JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 8 (2021) 57-65

Occurrence of Intestinal Parasites in Fruits and Vegetables from Markets of Northwest Mexico

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HIGHLIGHTS

- Parasitic contaminations were found in 45% of fruit and vegetable samples (n=400).
- The most prevalent parasitic pathogens were *Cryptosporidium* spp., *Cyclospora* spp., and *Blastocystis hominis*.
- Samples from open-air markets had higher parasitic contamination than those collected in closed establishments.
- Parasitic contamination in the fresh produce of this region of Mexico is a serious public health concern.

Article type
Original article

Keywords
Parasites
Intestinal Diseases, Parasitic
Fruit
Vegetables
Food Contamination
Mexico

Article history
Received: 14 Jan 2021
Revised: 9 Apr 2021
Accepted: 23 Apr 2021

Acronyms and abbreviations
ELISA=Enzyme-Linked Immunosorbent Assay

ABSTRACT

Background: Fruits and vegetables are potential vehicle of transmission of intestinal parasites. The main aim of this study was to determine prevalence of intestinal parasitic contamination in fruits and vegetables sampled from Caborca region, Northwest Mexico.

Methods: A total of 400 fruit and vegetable samples were collected from unregulated open-air markets and closed (i.e., regulated) markets in Caborca region of Northwest Mexico; including melon, peach, asparagus, and grapes. Faust, Kinyoun, and Enzyme-Linked Immunosorbent Assay (ELISA) techniques were used to detect and identify the genus and species of all parasites found in the examined samples. Data were statistically analyzed using STATA/SE (version 12.0).

Results: An overall prevalence (45%) of parasitic contamination was found in the 400 fruit and vegetable samples. *Endolimax nana* (27.5%) and *Entamoeba coli* (17.5%) were the most common nonpathogenic parasites, while the most prevalent parasitic pathogens were *Cryptosporidium* spp. (11.7%), *Cyclospora* spp., (11.0%), and *Blastocystis hominis* (9.2%). Asparagus (31%) and grapes (38.9%) had significantly (p<0.05) higher percentages of overall and multiple parasitic contamination than melon (10.6%) and peaches (19.4%). The fresh produce from the open-air markets had significantly (p<0.05) higher overall parasitic contamination (53.5%) than those of the closed establishments (36.5%).

Conclusion: The parasitic contamination in the fresh produce sold in the Northwest region of Mexico is a serious public health concern.

Introduction

Fruits and vegetables play critical role in healthy human nutrition due to their high content of vitamins, fiber, and minerals (Said, 2012). However, since they are often hand-manipulated or eaten raw, they are potential

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To cite: Morales-Figueroa G.G., Sánchez-Guerrero M.A., Castro-García M., Esparza-Romero J., López-Mata M.A., Quihui-Cota L. (2021). Occurrence of intestinal parasites in fruits and vegetables from markets of Northwest Mexico. *Journal of Food Quality and Hazards Control*. 8: 57-65.

DOI: 10.18502/jfqhc.8.2.6469 Journal website: http://www.jfqhc.com

[DOI: 10.18502/jfqhc.8.2.6469]

vehicles in transmission of intestinal parasites. Indeed, an increasing number of outbreaks of food-borne illness worldwide have been attributed to their consumption. It has been evidenced repeatedly that protozoan parasites are associated with diarrheal diseases (Li et al., 2020), and numerous studies have reported their presence in raw fruits and vegetables (CDC, 2020a).

Few studies have explored parasitic contamination in fruits and vegetables in Mexico (Canales et al., 2019). Gastrointestinal infections constitute a significant public health problem in the Caborca region of Mexico. Despite these figures, only one study has analyzed parasitic contamination in vegetables (especially asparagus) sold in Northwest Mexico. It reported a prevalence of 29% for *Cryptosporidium* spp. in asparagus (Morales-Figueroa et al., 2019), indicating that the fresh produce marketed is not well-regulated by the Sanitary Control authorities and the Department of Public Health in this country. This finding motivated us to investigate intestinal parasites in the fruits and vegetables marketed in Northwest Mexico.

Materials and methods

Study area

The Caborca region of Mexico is located 280 meters above sea level. It is bordered to the North by the United States and municipalities in the Puerto Peñasco and General Plutarco Elias Calles regions. The study area has an extreme semi-warm, dry climate with an annual rainfall of 209 mm, and an average maximum monthly temperature of 31.9 °C from June to September. The average annual temperature is 21 °C (website: https://es.climate-data.org/).

