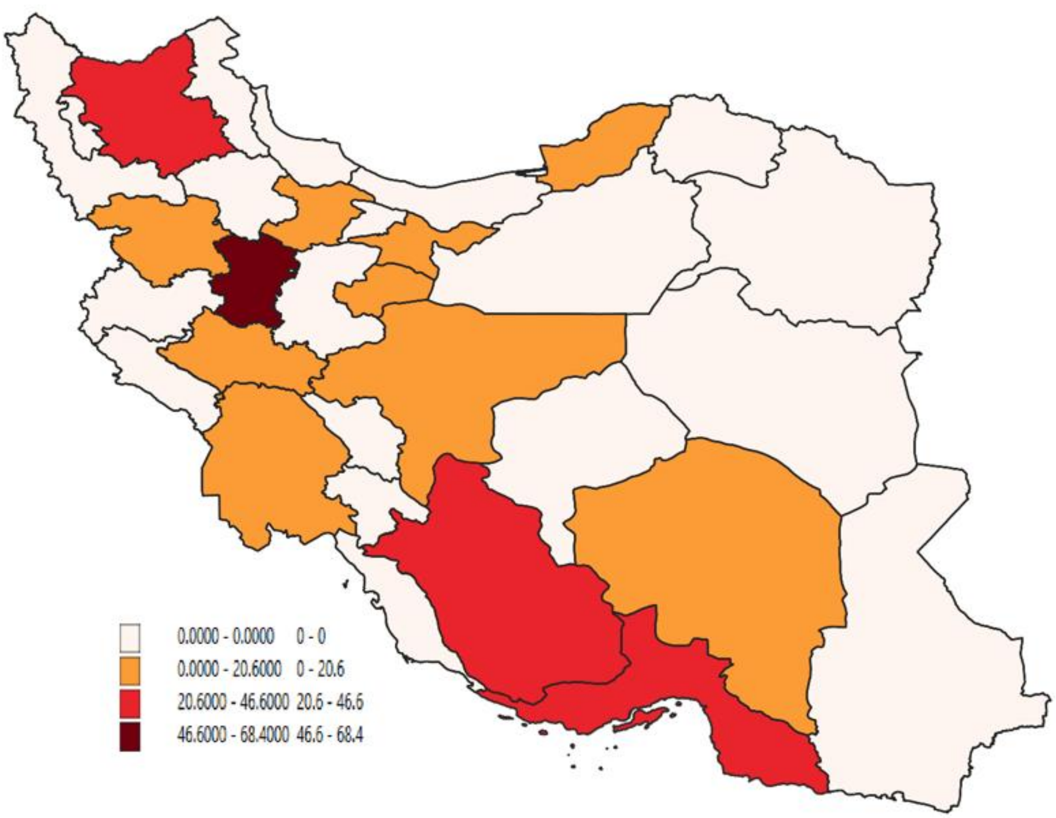

Figure 3: The prevalence and distribution of intestinal protozoan parasites among food handlers in different geographical regions of Iran based on included studies (https://qgis.org/en/site/).

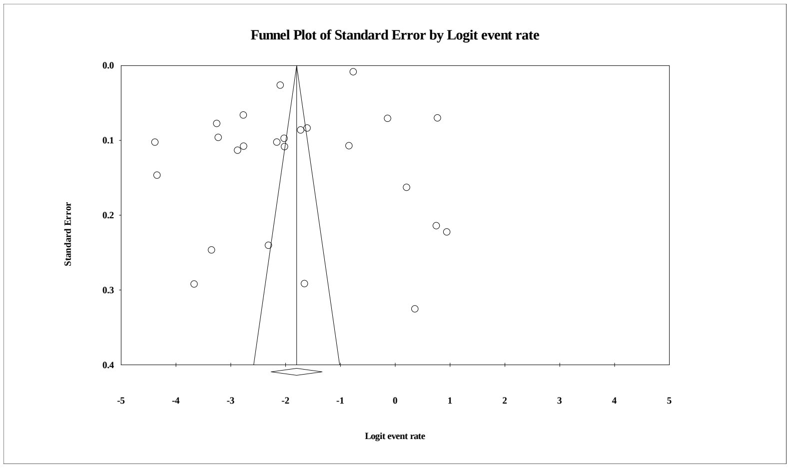

Figure 4: Egger's funnel plot was used to assess potential publication bias in estimating the prevalence of intestinal protozoan parasites among food handlers in Iran

