RESEARCH ARTICLE

REVISED Field study of parasitic contamination of fruits, vegetables and leafy greens in the Ecuadorian Andes [version 2; peer review: 4 approved]

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v2 **First published:** 23 May 2023, **12**:532
https://doi.org/10.12688/f1000research.132957.1
Latest published: 12 Mar 2024, **12**:532
https://doi.org/10.12688/f1000research.132957.2

Abstract

Background: Raw vegetables have been considered vehicles of enteroparasites. South American countries are among the most important exporters of fresh vegetables, including Ecuador, which has a tropical climate and soils rich in organic matter that allow it to harvest throughout the year for sale to different countries. The aim of the study was to assess the occurrence of the parasitic contamination of fruits, vegetables and leafy greens grown in an agricultural area of the Ecuadorian Andes.

Methods: A cross-sectional field study was conducted with snowball sampling on 1,416 samples (516 fruits, 488 vegetables, and 412 leafy greens). Each sample were washed with water, and the resulting solution after removing the vegetables, was subjected to 24-hour sedimentation. The concentrated sediment underwent microscopic analysis.

Results: The overall positivity for parasitic contamination was 63.4%, with leafy greens having the highest contamination rate (76.9%) (P<0.0001), surpassing vegetables (67.8%) and fruits (48.4%). Cabbage (100%), onions (84%), and strawberries (60.2%) emerged as the most

Open Peer Review

Approval Status ✓ ✓ ✓ ✓

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version 2 (revision) 12 Mar 2024			✓ view	✓ view
version 1 23 May 2023	✓ view	✓ view	? view	? view

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contaminated within their respective groups. Protozoa were more prevalent (49.6%) than helminths (15.5%) (P<0.0001). *Blastocystis* sp. (33.5%) ranked highest, followed by *Eimeria* spp. (26.3%), *Entamoeba* spp. (10.3%), *Giardia* spp. (8.3%), *Balantidium* spp. (6.9%), *Cryptosporidium* spp. (6.6%), *Cyclospora* spp. (4.4%), *Cystoisospora* spp. (0.5%), Strongylida (15.5%), and *Ascaris* spp. (0.4%).
Conclusion: The study reveals that vegetables and fruits for human consumption from this area of the Ecuadorian Andes are highly contaminated with various parasites, constituting a possible source of infection for humans and animals in this area, or in non-endemic areas where these products are marketed. The finding emphasizes the need for strict hygienic measures in agricultural crops, which will be properly achieved through the treatment of soil, manure and water used for cultivation.

Keywords
agricultural production, food, transmission, parasites, fruits, vegetables, leafy greens

This article is included in the Global Public Health gateway.

This article is included in the Agriculture, Food and Nutrition gateway.

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Competing interests: No competing interests were disclosed.

Grant information: This research was supported by Universidad Nacional de Chimborazo under Research Funds (FCS-006-2021). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
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How to cite this article: González-Ramírez LC, Djabayan-Djibeyan P, Prato JG *et al.* **Field study of parasitic contamination of fruits, vegetables and leafy greens in the Ecuadorian Andes [version 2; peer review: 4 approved]** F1000Research 2024, **12**:532 https://doi.org/10.12688/f1000research.132957.2

First published: 23 May 2023, **12**:532 https://doi.org/10.12688/f1000research.132957.1

REVISED **Amendments from Version 1**

The revised version of the manuscript incorporates the corrections suggested by the reviewers. The Introduction was expanded, incorporating current bibliographic references. The Discussion was synthesized, and current quotes that better explain the results were incorporated.

In the new version of the article, an extensive grammar check is done, improving the clarity and coherence of the manuscript.

Any further responses from the reviewers can be found at the end of the article

Introduction

Incorporating vegetables, leafy greens, and fruits into human diet is essential to ensure vital nutrients crucial to maintaining health. However, these foods can also serve as vehicles for enteroparasites, representing a paradox within nutritional health practices (Punsawad et al., 2019; Al Nahhas and Aboualchamat, 2020; Barlaam et al., 2021, 2022; Faria et al., 2023). The role of vegetables in the spread of pathogens is notably substantial. The external surfaces of these foods can retain infectious stages of various parasites, thereby posing a risk of direct transmission to humans when consumed raw or poorly washed (Mufida et al., 2022; Lucas et al., 2023; Moreno-Mesonero et al., 2023).

The importation of fresh vegetables from endemic to non-endemic regions has contributed to the spread of parasites. Diarrhoea epidemics have been reported from the consumption of berries, tomatoes, peppers, onions, carrots, lettuces, cabbage, radishes and mixed-salad packages (Dixon, 2016; Machado-Moreira et al., 2019; Barlaam et al., 2021, 2022; Temesgen et al. 2022). Leafy greens are highlighted by the potential role of spinach, lettuce, cabbage, watercress, basil, mint, coriander, and parsley as vehicles for food-borne parasites (Ahlinder et al., 2022; Lucas et al., 2023; Moreno-Mesonero et al., 2023). There is evidence that leafy green can carry and spread parasites as *Cyclospora cayetanensis*, *Cryptosporidium* spp. and *Giardia duodenalis*, *Toxoplasma gondii*, *Entamoeba histolytica*, *Blastocystis* sp., *Cystoisospora belli*, *Balantidium coli*, *Dientamoeba fragilis*, *Echinococcus* sp., *Dipylidium caninum*, *Ascaris* sp., *Trichuris* sp., and Nematode spp. Larvae (Dixon, 2016; Caradonna et al., 2017; Karshima, 2018; Robertson, 2018; Barlaam et al., 2021; Yahia et al., 2023).

The persistence and survival of parasites in soil (Qorom, et al. 2023; Falcone et al., 2023), vegetables (Falcone et al., 2023) and water (Kubina et al. 2023), has been proven. According to these studies, *Cryptosporidium* survives in lamb's lettuce for two months and its washing showed limited effectiveness in reducing parasite load and had no impact on the parasite's survival. Furthermore, chlorination of the wash water failed to improve the efficiency of the disinfection process. The situation is aggravated by cases of parasite resistance to certain chemical and physical inactivating agents (Ramos et al., 2013). This underlines the ability of parasites to persist and survive along the food chain, facilitating their transmission to humans, even far from the site of production (Dixon, 2016; Caradonna et al., 2017; Barlaam et al., 2021, 2022; Temesgen et al. 2022; Ahart et al., 2023).