Sampling

In this cross-sectional study, the sampling process for asparagus and melon was carried out in November 2019, peaches in April 2020, and grapes in June 2020. A total of 400 units (pieces, bundles, or bunches) of fruits and vegetables were sampled. Fifty pieces each of melon and peach, 50 bundles of asparagus, and 50 bunches of grapes were gathered from 8 unregulated open-air markets. The same numbers of melons, peaches, asparagus, and grapes were obtained from 8 closed (regulated) markets. All the fresh produce were selected using a simple random sampling technique. Each piece of melon and peach, each bundle of asparagus, and each bunch of grapes taken from the display racks at the open-air and closed markets were numbered and then removed using randomly-selected numbers. Separately, an additional 100 bundles of asparagus were collected from an agricultural field that produces for export. Of these, 50 were substandard (i.e., failed to meet international quality standards), the other 50 were of export-grade. The substandard and export bundles were selected randomly from different conveyor belts.

All sampling sites were located in the Northwest region of Mexico describe above. Each piece of melon and peach, each bundle of asparagus, and each bunch of grapes were placed hygienically in sterile, labeled sampling bags showing the collection date, address and type of market, and transported to the Centro de Investigación en Alimentación y Desarrollo, A.C., where they were kept at 4-6 °C for less than 24 h until analysis.

Sample processing

A modification of the method described by Ezatpour et al. (2013) was used to process the fresh produce. Each piece of melon and peach were washed in 1 L of 0.95% NaCl in the sterile sampling bags during sampling (Ezatpour et al., 2013). For the asparagus and grapes, a number of spears and bunches were taken randomly to complete a weight of ≈250 g per product. All the bundles of asparagus and bunches of grapes were subjected to the washing process just described. Next, the wash water was filtered by a vacuum pump using Whatman filters (1 µm). All glassware was washed with sterile distilled water to prevent contamination. Filtration was performed at a flow rate of 0.01 L/s. While still in the filter, part of the material was processed using the diagnostic techniques to detect the genus and species of parasitic infections. Another portion of the material was re-suspended and homogenized in 3 ml of sterile distilled water, transferred to 5-ml Eppendorf tubes, and then stored at -20 °C for Enzyme-Linked Immunosorbent Assay (ELISA) analyses.

Detection of parasites

-Faust technique

In this technique (Chejfec, 1999), sterile distilled water from the washing process was poured into a tube (100×13 mm). The sediment was suspended and centrifuged for 10 min at 2 500 rpm (700×*g*). The supernatant was discarded. This washing procedure was repeated 3 times. After that, 3.5 ml of aqueous $ZnSO_4$ solution (specific gravity 1.180) was added to 50.8 mm of the rim of the tube and the packed sediment was re-suspended. This suspension was centrifuged for 5 min at 2 500 rpm (700×g) and allowed to stand for 20 min. Two loops of the surface film were transferred to a drop of iodine solution (Weigert's solution) already placed on a glass slide for wet mount examination using 10× and 40× objectives to identify cysts of *Giardia intestinalis*, *Entamoeba histolytica/dispar/moshkovskii*, *Blastocystis hominis*, and

[DOI: 10.18502/jfqhc.8.2.6469]

the nonpathogens *Endolimax nana* and *Iodamoeba bütschlii* (CDC, 2020b).

-Kinyoun technique

In this case, 3 ml of each sample of sterile distilled water, recovered from the washing process, was centrifuged for 10 min at 2 500 rpm (700×g). The sediment was smeared on a slide and left to dry after applying methanol to fix the smear. Each smear was stained for 2 min with carbol-fuchsin. Sulfuric acid (10%) was used to decolorize the smears, and malachite green was applied to stain the decolorized smears again. The colored smears were dried and immersion oil was added for microscopic observation with the 100× objective. *Cryptosporidium* spp., *Cyclospora* spp., and *Isospora* spp. in the samples were observed as pink-stained oocysts against the heavily dark blue background (Garcia et al., 2018). The Kinyoun technique has shown good performance in detecting the abovementioned parasitic genera (Abou El-Naga and Gaafar, 2014).