Meta-regression analysis

The meta-regression showed a statistically significant association between publication year and the logit event rate (coefficient = 0.1310, 95% CI: −0.2431 to −0.0188, $P = 0.0240$). The model explained 20% of the between-study variance ($R^2 = 0.20$), although substantial residual heterogeneity remained ($I^2 = 99.66\%$, $P < 0.0001$). Therefore, publication year is a significant predictor of effect size at the $\alpha = 0.05$ level (Figure 5).

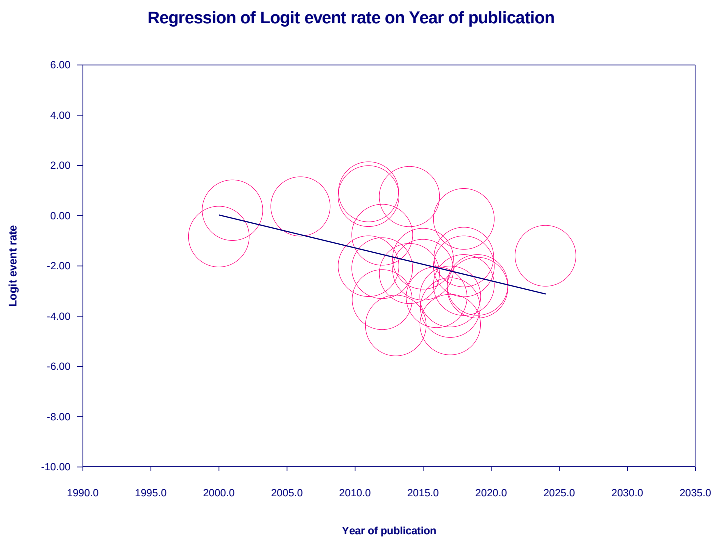

Figure 5. Meta-regression of intestinal protozoan parasites among food handlers in Iran based on year of publication

Quality assessment

Following the quality assessment, 14 of the 24 studies were classified as high quality (scores 7–9), and the remaining 10 as moderate quality (scores 4–6) (Table 4).

Table 4: Quality assessment using the Newcastle–Ottawa scale modified for cross sectional studies

Reference	*Author name*	*Publication years*	*Province*	*Selection (maximum of 5 stars)*	*Comparability (maximum of 2 stars)*	*Outcome (maximum of 3 stars)*	*Total Score*
(19)	Rouhani et al.	2000	Mazandaran	***	*	***	7
(20)	Ketabi et al.	2001	Isfahan	***	*	***	7
(21)	Neghab et al.	2006	Fars	***	*	**	6
(22)	Kheirandish et al.	2011	Lorestan	***	*	***	7
(23)	Fallah et al.	2011	Hamadan	***	*	***	7
(24)	Garedaghi and Mashaei	2011	East Azerbaijan	****	**	**	8
(25)	Saki et al.	2012	Khuzestan	***	*	**	6
(26)	Koohsar et al.	2012	Golestan	**	*	**	5
(27)	Safi et al.	2012	Khuzestan	****	**	**	8
(28)	Salari & Safizadeh	2013	Kerman	**	*	**	5
(29)	Garedaghi and Firouzivand	2014	East Azerbaijan	***	**	***	8
(30)	Kheirandish et al.	2014	Lorestan	***	*	**	6
(31)	Motazedian et al.	2015	Fars	***	*	***	7
(32)	Sharif et al.	2015	Mazandaran	****	**	**	8
(33)	Balarak et al.	2016	East Azerbaijan	**	*	**	5
(34)	Balarak et al.	2017	Qom	***	*	**	6
(35)	Hooshyar et al.	2017	Isfahan	**	*	**	5
(36)	Beiromvand et al.	2017	Khuzestan	****	**	**	8
(37)	Heydari-Hengami et al.	2018	Hormozgan	***	*	***	7
(38)	Khodabakhsh Arbat et al.	2018	Isfahan	**	*	**	5
(39)	Mohammadzadeh et al.	2018	East Azerbaijan	****	**	**	8
(40)	Hatami and Azarbarzin	2018	Tehran	****	**	***	9
(41)	Shahnazi et al.	2019	Qazvin	***	*	***	7
(42)	Shahnazi et al.	2019	Kurdistan	***	*	**	6