Fruits are also considered significant carriers of parasites because they are consumed raw and do not undergo disinfection treatments such as the use of vinegar (Honório Santos et al., 2019). The study of berries is particularly important since their consumption has increased recently due to their high nutritional value, as a source of bioactive compounds and antioxidants (Tefera et al., 2018; Barlaam et al., 2022; Lucas et al., 2023). Unfortunately, berries such as raspberries and strawberries can carry infective forms of pathogens due to their delicate and porous nature, facilitating the attachment and protection of parasites. Inappropriate practices in cultivation, harvesting and handling pose a significant risk to consumers (Tefera et al., 2018; Temesgen et al., 2022). Recent molecular studies demonstrated the persistence in berries of parasites such as *Cryptosporidium*, *Cyclospora*, *Giardia duodenalis*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Acanthamoeba*, *Vermamoeba vermiformis*, *Blastocystis* sp., and *Echinococcus* (Marques et al., 2020; Barlaam et al., 2021, 2022; Trelis et al., 2022; Temesgen et al., 2022; Moreno-Mesonero et al., 2023), highlighting their resistance to washing processes and disinfection (Temesgen et al., 2022; Kubina et al., 2023).

The presence of parasites in the vegetables is an indicator of the lack of adherence to good agricultural practices (Lucas et al., 2023). The main risk factors for the transmission of parasites through vegetables include the soil contamination with excrement from defecation or direct fertilization (Ercumen et al., 2017; Falcone et al., 2023) and the use of contaminated water for irrigation, pesticide dilution or equipment washing (Efstratiou et al., 2017; Karshima, 2018; Tefera et al., 2018).

Although parasites do not multiply in food, the transmission of infectious forms is closely linked to their resistance to survive in the environment attached to vegetables, as reported by studies carried out throughout Latin America: Argentina

(Falcone et al., 2023); Brazil (Luz et al. 2017; Honório Santos et al., 2019; De Farias et al., 2021); Bolivia (Rodríguez et al., 2015); Colombia (Polo et al., 2016); Cuba (Puig-Peña et al., 2013); Ecuador (Bracho-Mora et al., 2022); Peru (Pérez-Cordón et al., 2008; Benites Salcedo et al., 2019; Lucas et al., 2023); Mexico (Chávez-Ruvalcaba et al., 2021); Venezuela (Cazorla-Perfetti et al., 2013; Devera et al., 2021).

South American countries are among the most important exporters of fresh vegetables. Ecuador has tropical climate and soils rich in organic matter that allow it to harvest fruits, vegetables, and grains throughout the year. According to data from the Agriculture and Livestock Ministry, during 2014-2018 period, Ecuador raised more than $3,500 million by exporting 6 million tons of fruits and vegetables (Ministerio de Agricultura y Ganadería Ecuador, 2020). Unfortunately, Ecuador has serious health problems in rural Andean regions, especially those located at high altitudes, mostly inhabited by indigenous populations whose means of subsistence is agriculture, livestock and animal husbandry (González-Ramírez et al., 2021, 2022).

Moreover, Ecuadorian farmers often do not use good agricultural practices due to the lack of training, confidence, or economic resources, which is detrimental to food quality production. Two local reports have shown high level of parasitic contamination in vegetables: up to 82.3% in lettuce from Manabí province (Bracho-Mora et al., 2022) and up to 70.6% in fruits and vegetables of six rural communities in the parish of San Andrés, Chimborazo province (González-Ramírez et al., 2022).

Due to the alarming contamination data previously reported by our group, in this work we have evaluated the detailed parasitic contamination of all fruits, vegetables and leafy greens grown in the capital of San Andrés, an agricultural zone of the Ecuadorian Andes.

Methods

Study area

The study area was the community of San Andrés, Guano canton, Chimborazo province of Ecuador, located at 3,900 meters above sea level. The local temperature ranges between 5-18 °C, and rainfall varies between 500-1,000 mm/year. There are two rainy periods, February to May and October to November; the remaining months are transitional with moderate rains. Evapotranspiration affects the drought of the soil, which originates from volcanic ashes of variable textures, most of which are shallow silty loam, with a pH of 4.5 to 6.5. There are loamy soils in the areas with the highest agricultural production, but they are affected by chemical fertilizers. There are also sandy soils with low fertility because they do not retain moisture and nutrients; the latter and the action of steep slopes make them susceptible to erosive processes; consequently, crops and sowing grass are not abundant. However, agricultural activity is 34.5%, and cattle breeding activity is 50.4%; these two are the main means of financial income for the local population (PDOT San Andrés, 2015).

Government records indicate that 47.9% of the rural population of Ecuador lives in poverty, with an average monthly family income of $84.05, and 27.5% living in extreme poverty, with an average income of $47.70. The province of Chimborazo has an illiteracy rate of 13.5%, and the community of San Andrés has an indigenous population of 36.9% (INEC, 2020). Hence, their training is based on habits and customs acquired from their ancestors, which may contribute to as a lack of basic hygiene and sanitary measures. The most remote communities have built septic tanks, and the communities closest to the capital have sewers; however, both drain wastewater into rivers and streams (PDOT San Andrés, 2015).

Investigation design

A field study, cross-sectional, observational and descriptive, was carried out during 1 month of rain and 7 months of drought. The snowball sampling technique was applied, whereby a grower helped locate the nearest farm and so on. All types of products found were included in the sampling (1,416 samples in total); the inclusion criteria were that all agricultural products must come from San Andrés fields and those not cultivated in the community were excluded.

Sampling

The total of 1,416 samples analyzed included 516 fruits of 8 types: *Fragaria ananassa* (strawberry), *Rubus glaucus* (blackberry), *Physalis peruviana* (uvilla), *Prunus persica* (peach), *Citrus limon* (lemon), *Psidium guajava* (guava), *Ficus carica* (fig), and *Solanum lycopersicum* (tomato); 488 vegetables of 9 types: *Allium cepa* var. rosum (red onions) and *Allium cepa L* (white onions), *Solanum tuberosum* (potato), *Daucus carota* (carrot), *Raphanus sativus* (radish), *Beta vulgaris* (beet), *Capsicum annuum* (sweet pepper), *Capsicum frutescens* (chili pepper), and *Lupinus mutabilis* (bean chochos) and 412 leafy greens of 8 types: *Medicago sativa* (alfalfa), *Lactuca sativa* (lettuce), *Brassica oleracea* (cabbage), *Beta vulgaris* (chard), *Petroselinum crispum* (parsley), *Coriandrum sativum* (cilantro), *Apium graveolens* (celery), and *Nasturtium officinale* (watercress).