-ELISA technique

ELISA technique was applied to diagnose species of *Cryptosporidium* and *Entamoeba*. The DRG ELISA kit (DRG Diagnostics, Germany) was used to detect *Cryptosporidium parvum* antigens. In this procedure, 1 ml of wash water was homogenized and transferred to cryogenic vials (2 ml), previously labeled, and then stored at -20 °C until analysis. The samples were allowed to thaw at room temperature (24 °C) and 5 ml of anti *C. parvum* solution was added to each vial. The content was homogenized and 200 µl of a second anti *C. parvum* solution was added to form a "sandwich" with the *C. parvum* antigen bound by the first antibody. Next, a *C. parvum* antibody was added to bind a peroxidase conjugate with chromogenic tetramethylbenzidine against the second *C. parvum* antibody, and then a blue color developed. This reaction was stopped by adding phosphoric acid. This produced a yellow color that was read at a wavelength range of 450-650 nm using 680 microplate reader (Bio-Rad Laboratories, Hercules, USA). Positive and negative standard references were included in each run for quality control. A reading >0.150 was considered a positive result based on the manufacturer's instructions. The aforementioned DRG ELISA kit had sensitivity of 93% and specificity of 98% for detecting *C. parvum* antigens.

The *Cryptosporidium* test (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) was used to identify *Cryptosporidium hominis*. A 100-µl sample of wash water was examined together with control specimens (negative and positive), which were poured into the well of a microwell plate with biotinylated anti-*Cryptosporidium* antibodies (Conjugate 1) for incubation at room temperature (20-25 °C). After washing, 100 µl of streptavidin poly-peroxidase conjugate (Conjugate 2) were added and incubated at room temperature (20-25 °C). The *C. hominis* antigens in the sample, the immobilized antibodies, and the conjugated antibody formed a complex sandwich. Another wash step removed the unattached streptavidin poly-peroxidase conjugate. After adding 100 µl of the substrate, the attached enzyme changed the color of the previously colorless solution to blue in the wells of the microwell plate for the positive results. Adding the stop reagent (50 µl) changed the color from blue to yellow. The optical density (O.D.) at 450 nm for the negative control was <0.2, while for the positive control it was >0.8. This test has a sensitivity of 92-96% and a specificity of 95-100%.

The ProSpecTTM *E. histolytica* microplate assay (Oxoid Ltd., Wade Road, Basingstoke Hants, RG24 8PW UK) was used to detect *E. histolytica*. A sample of wash water was allowed to thaw at room temperature (24 °C) and added to breakaway microwell plate on which anti *E. histolytica* antibodies were bound. If the *E. histolytica*-specific antigen (EHSA) was present, it was captured by the bound antibody. The wells were incubated and then washed to remove all unbound material. The enzyme conjugate (anti-EHSA antibody labeled with horse radish peroxidase enzyme) was added (200 µl) and the wells were incubated and washed again to remove any unbound enzyme conjugate. Next, the substrate for the enzyme 3,3',5,5'-tetramethylbenzidine (TMB) was added (200 µl) and a yellow-colored reaction was developed and read spectrophotometrically at 450 nm in a model 680 microplate reader (Bio-Rad Laboratories, Hercules, USA). Positive and negative controls were used during each run. The OD of the positive samples should be ≥0.050 at 450 nm after subtracting the OD from the negative control (following the manufacturer's instructions). The same ProSpecTTM *E. histolytica* microplate assay was used at a sensitivity of 87% and a specificity of 99% for detecting *E. histolytica* antigens.

Statistical analysis

The prevalence of parasites in the fresh produce was used as the dependent variable, while the type of market was the independent variable. The positive case was the sample with at least one parasite detected by microscopy and/or the ELISA technique. The case was a dichotomous variable assigned as (1) presence or (0) absence. Prevalence of parasites was calculated as the percentage of positive cases per genus or species of parasite in relation to the total number of experimental units sampled in each comparison group; 95% confidence intervals were estimated. The two-proportion ($\chi 2$ test) technique was used to compare the prevalence rates of parasitic

[DOI: 10.18502/jfqhc.8.2.6469]

contamination between the two types of markets. Pearson's Chi-square ($\chi 2$ test) and Student-Newman-Keuls tests were performed to determine differences in the prevalence of the intestinal parasites isolated from the types of fresh produce sampled (experimental units). Data were statistically analyzed using STATA/SE (v. 12.0). Significance level was set at $p<0.05$.