*Indicates one criteria was followed, ** two criteria were followed,
three criteria were followed, ****four criteria were followed, and ** five criteria were followed

Discussion

This systematic review and meta-analysis, encompassing 24 studies with 110,404 food handlers across Iran, provides comprehensive evidence to date on the burden of intestinal protozoan parasites in this high-risk occupational group. The overall pooled prevalence of 14.1% indicates that approximately one in seven food handlers in Iran harbors at least one intestinal protozoan. This figure is like the global pooled prevalence of 14.3% reported by Eslahi et al. (8), suggesting that Iran does not deviate substantially from the worldwide pattern. However, this overall estimate masks profound heterogeneity across provinces and parasite species, underscoring the need for geographically tailored interventions rather than one-size-fits-all policies.

At the national level, a previous meta-analysis by Teimouri and colleagues (43) reported an overall prevalence of 19.3% for intestinal parasites among food handlers in Iran, although this estimate included both protozoa and helminths and was based on studies published up to 2020. The observed prevalence of 14.1% among Iranian food handlers carries significant public health implications. Unlike the general population, where intestinal protozoan infections are often symptomatic and prompt healthcare-seeking behavior, food handlers frequently remain asymptomatic carriers, unknowingly perpetuating transmission cycles (4,5). This asymptomatic carriage is particularly concerning because food handlers are in direct contact with ready-to-eat foods, beverages, and food preparation surfaces, creating multiple opportunities for contamination (6). Food handlers with untrimmed fingernails, lack of access to handwashing facilities, or inadequate food safety knowledge are significantly more likely to harbor intestinal parasites (7). A prevalence rate of 14.1% implies that in an average restaurant or cafeteria employing ten food handlers, at least one person could be actively excreting protozoan cysts or oocysts into the food chain. This finding highlights the critical importance of implementing regular screening programs as a public health priority.

The predominance of *B. hominis* (11.2%) as the most frequently detected protozoan aligns with global trends (8) and challenges the lingering misconception that this organism is merely a commensal (44). Given its significant associations with irritable bowel syndrome (IBS) and chronic urticaria (45), the presence of *B. hominis* in food handlers many of whom are asymptomatic represents a silent but potentially consequential public health concern (46). Similarly, the high pooled prevalence of *E. coli* (8.2%), a non-pathogenic but fecal-indicative species, serves as a proxy for poor sanitary practices and fecal contamination of the food supply chain (47). Such findings directly reflect inadequate hand hygiene and lack of protective equipment among food workers (8).

The identical pooled prevalence of *G. lamblia* and *C. mesnili* is noteworthy. While *G. lamblia* is a well-recognized pathogen capable of causing malabsorption and growth stunting in children (12), *C. mesnili* is generally non-pathogenic (48). Their co-occurrence at equivalent rates further reinforces that fecal–oral transmission routes are active, likely due to contaminated water or surfaces (49). The very low prevalence of *Cryptosporidium* spp. (0.5%) and *Dientamoeba fragilis* (0.3%) each derived from only a single study should be interpreted with extreme caution. These estimates likely underestimate true prevalence because diagnosing *Cryptosporidium* often requires acid-fast staining or molecular methods (15,16), and therefore do not reflect the potential public health impact, particularly in immunocompromised consumers.

The relatively low prevalence of pathogenic species such as *E. histolytica/dispar* (1.9%) and *Cryptosporidium* spp. (0.5%) should not be interpreted as a low public health risk. Even a single infected food handler can contaminate large batches of food, potentially affecting hundreds of consumers (15). Furthermore, the diagnosis of

Cryptosporidium requires specific staining techniques (e.g., modified Ziehl-Neelsen or auramine-rhodamine) or molecular methods such as PCR, not routinely employed in most Iranian laboratories (16). Therefore, the actual prevalence of *Cryptosporidium* infection among food handlers is probably underreported in the reviewed studies, since the majority employed only direct wet mount or formalin-ether concentration methods. This methodological constraint constitutes a significant shortcoming in food safety monitoring and implies that the true burden of foodborne cryptosporidiosis may be considerably greater than what existing data suggest (8).