All samples were obtained from the owners' fields and stored in hermetically sealed propylene bags. Each sample was labelled indicating the plant species name, origin, date, and time of collection. The samples were immediately transported in their containers with cooling gels to the Laboratorio de Investigación de la Facultad de Ciencias de la Salud, Universidad Nacional de Chimborazo, to be processed within one hour of collection.

Ethical considerations

The sampling was carried out with the appropriate permission of the Cantonal and Parochial Decentralized Autonomous Governments. All farmers collected samples of their own crops (as they always do), knowing that the study benefits the community, without compromising the health of the population with respect to bioethical principles.

Parasitological analysis

The processing protocol for the parasitological analysis of all samples, previously described by Rivero de Rodríguez *et al.* (1998), was utilized. For the processing of the samples, 75 g of vegetables, fruits or green leaves were taken and added to 500 mL of previously filtered and boiled water. The contents were stirred with the help of a magnetic stirrer for 1 hour, the remains of the vegetable were removed and the solution was left to stand for 24 hours. Subsequently, the solution was decanted and the first fraction was collected in 15 mL tubes to be subjected to centrifugation for 5 min at 800 xg. Once the concentrate or sediment was separated, the supernatant was discarded and the precipitate was reconstituted in 400 µL of saline (0.85%). Each sample was observed under a light microscope (Nikon E200) using 10x and 40x objectives. In addition, iodized solution and the ocular micrometer were used when necessary, for stain parasitic structures or to measure the dimensions for their recognition. Additionally, a smear was made with one drop from the pellet and prepared for acid-fast staining (using a modified Zielh-Neelsen technique) for coccidia oocyst detection and identification after measurement, mainly *Crytosporidium* and *Cyclospora*, and subsequent microscopic assessment (100×) (García *et al.*, 1983).

Statistical analysis

The database made in Microsoft Excel was exported to SPSS Statistic 26.0 software (IBM, New York, NY, USA). The difference in parasitic contamination between the various categories of plant products and the predominant parasite type in each plant species were compared using Pearson's chi-square test (χ^2) and Fisher's exact test, when appropriate. A P value <0.05 was considered statistically significant.

Results

When analyzing the different crop products, a total of 898 (63.4%) were contaminated by parasites. Noteworthy, every sample analyzed showed more than one associated parasite, i.e. 100% multiparasitism was detected. A statistically significant difference between the overall contamination rates, was observed with the leafy greens (76.9%) being more contaminated than vegetables (67.8%) and both, more contaminated than fruits (48.4%) (P<0.0001). In total, 15 protozoa and 2 helminth nematodes were identified, with protozoa also showing a higher prevalence (49.6%) than nematodes (15.5%) (P<0.0001). *Blastocystis* sp. was outstanding (33.5%) (P<0.0001), showing central body, granular, and resistance forms, whereas dividing, globular, or amoeboid forms were not observed. Other protozoa identified include *Eimeria* spp. (26.3%), *Entamoeba* spp. (10.3%), *Giardia* spp. (8.3%) and *Cryptosporidium* spp. (6.6%). Regarding the nematodes, Strongylida was more frequent than *Ascaris* spp. (P<0.0001) (see Table 1).

When analyzing the percentages of parasites in the three groups of samples, the statistical analysis revealed a high prevalence in fruits of *Blastocystis* (37.4%) (P=0.0018), *Cryptosporidium* (7.6%) (P<0.0001), *Cyclospora* (6%) (P<0.0001) and *Endolimax nana* (6%) (P=0.0028). In contrast, vegetables were mostly contaminated by helminths (24.2%) (P<0.0001), represented mainly Strongylida (23.6%) (P<0.0001). Finally, the leafy greens showed high contamination with *Eimeria* (33.5%) (P=0.0002), *Entamoeba* spp. (16.7%) (P<0.0001), *Balantidium* (15.0%) (P<0.0001) and *Giardia* (12.6%) (P=0.0002). Overall, a total parasitic contamination of 76.9% (P<0.0001) with 61.4% (P<0.0001) being protozoa was obtained (see Table 1).

Table 2 summarizes the results according to the type of fruit, the highest number of protozoa was found in strawberries (60.2%) (P<0.0001), with *Blastocystis* sp. (59.2%) (P<0.0001), *E. nana* (17.4%) (P<0.0001) and *Cyclospora* spp. (14.3%) (P=0.0011) the most frequent. In contrast, peaches were more often contaminated with helminths (30%) (P<0.0001).

Parasitic contamination in vegetables is detailed in Table 3. The highest frequency of contamination was found in red (84%) and white (82.4%) onions, followed by chili pepper (78%) (P<0.0001). It is important to highlight the level of contamination detected in other vegetables that are eaten raw such as carrot (66%), radish (72.1%) and pepper (44%). When compared vegetables for the type of parasites, a higher frequency of protozoa (47.1%) than helminths (24.2%) was observed (P<0.0001).

Table 1. Distribution of parasites according to the plant type analysed.