Results

Overall parasitic contamination

An overall prevalence of 45% (180 out of 400; 95% CI=40.1-49.8) of parasitic contamination (i.e., presence of at least one genus or species of parasite) was found in the samples analyzed. In addition, 12 types of intestinal parasites were identified in the fresh produce collected (peaches, asparagus, melons, grapes) as shown in Table 1. *E. nana* was the parasite detected most often ($p<0.05$). *E. nana* and *Entamoeba coli* were the predominant nonpathogenic parasites found as contaminants in these perishables. No statistical differences ($p=0.510$) were found in the prevalence rates among the predominant genera of the pathogens. *Cryptosporidium* spp. (11.7%), *Cyclospora* spp. (11.0%), and *B. hominis* (9.2%), *I. bütschlii* (2.5%), *G. intestinalis* (1.5%), *Chilomastix mesnili* (1.2%), and *Isospora* spp. (0.5%) were the intestinal parasites isolated least often (Table 1).

Contamination of various fresh produce samples

The grapes (38.9%) and asparagus (31.1%) had significantly ($p=0.001$) higher percentages of overall protozoan parasitic contamination than melons (10.6%) and peaches (19.4%) which is illustrated in Table 2. Similarly, the prevalence of multiparasitic contamination in the asparagus was significantly ($p<0.05$) higher than in melons and peaches; while the prevalence of multiple parasitic contamination in the grapes was significantly ($p<0.05$) higher than in melons and peaches.

Also we found that 30% of the total samples were contaminated with two species of parasites, 15.5% with three species of parasites, 7.8% and 2.2% with four and five parasitic contaminations, respectively. Grapes had the highest contamination by two parasite spp. (Table 2) and they were mainly contaminated with *E. nana*, *I. bütschlii*, and *Cyclospora* spp.; asparagus had the highest level of contamination with *E. coli*, *Entamoeba* spp., and *Cryptosporidium* spp. showing significant ($p<0.05$) differences (Table 3). Asparagus and grapes had the same prevalence of contamination by *B. hominis*. No differences ($p>0.05$) were found in the prevalence rates of the other genera or species in the perishables analyzed.

Parasitic contamination in samples from open and closed-air markets

As revealed in Table 4, the overall prevalence of parasitic contamination was significantly ($p=0.001$) higher in the fresh produce collected from the open-air markets (53.5%; 95% IC=46.0-60.0) than the closed establishments (36.5%; 95% IC=29.3-42.6). However, per genera or species, no statistical differences ($p=0.347$) were found in the prevalence of the *Cryptosporidium* spp. between the two kinds of markets (13.5%; 95% IC=8.3, 18.0) and closed-air markets (10%; 95% IC=6.0, 14.1). Similarly, no differences ($p>0.05$) were found in the prevalence of *C. parvum* and *C. hominis* between the markets. Prevalence of the pathogen *B. hominis* was significantly ($p=0.01$) higher in the open-air markets (13.0%: 95% IC=8.3-17.6 vs.5.5%: 95% IC=1.9-8.0).

Parasitic contamination in asparagus

The prevalence of parasitic contamination by the nonpathogenic protozoa (*E. nana* and *E. coli*) was significantly ($p<0.05$) higher in the asparagus collected from open-air markets (42%, 95%CI=28.1-56.0 and 38%, 95%CI=24.3-51.6, respectively) than the closed-air markets (26%, 95%CI=13.6-38.3 and 20%, 95%CI=8.7-31.3, respectively). Low contamination by these parasites was observed in the asparagus from the agricultural export field, where only *I. bütschlii* was detected (8%) in the sub-standard asparagus (Table 5).

Discussion

Totally, parasitic contaminations were found in 45% of fruit and vegetable samples (n=400). *E. nana* (27.5%) and *E. coli* (17.5%) were the most common nonpathogenic parasites contaminating the collected perishables, while the most prevalent pathogens were *Cryptosporidium* spp. (11.7%), *Cyclospora* spp. (11.0%), and *B. hominis* (9.2%). On the contrary, *I. bütschlii* (4.3%), *E, histolytica* (2.5%), *G. intestinalis* (1.5%), *C. mesnili* (1.2%), and *Isospora* spp. (0.5%) were the least common parasites. It could be stated that persistent poor hygiene in sites of sale, polluted environment, different storage conditions, and improper handling and transportation are apparently the common factors which contribute to the high parasitic contamination of fresh produce in the study area.

In a research conducted in Southwest Ethiopia, the overall prevalence of parasitic contamination in 360 fruits and vegetables samples was reported as 57.8% (Tefera et al., 2014) which was higher than that of our study. In addition, these researchers showed that the most common isolated protozoa were *Cryptosporidium* spp.