The most revealing finding of this meta-analysis is not the national pooled prevalence of 14.1%, but the extreme provincial disparities that it masks. Our data show a 67-percentage-point gap between the highest-prevalence province, Hamadan (68.4%), and the lowest, Kerman (1.2%) a spread that far exceeds what could be explained by chance or methodological variation alone. A clear high-prevalence cluster emerges in western and southern provinces (Hamadan 68.4%, Hormozgan 46.6%, East Azerbaijan 30.8%), while a low-prevalence cluster appears in central and northern regions (Kerman 1.2%, Golestan 3.4%, Qom 3.8%). Importantly, the presence of very high heterogeneity across most provinces means these pooled estimates should be interpreted as averages of diverse studies rather than precise epidemiological constants. From a policy perspective, this provincial mapping argues strongly for geographically targeted interventions: high-prevalence provinces require mandatory quarterly stool screening and intensive hygiene inspections, while low-prevalence provinces may suffice with annual monitoring (50,51).

This meta-analysis has several key limitations. First, the distribution of studies across provinces is highly uneven, with most provinces contributing only one or two studies including those with extreme prevalence estimates which makes the pooled results susceptible to the influence of outliers. Moreover, a number of provinces have no published data at all, which restricts the generalizability of our findings to the entire country. Second, the provincial pooled estimates are ecological in nature and do not account for individual-level determinants of infection. For instance, a food handler working in a province with high prevalence could still maintain strict hygienic practices and remain uninfected, whereas another in a low-prevalence area might contract the infection. In the absence of individual-level covariate information, attributing infection risk solely to provincial factors remains speculative. Despite these constraints, this work represents the most comprehensive synthesis of intestinal protozoan infections among food handlers in Iran to date.

The results of this meta-analysis indicated the necessity to conduct intensified surveys and studies on poor represented areas of Iran for intestinal protozoan infection. Sistan and Baluchestan, Chaharmahal and Bakhtiari, Kohgiluyeh and Boyer-Ahmad, and Ilam provinces was not present in any study conducted by others so we propose the use of targeted and intensive surveys in these provinces, especially in the hot province, or in provinces near the border areas with high mobility of their people, as they can bear a substantial burden of intestinal protozoa infection in the population under study, and also to develop local control methods in the mentioned areas. Furthermore, in order to estimate the actual prevalence rate and to distinguish the pathogenic from the non-pathogenic parasitic organisms, future studies should involve the use of molecular methods to determine different types of the organisms and analyze of their DNA polymorphism for the most frequently observed intestinal protozoa, *G. lamblia*, *E. histolytica*, and *B. hominis* within the food handlers in the population (8), and ultimately to control intestinal protozoa infection among food handler, the cooperation from government health services, education, labors and Municipality department of the state should be obtained to establish guidelines of food security programs and evaluating outcomes over the years.

Conclusion

Intestinal protozoan parasites are considerably prevalent among food handlers in Iran, with marked geographical disparities across provinces. These findings highlight an urgent need for targeted interventions rather than one-size-fits-all policies. First, public awareness campaigns must be launched to inform both food handlers and the general population about the routes of protozoan transmission and the potential health consequences. Second, periodic health inspections of food handlers by sanitary authorities should be conducted regularly and mandatorily, with higher frequency in high-prevalence provinces, including stool screening and verification of proper hygiene practices. Third, public education programs should be systematically delivered to all individuals involved in food preparation and distribution, emphasizing correct handwashing techniques, fingernail trimming, use of protective equipment, and safe food handling protocols. Without the simultaneous implementation of these three measures, the burden of foodborne protozoal diseases in Iran is unlikely to decline substantially.

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Conflict of Interests

All authors declare that there are no existing or potential conflicts of interest relevant to this work.

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Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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