Parasites	Fruits			Vegetables			Leafy Greens			Total		
	n=516	%	IC	n=488	%	IC	n=412	%	IC	n=1416	%	IC
Blastocystis sp.	193	37.4	(33.2-41.6)	134	27.5	(23.5-31.4)	148	35.9	(31.3-40.6)	475	33.5	(31.1-36)
Entamoeba spp.	29	5.6	(3.6-7.6)	48	9.8	(7.2-12.5)	69	16.7	(13.1-20.4)	146	10.3	(8.7-11.9)
E. coli	9	1.7	(0.6-2.9)	11	2.3	(0.9-3.6)	3	0.7	(0-1.6)	23	1.6	(1-2.3)
E. hartmanni	2	0.4	(0-0.9)	2	0.4	(0-1)	1	0.2	(0-0.7)	5	0.4	(0-0.7)
Endolimax nana	31	6	(4.0-8.1)	9	1.8	(0.7-3.0)	15	3.6	(1.8-5.5)	55	3.9	(2.9-4.9)
Iodamoeba buetschlii	2	0.4	(0-0.9)	2	0.4	(0-1)	1	0.2	(0-0.7)	5	0.4	(0-0.7)
Giardia spp.	26	5	(3.2-7)	40	8.2	(5.8-10.6)	52	12.6	(9.4-15.8)	118	8.3	(6.9-9.8)
Chilomastix spp.	6	1.2	(0.3-2.1)	8	1.6	(0.5-2.8)	3	0.7	(0-1.6)	17	1.2	(0.6-1.8)
Retortamonas spp.	0	0	0	1	0.2	(0-0.6)	0	0	0	1	0.1	(0-0.2)
Enteromonas spp.	3	0.6	(0-1.3)	0	0	0	1	0.2	(0-0.7)	4	0.3	(0-0.6)
Cryptosporidium spp.	39	7.6	(5.3-9.9)	30	6.1	(4.0-8.3)	24	5.8	(3.6-8.1)	93	6.6	(5.3-7.9)
Cyclospora spp.	31	6	(4.0-8.1)	20	4.1	(2.3-5.9)	12	2.9	(1.3-4.5)	63	4.4	(3.4-5.5)
Cystoisospora spp.	3	0.6	(0-1.2)	2	0.4	(0-1)	2	0.5	(0-1.2)	7	0.5	(0.1-0.9)
Eimeria spp.	112	21.7	(18.1-25.3)	123	25.2	(21.4-29.1)	138	33.5	(28.9-38.1)	373	26.3	(24.0-28.6)
Balantidium spp.	5	1	(0.1-1.8)	30	6.1	(4.0-8.3)	62	15	(11.6-18.5)	97	6.9	(5.5-8.2)
Protozoa	220	42.6	(38.4-46.9)	230	47.1	(42.7-51.6)	253	61.4	(56.7-66.1)	703	49.6	(47-52.3)
Ascaris spp.	0	0	0	3	0.6	(0-1.3)	2	0.5	(0-1.2)	5	0.4	(0-0.7)
Strongylida	32	6.2	(4.1-8.3)	115	23.6	(20.4-28.0)	70	17	(13.4-20.6)	217	15.3	(13.7-17.4)
Helminths	32	6.2	(4.1-8.3)	118	24.2	(20.4-28.0)	70	17	(13.4-20.6)	220	15.5	(13.7-17.4)
Total	250	48.4	(44.1-52.7)	331	67.8	(63.7-71.9)	317	76.9	(72.8-81)	898	63.4	(60.9-65.9)

n = number of studied; IC = Confidence interval.

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Table 2. Distribution of parasites according to the fruit type analysed.

Parasites	Strawberry		Blackberry		Uvilla		Peach		Lemon		Guava		Fig		Tomato		Total	
	n=98	%	n=83	%	n=56	%	n=50	%	n=56	%	n=57	%	n=50	%	n=66	%	n=516	%
Blastocystis sp.	58	59.2	35	42.2	19	33.9	24	48	14	25	19	33.3	15	30	9	13.6	193	37.4
Entamoeba spp.	4	4.1	5	6	0	0	2	4	0	0	9	15.8	3	6	6	9.1	29	5.6
E. coli	4	4.1	4	4.8	0	0	0	0	0	0	1	1.8	0	0	0	0	9	1.7
E. hartmanni	0	0	1	1.2	0	0	0	0	0	0	0	0	0	0	1	1.5	2	0.4
Endolimax nana	17	17.3	6	7.2	0	0	3	6	1	1.8	3	5.3	0	0	1	1.5	31	6
Iodamoeba buetschlii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	2	0.4
Giardia spp.	10	10.2	4	4.8	1	1.8	1	2	1	1.8	1	1.8	2	4	6	9.1	26	5
Chilomastix spp.	0	0	3	3.6	0	0	0	0	0	0	0	0	2	4	1	1.5	6	1.2
Retortamonas spp.	3	3.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0.6
Enteromonas spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptosporidium spp.	11	11.2	11	13.3	4	7.1	4	8	1	1.8	4	7	1	2	3	4.5	39	7.6
Cyclospora spp.	14	14.3	7	8.4	5	8.9	0	0	1	1.8	3	5.3	0	0	1	1.5	31	6
Cystoisospora spp.	1	1	1	1.2	1	1.8	0	0	0	0	0	0	0	0	0	0	3	0.6
Eimeria spp.	20	20.4	23	27.7	24	42.9	10	20	1	1.8	5	8.8	17	34	12	18.2	112	21.7
Balantidium spp.	3	3.1	0	0	1	1.8	0	0	0	0	0	0	0	0	1	1.5	5	1
Protozoa	59	60.2	46	55.4	24	42.9	16	32	3	5.4	20	35.1	23	46	29	43.9	220	42.6
Ascaris spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strongylida	0	0	0	0	9	16.1	15	30	5	8.9	0	0	1	2	2	3	32	6.2
Helminths	0	0	0	0	9	16.1	15	30	5	8.9	0	0	1	2	2	3	32	6.2
Total	59	60.2	46	55.4	32	57.1	31	62	7	12.5	20	35.1	24	48	31	47	250	48.4

n = number of studied; IC = Confidence interval.

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Table 3. Distribution of parasites according to the vegetable type analysed.