[DOI: 10.18502/jfqhc.8.2.6469]

Table 1: General prevalence of protozoan parasitic contamination in 400 collected fruits and vegetables from markets of Northwest Mexico (Caborca Sonora) during November 2019-June 2020

Parasites	General prevalence	
	n (%)	95% CI
Entamoeba nana	110 (27.5)	23.1-31.8
Entamoeba coli	70 (17.5)	13.7-21.2
Cryptosporidium spp. *	47 (11.7)	8.5-15.0
Cryptosporidium parvum	14 (3.5)	2.0-5.3
Cryptosporidium. hominis	20 (5.0)	2.9-7.2
Cyclospora spp. ***	44 (11.0)	8.0-14.0
Blastocystis hominis	37 (9.2)	6.3-12.1
Entamoeba spp. **	22 (5.5)	3.2-7.7
Entamoeba dispar/Entamoeba moshkovskii	12 (3.0)	1.8-4.9
Entamoeba histolytica	10 (2.5)	0.1-4.0
Iodamoeba bütschlii	17 (4.3)	2.4-6.0
Giardia intestinalis	6 (1.5)	0.3-2.6
Chilomastix mesnili	5 (1.2)	0.1-2.3
Isospora spp. ***	2 (0.5)	0.1-1.1

Total size of the fruit and vegetable samples was 400.
95% CI=95% Confidence interval.
n=contaminated cases.
*Only *Cryptosporidium parvum*, *Cryptosporidium hominis*.
** Only *Entamoeba. histolytica* was identified.
*** Species were not identified.

Table 2: Multiple parasitic contaminations in various types of fruits and vegetables collected from markets of Northwest Mexico (Caborca, Sonora) during November 2019-June 2020

Produce	Sample size	Contaminated number (%)	Number of detected parasite species						
			One	Two	Three	Four	Five	Six	Seven
Melon	100	19 (10.6)	10	3	2	0	0	0	0
Peach	100	35 (19.4)	15	12	6	1	0	0	1
Asparagus	100	56 (31.1)	22	14	8	9	3	0	0
Grape	100	70 (38.9)	27	26	12	4	1	0	0
Total (%)	400	180 (45.0)	74 (41.1)	55 (30.5)	28 (15.5)	14 (7.8)	4 (2.2)	0 (0.0)	1 (0.5)

Table 3: Percentage (%) of protozoan parasites in fresh produce collected from Northwest Mexico (Caborca Sonora) during November 2019-June 2020

Parasite spp.	Melon n=100	Peach n=100	Asparagus n=100	Grape n=100
Endolimax nana	12 ^{a}	14 ^{a}	34 ^{b}	50 ^{c}
Entamoeba coli	5 ^{a}	16 ^{b}	29 ^{c}	20 ^{bc}
Iodamoeba bütschlii	1 ^{a}	0 ^{a}	0 ^{a}	9 ^{b}
Cryptosporidium spp. *	4 ^{a}	10 ^{a}	23 ^{b}	10 ^{a}
Cryptosporidium parvum	1 ^{a}	4 ^{a}	8 ^{b}	1 ^{a}
Cryptosporidium hominis	1 ^{a}	5 ^{a}	14 ^{b}	3 ^{a}
Cyclospora spp. **	1 ^{a}	5 ^{a}	8 ^{a}	30 ^{b}
Blastocystis hominis	0 ^{a}	7 ^{b}	15 ^{b}	15 ^{b}
Chilomastix mesnili	0 ^{a}	3 ^{a}	2 ^{a}	0 ^{a}
Giardia intestinalis	0 ^{a}	2 ^{a}	4 ^{a}	0 ^{a}
Entamoeba spp. ***	0 ^{a}	9 ^{b}	12 ^{b}	1 ^{a}
Entamoeba dispar/Entamoeba moshkovskii	0 ^{a}	7 ^{b}	4 ^{b}	0 ^{a}
Entamoeba histolytica	0 ^{a}	2 ^{b}	8 ^{b}	1 ^{a}
Isospora spp. **	0 ^{a}	0 ^{a}	0 ^{a}	2 ^{a}

*Only *Cryptosporidium parvum* and *Cryptosporidium hominis* were identified.
** Species were not identified.
*** Only *Entamoeba histolytica* was identified.
-Different superscript letters in rows mean difference (Student-Newman-Keuls). Significant level was set at $p<0.05$.

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Table 4: Prevalence of protozoan parasites in open and closed-air markets of fresh produce in Northwest Mexico (Caborca, Sonora) during November 2019-June 2020

Parasites	Open-air markets (n=200)		Closed-air market (n=200)		*p*
	n (%)	95%CI	n (%)	95% (CI)	
Endolimax nana	69 (34.5)	27.4-40.5	41 (20.5)	14.4-25.5	0.001
Entamoeba coli	51 (25.5)	18.9-31.0	19 (9.5)	5.0-12.9	0.001
Iodamoeba bütschlii	2 (1.0)	0.4-2.4	8 (4.0)	1.2-6.7	0.055
Cryptosporidium spp. *	27 (13.5)	8.3-18.0	20 (10)	6.0-14.1	0.347
Cryptosporidium parvum	8 (4.0)	1.2-7.0	6 (3.0)	1.0-5.3	0.586
Cryptosporidium hominis	3 (1.5)	0.3-2.3	2 (1.0)	0.4-2.4	0.652
Cyclospora spp. **	22 (11.0)	6.6-15.3	22 (11.0)	6.6-15.3	0.999
Blastocystis hominis	26 (13.0)	8.3-17.6	11 (5.5)	1.9-8.0	0.010
Chilomastix mesnili	1 (0.5)	0.4-1.4	4 (2.0)	0.0-3.9	0.177
Giardia intestinalis	3 (1.5)	0.3-2.3	3 (1.5)	0.3-2.3	0.999
Entamoeba spp. ***	17 (8.5)	4.2-11.7	5 (2.5)	0.0-3.9	0.006
Entamoeba dispar/Entamoeba moshkovskii	9 (4.5)	1.1-6.8	3 (1.5)	0.4-2.6	0.052
Entamoeba histolytica	8 (4.0)	1.2-7.0	2 (1.0)	0.4-2.4	0.055
Isospora spp. **	2 (1.0)	0.3-2.3	0 (0.0)	-	0.156
Units with at least one parasite	107 (53.5)	46.0-60.0	73 (36.5)	29.3-42.6	0.001

95% CI=95% Confidence Interval
n=Contaminated cases
* Only *Cryptosporidium parvum* and *Cryptosporidium hominis* were identified.
** Species were not identified.
*** Only *Entamoeba histolytica* was identified.

Table 5: Comparison of the prevalence of protozoan parasites isolated from asparagus collected from Northwest Mexico (Caborca, Sonora) during November 2019-June 2020

Parasites	Asparagus of open-air-market (n=50)		Asparagus of closed-air-market (n=50)		Asparagus of an agricultural export field			
					γ Sub-standard (n=50)		Export (n=50)	
	n (%)	95%CI	n (%)	95% (CI)	n (%)	95% (CI)	n (%)	95% (CI)
Endolimax nana	21 (42) ^{a}	28.1-56.0	13 (26.0) ^{b}	13.6-38.3	3 (6.0) ^{c}	1.2-12.0	0 (0.0) ^{c}	-
Entamoeba coli	19 (38.0) ^{a}	24.3-51.6	10 (20.0) ^{b}	8.7-31.3	0 (0.0) ^{c}	-	0 (0.0) ^{c}	-
Iodamoeba bütschlii	0 (0.0) ^{a}	-	0 (0.0) ^{a}	-	4 (8.0) ^{b}	0-3-16.6	0 (0.0) ^{a}	-
Cryptosporidium spp. *	16 (32.0) ^{a}	18.8-45.1	7 (14.0) ^{b}	4.2-23.7	17 (34.0) ^{a}	20.6-47.3	2 (4.0) ^{b}	1.5-9.5
Cryptosporidium. parvum	7 (14.0) ^{a}	4.2-23.7	1 (2.0) ^{b}	1.1-5.9	3 (6.0) ^{bc}	0.8-12.7	0 (0.0) ^{c}	-
Cyclospora spp. **	4 (8.0) ^{a}	0.4-15.6	4 (8.0) ^{a}	0.4-15.6	1 (2.0) ^{a}	0.2-5.9	0 (0.0) ^{a}	-
Blastocystis hominis	11 (22.0) ^{a}	10.3-33.7	4 (8.0) ^{b}	0.4--15.6	1 (2.0) ^{b}	0.2-5.9	0 (0.0) ^{b}	-
Chilomastix mesnili	0 (0.0) ^{a}	-	2 (4.0) ^{a}	0.2-9.5	0 (0.0) ^{a}	-	0 (0.0) ^{a}	-
Giardia intestinalis	2 (4.0) ^{a}	0.2-9.5	2 (4.0) ^{a}	0.2-9.5	2 (4.0) ^{a}	0.2-9.5	0 (0.0) ^{a}	-
Entamoeba dispar/Entamoeba moshkovskii	3 (1.5) ^{a}	0.3-2.3	1 (2.0) ^{b}	1.1-5.9	0 (0.0) ^{b}	-	0 (0.0) ^{b}	-
Entamoeba histolytica	8 (22.0) ^{a}	1.2-7.0	0 (0.0) ^{b}	-	0 (0.0) ^{b}	-	0 (0.0) ^{b}	-