Parasites	Red onion		White onion		Potato		Carrot		Radish		Beet		Pepper		Chili pepper		Beans chocho		Total	
	n=50	%	n=51	%	n=52	%	n=53	%	n=61	%	n=51	%	n=50	%	n=50	%	n=70	%	n=488	%
Blastocystis sp.	28	56	16	31.4	11	21.2	12	22.6	22	36.1	9	17.6	6	12	14	28	16	22.9	134	27.5
Entamoeba spp.	3	6	1	2	6	11.5	6	11.3	8	13.1	13	25.5	1	2	7	14	3	4.3	48	9.8
E. coli	0	0	2	3.9	3	5.8	1	1.9	5	8.2	0	0.0	0	0	0	0	0	0	11	2.3
E. hartmanni	0	0	1	2	1	1.9	0	0	0	0	0	0.0	0	0	0	0	0	0	2	0.4
Endolimax nana	0	0	4	7.8	1	1.9	2	3.8	0	0	0	0.0	0	0	2	4	0	0	9	1.8
Iodamoeba buetschlii	0	0	0	0	0	0	1	1.9	0	0	0	0.0	1	2	0	0	0	0	2	0.4
Giardia spp.	8	16	6	11.8	1	1.9	3	5.7	3	4.9	2	3.9	7	14	7	14	3	4.3	40	8.2
Chilomastix spp.	4	8	2	3.9	1	1.9	0	0	0	0	0	0	0	0	1	2	0	0	8	1.6
Retortamonas spp.	0	0	0	0	0	0	1	1.9	0	0	0	0	0	0	0	0	0	0	1	0.2
Enteromonas spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptosporidium spp.	3	6	7	13.7	2	3.8	6	11.3	3	4.9	2	3.9	0	0	2	4	5	7.1	30	6.1
Cyclospora spp.	2	4	2	3.9	1	1.9	5	9.4	3	4.9	6	11.8	0	0	0	0	1	1.4	20	4.1
Cystoisospora spp.	0	0	0	0	1	1.9	1	1.9	0	0	0	0	0	0	0	0	0	0	2	0.4
Eimeria spp.	10	20	10	19.6	15	28.8	13	24.5	22	36.1	17	33.3	12	24	17	34	7	10	123	25.2
Balantidium spp.	1	2	3	5.9	3	5.8	4	7.5	7	11.5	6	11.8	1	2	3	6	2	2.9	30	6.1
Protozoa	24	48	28	54.9	27	51.9	25	47.2	33	54.1	28	54.9	19	38	27	54	19	27.1	230	47.1
Ascaris spp.	0	0	0	0	0	0	0	0	0	0	2	3.9	1	2	0	0	0	0	3	0.6
Strongylida	16	32	28	54.9	22	42.3	7	13.2	15	24.6	11	21.6	1	2	15	30	0	0	115	23.6
Helminths	16	32	28	54.9	22	42.3	7	13.2	15	24.6	13	25.5	2	4	15	30	0	0	118	24.2
Total	42	84	42	82.4	40	76.9	35	66.0	44	72.1	37	72.5	22	44	39	78	30	42.9	331	67.8

n = number of studied; IC = Confidence interval.

Table 4. Distribution of parasites according to the leafy green type analysed.

Parasites	Alfalfa		Lettuce		Cabbage		Chard		Parsley		Coriander		Celery		Watercress		Total	
	n=51	%	n=58	%	n=52	%	n=50	%	n=51	%	n=50	%	n=50	%	n=50	%	n=412	%
Blastocystis sp.	13	25.5	24	41.4	20	38.5	12	24	29	56.9	19	38	12	24	19	37.3	148	35.9
Entamoeba spp.	4	7.8	21	36.2	16	30.8	3	6	2	3.9	6	12	7	14	10	19.6	69	16.7
E. coli	0	0	1	1.7	1	1.9	0	0	1	2	0	0	0	0	0	0	3	0.7
E. hartmanni	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	1	0.2
Endolimax nana	5	9.8	0	0	7	13.5	0	0	2	3.9	1	2	0	0	0	0	15	3.6
Iodamoeba buetschlii	0	0	0	0	1	1.9	0	0	0	0	0	0	0	0	0	0	1	0.2
Giardia spp.	5	9.8	5	8.6	9	17.3	4	8	7	13.7	8	16	4	8	10	19.6	52	12.6
Chilomastix spp.	0	0	0	0	0	0	1	2	2	3.9	0	0	0	0	0	0	3	0.7
Retortamonas spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enteromonas spp.	0	0	0	0	1	1.9	0	0	0	0	0	0	0	0	0	0	1	0.2
Cryptosporidium spp.	1	2	3	5.2	2	3.8	1	2	5	9.8	3	6	4	8	5	9.8	24	5.8
Cyclospora spp.	0	0	1	1.7	4	7.7	0	0	1	2	2	4	4	8	0	0	12	2.9
Cystoisospora spp.	0	0	0	0	1	1.9	1	2	0	0	0	0	0	0	0	0	2	0.5
Eimeria spp.	26	51	17	29.3	28	53.8	14	28	13	25.5	9	18	17	34	14	27.5	138	33.5
Balantidium spp.	7	13.7	8	13.8	11	21.2	5	10	5	9.8	8	16	9	18	9	17.6	62	15
Protozoa	33	64.7	37	63.8	45	86.5	23	46	33	64.7	25	50	30	60	27	52.9	253	61.4
Ascaris spp.	1	2	0	0	0	0	0	0	0	0	0	0	1	2	0	0	2	0.5
Strongylida	13	25.5	11	19	11	21.2	6	12	8	15.7	6	12	9	18	6	12.0	70	17
Helminths	13	25.5	11	19	11	21.2	6	12	8	15.7	6	12	9	18	6	12.0	70	17
Total	46	90.2	43	74.1	52	100	30	60	42	82.4	31	62	39	78	34	66.7	317	76.9

n = number of studied; IC = Confidence interval.

Table 5. Comparation of parasitic contamination between fruits, vegetables and leafy greens.

Parasites	Fruit			Vegetables + Greens leafy		
	n=516	%	IC	n=900	%	IC
Blastocystis sp.	193	37.4	(33.2-41.6)	282	31.3	(28.3-34.3)
Entamoeba spp.	29	5.6	(3.6-7.6)	117	13	(10.8-15.2)
E. coli	9	1.7	(0.6-2.9)	14	1.6	(0.8-2.4)
E. hartmanni	2	0.4	(0-0.9)	3	0.3	(0-0.7)
Endolimax nana	31	6	(4.0-8.1)	24	2.7	(1.6-3.8)
Iodamoeba buetschlii	2	0.4	(0-0.9)	3	0.3	(0-0.7)
Giardia spp.	26	5	(3.2-7.0)	92	10.2	(8.2-12.2)
Chilomastix spp.	6	1.2	(0.3-2.1)	11	1.2	(0.5-1.9)
Retortamonas spp.	0	0	0	1	0.1	(0-0.3)
Enteromonas spp.	3	0.6	(0-1.3)	1	0.1	(0-0.3)
Cryptosporidium spp.	39	7.6	(5.3-9.9)	54	6	(4.4-7.6)
Cyclospora spp.	31	6	(4.0-8.1)	32	3.6	(2.4-4.8)
Cystoisospora spp.	3	0.6	(0-1.2)	4	0.4	(0-.80)
Eimeria spp.	112	21.7	(18.1-25.3)	261	29	(26.0-32.0)
Balantidium spp.	5	1	(0.1-1.8)	92	10.2	(8.2-12.2)
Protozoa	220	42.6	(38.4-46.9)	483	53.7	(50.4-57.0)
Ascaris spp.	0	0	0	5	0.6	(0.1-1.1)
Strongylida	32	6.2	(4.1-8.3)	185	20.6	(18.2-23.6)
Helminths	32	6.2	(4.1-8.3)	188	20.9	(18.2-23.6)
Total	250	48.4	(44.1-52.7)	648	72	(69.1-74.9)

n = number of studied; IC = Confidence interval.