95% CI=95% Confidence Interval
n=Contaminated cases
* Only *Cryptosporidium parvum* and *Cryptosporidium hominis* were identified.
** Species were not identified.
-Different superscript letters in rows mean difference (Student-Newman-Keuls). Significant level was set at $p<0.05$.
γ=Product that fails to pass international quality control standards.

(12.8%), *Giardia lamblia* (7.5%), *E. histolytica*/*dispar* (5.3%), and also *Cyclospora* spp. (5.0%). In the United Arab Emirates (UAE), El Bakri et al. (2020) have analyzed 218 fresh vegetable samples (including fennel, green pepper, chard, rocket, watercress, lettuce, spring onion, tomato, radish, broccoli, parsley, mint, carrots, and also cucumber) using the sedimentation techniques. The most isolated parasites were *E. histolytica*/*dispar*/*moshkovskii* (30.3%), *E. coli* (18.2%), *Ascaris lumbricoides* (9.1%), *E. nana* (6.1%), *Enterobius vermicularis* (6.1%), *G. lamblia* (3.0%), *Hymenolepis nana* (3.0%), etc. The authors recognized that the origin and surface shape and texture of the vegetables, the techniques used, ample size, geographical region, type of water, night soil, farming practices, and handling measures are factors responsible of the different prevalence of parasitic contamination found in the fresh vegetables of UAE. In Bahir Dar city of Ethiopia, Alemu et al. (2020) used iodine wet mount smear and the modified Ziehl-Neelsen to examine a total of 384 fruits and

[DOI: 10.18502/jfqhc.8.2.6469]

vegetables (lettuce, spinach, cabbage, carrot, tomato, mango, and green pepper) acquired in local markets. Of those samples, 150 (39.1%) were contaminated with at least one parasite spp. which is comparable with our finding. Apparently, lack of hygiene practices by vendors in the markets was the factor that contributed to parasitic contamination of fresh produces. Contrary with the results of this research, a recent survey showed that *G. lamblia* (21.9%) and *E. histolytica* (21.4%) were the most prevalent parasites isolated from vegetable samples distributed in Ramadi, Iraq (Mohemeed et al., 2021).

In the present study, grapes and asparagus had higher percentages of overall parasitic and multiple parasitic contaminations than melon and peach. Asparagus has an uneven surface texture that permits better attachment by parasitic protozoa in different stages of development. This likely explains its higher parasitic contamination compared to the smooth surfaces of melon and peach samples. Although grapes also have a smooth surface, during sampling, we noted a sugary covering that gave a sticky consistency. This is evidence of poor hygiene and inadequate handling practices by vendors that may well explain the high levels of parasitic contamination. Similarly, Tefera et al. (2014) stated that uneven surfaces (salad, cabbage, and carrots) are more susceptible to parasitic contamination because they permit better adherence, while smooth surfaces (tomatoes and mango) are slippery and make it more difficult for microorganisms to anchor there. Alemu et al. (2020) found that multiple parasitic contaminations were more common in vegetables than fruits. They stated that lettuce, spinach, and cabbage have rough, uneven surfaces that permit easier attachment of parasites than the smooth surfaces of many fruits. Mohemeed et al. (2021) explained that *Lactuca sativa* plant is more in contact with soil surface and the abundance of leafy folds in *Lactuca* is the perfect shelter of parasites. Also, El Bakri et al. (2020) concluded that lettuce, spring onions, watercress, and parsley have uneven surfaces that make them most vulnerable to highest parasitic contamination as opposed to smooth-surfaced vegetables.

We detected no helminths, but this may be related to climate since the Caborca region of Mexico has extremely semi-warm dry weather almost year round with only 209 mm of rainfall. These conditions may contribute to the absence of helminth spp., organisms that are frequently found in countries or regions with tropical or subtropical climates (Sturrock et al., 2017). In a study conducted in Thailand, Punsawad et al. (2019) estimated an overall prevalence of intestinal parasites of 35.1% in 265 samples of various vegetables (mint, lettuce, coriander, celery, leek, and basil) acquired in open markets. The predominant parasite they found was the hookworm helminth (42.9%). It is important to note that the Thai city of Sakhon Si Thammarat has a tropical climate with no dry season and average annual temperature of 27.2 °C, and annual rainfall of at least 2 292 mm. In the present study, the overall prevalence of parasitic contamination was higher in the perishables collected from open-air markets (53.5%) than closed-air markets (36.5%). In the study of Duedu et al. (2014) in Accra, Ghana, the overall prevalence of parasitic contamination was higher in the vegetables collected from the open-air markets than in those gathered in the closed supermarkets. They concluded that this difference may result from greater handling of the produce by vendors and consumers. Ismail (2016), in contrast, found no difference in prevalence of parasitic contamination in 133 samples (cucumber, parsley, tomato, and lettuce) obtained from open-air and closed markets in Jordan (31.8% and 26.9%, respectively). They concluded that all examined markets had used the same contaminated water for washing vegetables.

We observed poor hygienic and environmental conditions, such as the presence of dust, varied storage temperatures, distinct kinds and qualities of produce, and improper handling and transport to market, all of which likely contribute to the differences in the levels of overall parasitic contamination of fresh produce found among the markets examined in the current work. However, it is difficult to explain the absence of differences in the prevalence rates of specific genera and species, such as *I. bütschlii*, *C. mesnili*, *Cryptosporidium* spp., *E. histolytica*, *Cyclospora* spp., *Isospora* spp., and *G. intestinalis* between the markets. Finally, it is important to emphasize that asparagus from the open-air markets had a higher prevalence of parasitic contamination (72%) than the product from the closed markets, and both the sub-standard and export-quality asparagus collected from the export field where strict international quality standards are imposed. This is evidence of the poor hygiene practices at which the asparagus is sold in the markets of the Caborca region.

In the current study, we used some different techniques (except molecular technique) to obtain exact data about situation of parasitic contaminations in the fruits and vegetables distributed in the Caborca region. However, it should be noted that molecular technique is one of the most important and useful approaches to identification of the parasitic species in the food samples (Bahreh et al., 2021; Deksne et al., 2020; Eslami et al., 2017; Zolfaghari Emameh et al., 2018).

Conclusion

A high overall prevalence of parasitic contamination was found in the fruits and vegetables analyzed in Caborca region (Northwest Mexico), showing a serious

[DOI: 10.18502/jfqhc.8.2.6469]

public health concern. This parasitic prevalence rate of samples was higher in the open-air markets than the closed markets. *E. nana* and *E. coli* were the predominant nonpathogenic species of parasites, while *Cryptosporidium* spp., *Cyclospora* spp., and *B. hominis* were the predominant pathogenic genera. Asparagus and grapes had higher parasitic contamination than melon and peach.

Finally, educational interventions should be carried out to create or increase local people's awareness of the importance of adequate hygiene practices and the appropriate handling of fruits and vegetables, as this will positively impact the health of the general population. Future studies in the Caborca region should be used not only common parasitological techniques but also immunological and molecular methodologies to obtain more realistic views of this problem. In addition, the current Mexican Official Standard must be updated to regulate the presence of intestinal parasites in fruits and vegetables marketed.

Author contributions

L.Q-C. and G.G.M-F. designed the research on line, analyzed the data, conducted the experiment, and wrote the manuscript; M.A.S-G. and M.C-G. conducted the experiment and analyzed the data; J.E-R. did the statistical analysis; M.A.L-M. wrote the manuscript. All authors read and approved the final revised manuscript.

Conflicts of interest

The authors declared no conflict of interest.

Acknowledgements

The authors thank Mexico's Consejo Nacional de Ciencia y Tecnología and the Centro de Investigación en Alimentación y Desarrollo A.C. for their financial support. Also, we hank the owners of the open and closed air markets, and the agricultural fields, the Unidad de Control Sanitario, the Junta Local de Sanidad Vegetal and the Comité Estatal de Sanidad Vegetal de Sonora for the facilities provided for this study.

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