Regarding the parasitic contamination of leafy greens, parasites were found in almost each specimen analyzed of cabbage (100%), alfalfa (90.2%) and parsley (82.4%). Cabbage had high contamination with *Eimeria* (53.8%) (*P*<0.0001) and with *Endolimax nana* (13.5%) (*P*=0.0002), whereas lettuce was mainly contaminated with *Entamoeba* spp. (36.2%) (*P*<0.0001), and parsley with *Blastocystis* (56.9%) (*P*=0.0071) (Table 4).

The comparative analysis of parasitic contamination rates (Table 5) showed higher parasites percentages in vegetables + leafy greens: total (72%) (*P*<0.0001), protozoa (53.7%) (*P*<0.0001) and helminths (20.9%) (*P*<0.0001). A higher prevalence of *Eimeria* (29%) (*P*=0.0027), *Entamoeba* spp. (13%) (*P*<0.0001), *Giardia* (10.2%) (*P*=0.0007), and *Balantidium* (10.2%) (*P*<0.0001) was found respect to the fruits. In contrast, higher percentages of *Blastocystis* (37.4%) (*P*=0.0199) and *Cyclospora* (6%) (*P*=0.0313) were found in fruits respect to vegetables.

Finally, when parasitic contamination was compared between leafy greens (76.9%) and vegetables (67.8%), a statistically significant difference was found (*P* =0.0024) (see Table 6), including the highest contamination of leafy greens with

Table 6. Comparation of parasitic contamination between vegetables and leafy greens.

Parasites	Vegetables			Leafy Greens		
	Total			Total		
	n=488	%	IC	n=412	%	IC
Blastocystis sp.	134	27.5	(23.5-31.4)	148	35.9	(31.3-40.6)
Entamoeba spp.	48	9.8	(7.2-12.5)	69	16.8	(13.1-20.4)
E. coli	2	0.4	(0-1)	1	0.2	(0-0.7)

Table 6. *Continued*

Parasites	Vegetables			Leafy Greens		
	Total			Total		
	n=488	%	IC	n=412	%	IC
E. hartmanni	11	2.3	(0.9-3.6)	3	0.7	(0-1.6)
Endolimax nana	2	0.4	(0-1)	1	0.2	(0-0.7)
Iodamoeba buetschlii	9	1.8	(0.7-3.0	15	3.6	(1.8-5.5)
Giardia spp.	40	8.2	(5.8-10.6)	52	12.6	(9.4-15.8)
Chilomastix spp.	8	1.6	(0.5-2.8)	3	0.7	(0-1.6)
Retortamonas spp.	1	0.2	(0-0.6)	0	0	(0-0)
Enteromonas spp.	0	0	(0-0)	1	0.2	(0-0.7)
Cryptosporidium spp.	30	6.2	(4.0-8.3)	24	5.8	(3.6-8.1)
Cyclospora spp.	20	4.1	(2.3-5.9)	12	2.9	(1.3-4.5)
Cystoisospora spp.	2	0.4	(0-1)	2	0.5	(0-1.2)
Eimeria spp.	123	25.2	(21.4-29.1)	138	33.5	(28.9-38.1)
Balantidium spp.	30	6.2	(4.0-8.3)	62	15.1	(11.6-18.5)
Protozoa	230	47.1	(42.7-51.6)	253	61.4	(56.7-66.1)
Ascaris spp.	3	0.6	(0-1.3)	2	0.5	(0-1.2)
Strongylida	115	23.6	(20.4-28)	70	17	(13.4-20.6)
Helminths	118	24.2	(20.4-28)	70	17	(13.4-20.6)
Total	331	67.8	(63.7-72)	317	76.9	(72.8-81.0)

n = number of studied; IC = Confidence interval.

Blastocystis (35.9%) (P=0.0064), *Eimeria* (33.5%) (P=0.0063), *Balantidium* (15.1%) (P<0.0001), *Entamoeba* spp. (16.8%) (P=0.0021) and *Giardia* (12.6%) (P=0.0290). In contrast, vegetables were found to be more contaminated by helminths than leafy greens (24.2%) (P=0.0082), mainly represented by Strongylida (23.6%) (P=0.0150).

Discussion

This study uncovers significant parasitic contamination in fruits (48.4%), vegetables (67.8%), and leafy greens (76.9%), from San Andrés a principal agricultural hub in the Ecuadorian Andes, attributed to poor hygiene practices in agriculture. The detection of multiple enteric parasites in these foods highlight the potential risk of transmitting infections if consumed without adequate sanitation. The local, national and international distribution of these foods, amplifies the risk of disseminating parasites to non-endemic regions, thereby increasing the likelihood of disease outbreaks as it was shown in studies on leafy greens and berries (Tefera et al., 2018; Marques et al., 2020; Barlaam et al., 2021, 2022; Faria et al., 2023).

Direct contact with human and animal excrements is a potential source of contamination of anthroponotic and zoonotic parasites for vegetables. It is also possible that free-living parasites (Strongylida) contaminate the crop products, being considered an insignificant finding in comparison with parasite prevalence's reaching 97.3%. in humans (González et al., 2022) and 90.3% in animals (González et al., 2021).

When comparing the results of vegetable contamination from the San Andres capital, with an overall prevalence of 63.4%, (fruits 48.4% and vegetables 67.8%), was lower than the detected in provinces located at high altitudes and more indigenous populated with overall prevalence of 70.6% (fruits 67.1% and vegetables 73.6%) (González-Ramírez et al., 2022). Urban area used to have access to better methods of sanitation, cleaner restrooms with proper septic tanks, drinking water, and overall, more preventive education and information on food handling than rural areas. This could explain why central town of San Andres showed lower percent of parasitic contamination in their vegetable products when compared to the contamination rate determined in products from rural provinces located at high altitudes (63.4% *vs* 70.6%) (González-Ramírez et al., 2022).

In the present study, leafy greens were more contaminated (76.9%) than vegetables and fruits, probably since these maintain contact with the soil and organic fertilizers from the beginning as seedlings until they are fully grown, and external leaves allow protection for internal plant parts in contact with contaminated soil. The greater parasitic

contamination of leafy greens has been explained by the irregularities of their leaves and the roughness of their surface that allows the adhesion of infectious parasitic forms that persist in the environment (Tefera et al., 2018; Temesgen et al., 2022; Falcone et al., 2023).

Vegetables were the second most contaminated products after leafy greens, surpassing fruits, which is explained by the greater contact they maintain with the soil. The rooted vegetables (tubercle) were found to be highly parasitized by nematodes (24.3%), possibly because they grew under the ground. Noteworthy, onions (54.9%), carrots (13.2%) and radishes (24.6%) are frequently consumed raw and can function as efficient vehicles for parasites. Evidence of these tubers exhibit significant rates of parasitic contamination has been previously reported elsewhere (Puig-Peña et al. 2013; Yahia et al. 2023).

Fruits growing on trees or bushes were found less contaminated than creeping fruits. It is possible that these fruits have been in direct contact with the irrigation water (Esteban et al., 2002; González-Ramírez et al., 2020), organic fertilizers and the soil (Dixon, 2016; Barlaam 2021, 2022; Falcone et al., 2023). However, the roughness of its surface is also a condition that can also influence the contamination of blackberry and peach (Tefera et al., 2018), the texture of its surface allows the adhesion of parasites dispersed by wind, insects or farmers' hands (Dixon, 2016; Machado-Moreira et al. 2019).

Animal feces are a nutrient-rich fertilizer for agricultural systems and offer a low-cost solution (Daniels et al., 2016). However, without prior treatment (composting, storage, chemical treatment, drying, fermentation), it is a vehicle for microorganisms (Amissah-Reynolds et al., 2020). This risk factor was identified in the agricultural practice of San Andrés. (González-Ramírez et al., 2021), suboptimal crop management practices, including open defecation near crops without handwashing by farmers due to a lack of portable toilets, irrigation of crops with contaminated water and persistent unsatisfactory sanitary conditions in the areas where they sell their products (González-Ramírez et al., 2021, 2022).

Contaminated water from canals and wells (Esteban et al., 2002; González-Ramírez et al., 2020), spreads parasites and carries a high health risk, when is utilized for crop irrigation, supply animals, dilution of fertilizers and fungicides, washing machinery, equipment, and utensils work (Dixon 2016). Rain and sprinkler irrigation transport microorganisms from soil to plants when drops splash (Efstratiou et al., 2017). Besides, the wind lifts particles of dust from the ground that aid adherence of parasitic to the vegetables of trees or shrubs (Machado-Moreira et al., 2019), which explains the finding of Strongylida on the woolly surface of peaches.

Insects and animals' action, must be considered (González-Ramírez et al., 2021, 2022). However, the greatest influence is exerted by the agricultural activities carried out by farmers, when handle vegetables without hygienic measures, during planting, harvesting, transporting, storage, and washing (Dixon, 2016; Machado-Moreira et al., 2019). Parasitic contamination of vegetables harvested in this area could be one of the causes of the high prevalence in humans (98.2%), without ruling out the action of water contamination (57-100%), mechanical vectors (52.7%), and animals (90.3%) in these communities (González-Ramírez et al., 2020, 2021, 2022).

After evaluating the crop contamination in the area, we warn about the need to sanitize the products before consuming them raw, because the contamination detected in this Andean area can also occur in other countries where producers do not apply hygienic measures (Falcone et al., 2023). These results suggest the need to integrate parasites to the list of contaminants that are managed in the microbiological criteria required by the Ecuadorian Technical Standard (INEN, 2016). Monitoring only Escherichia coli in vegetables is not a good indicator to guarantee food safety, due low infectious doses of parasites constitute a risk (Barlaam et al., 2022).

It is advisable to consider the potential effects of productive activities on food security; these include identifying and minimizing contamination of soil, water, or any other agent used in production, and monitoring animal health so that it does not represent threats (Tefera et al., 2018). Authorities must develop mitigation plans that involve hygiene education programs for producers and consumers. In addition, facilitate the implementation of more advanced technological procedures to improve the diagnosis of microorganisms in laboratories, as well as field routines to improve the quality and safety of these foods in accordance with standards (Temesgen et al., 2022).

In developing countries, where molecular analyzes cannot be done, due to their high cost and the difficulty in permission to transport samples to a molecular laboratory. The sedimentation technique, staining, and micrometric measurement allow the identification of parasites at low cost (it being essential that analysts are trained). We are aware of the importance of determining parasitic species by molecular methods, for epidemiological control and we recognize, the limitation of the

present study in which the analyzes were carried out by microscopic diagnosis, although insufficient for specific identification, was relevant due to the percentage of parasite genera detected.

The prevalence of our study (63.4%) has been one of the highest, when compared to those described in Ethiopia 54.4% (Bekele et al., 2017); Brazil 50.9% (Luz et al. 2017); Ghana 57.5% (Kudah et al., 2018); Thailand 35.1% (Punsawad et al., 2019); Syria 34.4% (Al Nahhas and Aboualchamat, 2020); Peru 45.3% (Lucas et al., 2023); and Argentina 58.6% (Falcone et al., 2023). The parasite detected are similar to those reported in Andean area of Peru (Pérez-Cordón et al.,2008), with a greater number of protozoa than helminths.

Our results differ from those obtained in Brazil (Honório Santos et al., 2019), with prevalence of 70% in fruits: guava (90%), lemon and apple (70%) and grape (50%). The highest prevalence in this study was of the helminths *A. lumbricoides*, Ancylostomids, *Taenia* spp., and *E. vermicularis*, followed by *B. coli* and *E. coli.* These differences might be due to the high altitude of San Andrés (3,020–6,310 m above sea) could affect the evolution of soil-transmitted helminths due to the extreme environmental conditions such as low temperatures (0–19 °C), intense solar radiation and low rainfall (250 and 500 mm/year). In addition, these conditions affect the soil composition which is constituted by very thin layers of lithic materials of volcanic origin (González-Ramírez et al., 2022). Effect of altitude on helminths has been reported elsewhere (Chammartin et al., 2013).

Interestingly, in San Andrés, there were significant differences between contamination in leafy green types, which is consistent with the results that indicate highest-contaminated in lettuce, reaching rates of 83% Bolivia (Rodríguez et al., 2015); 54.2% Ghana (Kudah et al., 2018); 29.5% Syria (Al Nahhas and Aboualchamat, 2020); 80% Brazil (De Farias et al., 2021); 82.3% Ecuador (Bracho-Mora et al., 2022); 64.7% Argentina (Falcone et al., 2023); 23.8% Portugal (Faria et al., 2023).

Food-borne transmission of parasites is an emerging issue in countries around the world, although, verifying the transmission of parasites through food is not easy, there is a report from the Center for Science in the Public Interest in the United States, which found that, between 2004 and 2013, the consumption of fresh produce was associated with a total of 193,754 illnesses across 9,626 outbreaks. Of the total number of reported outbreaks, the U.S. Centers for Disease Control and Prevention were able to identify both the food source and the contaminant in fewer than 40 percent (CSPI, 2015).

Warning about contamination of unpasteurised apple juice, onions, salads, lettuce, basil, sandwiches, fruit salads, and raspberries with *Giardia*, *Cryptosporidium*, and *Cyclospora* (Dixon, 2016). Outbreaks, associated with the consumption of berries, specifically noting *Cyclospora* and *Trypanosoma*, this latter one associated with the consumption of açaí berries or their beverages (Tefera et al., 2018). Barlaam et al. (2021, 2022) confirm the contamination of produces exported from endemic to non-endemic countries by detecting *C. cayetanensis*, *E. histolytica*, and *Cryptosporidium* in berries imported to Italy from Peru, indicating a serious risk from contaminated produce.

Detection of foodborne parasites in produced has been reported in Latin American countries using the spontaneous sedimentation technique and optical microscopy. Contamination rates include: 77.78% vegetables in Venezuela (Cazorla-Perfetti et al., 2013), 83% lettuces in Bolivia (Rodríguez et al., 2015), 100% lettuces in Colombia (Polo et al., 2016), 50.9% vegetables in Brazil (Luz et al., 2017), 56.7% vegetables in Peru (Benites Salcedo et al., 2019), 82.3% lettuces in Ecuador (Bracho-Mora et al., 2022), 70.6% fruits-vegetables in Ecuador (González-Ramírez et al., 2022), 45.3% lettuces in Peru (Lucas et al., 2023), and 58.6% leafy vegetables in Argentina (Falcone et al., 2023).

In Europe, studies using molecular techniques have reported lower prevalences of parasites in fresh produce compared to Latin America. In Italia, Caradonna et al. (2017) detected *G. duodenalis* (0.6%), *T. gondii* (0.8%), *Cryptosporidium* spp. (0.9%), *C. cayetanensis* (1.3%), *B. hominis* (0.5%), and *D. fragilis* (0.2%) to overall contamination of 4.2% in salads. Barlaam et al. (2021, 2022) identified *G. duodenalis* (4.6%), *Entamoeba histolytica* (1%), and *Cryptosporidium* spp. (5.1%) in berries and salad. *E. multilocularis* (1.39%) in salad. Temesgen et al. (2022) identified *T. gondii* (2.9%), *C. cayetanensis* (6.6%), and *Cryptosporidium* spp. (8.3%) in berries.

On the contrary, in Spain, Trelis et al. (2022) demonstrated higher levels of contamination in green leafy vegetables, with *G. duodenalis* (23.3%) and *Cryptosporidium* spp. (7.8%), marketed in the city of Valencia. In the same city, Moreno-Mesonero et al. (2023) identified a greater variety of species than Trelis et al., in leafy greens and strawberries: *Acanthamoeba* (65.5%), *T. gondii* (37.2%), *Vermamoeba vermiformis* (17.3%), *C. cayetanensis* (12.7%), *Cryptosporidium* spp. (6.8%), *Blastocystis* sp. (1.8%), and *Giardia* sp. (1.7%). Similarly, in Portugal, Marques et al. (2020) have documented a contamination rate of 40% in fruits and vegetables with *T. gondii.*

When comparing findings from agricultural products from industrialized nations with our study in Ecuador, we obtained a higher prevalence and diversity of human and veterinary parasitic species. For example, in Italy (Barlaam et al. 2021, 2022), Spain (Trelis et al., 2022); Portugal (Marques et al., 2020), Norway (Temesgen et al., 2022) and Sweden (Ahlinder et al., 2022), coccidia were mainly identified. Notably, species prioritized in Europe such as *Echinococcus*, *T. gondii*, *Toxocara,* and *Fasciola* were not detected in our research (Bouwknegt et al., 2018). However, European studies used to be done on fruits and vegetables from supermarkets, which are pre-washed or disinfected prior to sale, in contrast to our agricultural products directly obtained from farmers' fields. This is a factor that likely influences the observed prevalence rates.

The place sampling was identified as critical nodes for contamination (Lucas et al., 2023), this is linked to suboptimal crop management practices, including open defecation, the absence of handwashing due to a lack of portable toilets in the fields; and the use of fresh animal excrement as fertilizer (Amissah-Reynolds et al., 2020). Farmers neglect to sanitize work tools like shovels, picks, rakes, and wheelbarrows, facilitating the transfer of parasites between different crops. Furthermore, unsatisfactory sanitary conditions persist in the areas where they sell their products (González-Ramírez et al., 2022).

In Latin America there are the highest records of contamination in vegetables (Cazorla-Perfetti et al., 2013; Rodríguez et al., 2015; Polo et al., 2016; Luz et al., 2017; Benites Salcedo et al., 2019; Bracho-Mora et al., 2022; González-Ramírez et al., 2022; Lucas et al., 2023; Falcone et al., 2023), being countries endemic for parasites, from there they spread to other countries nonendemic, through fresh vegetables. Developing countries have not been able to control their parasites due to low socioeconomic and hygienic levels, and the inability to offer adequate health and education infrastructure that can change people's habits and prevent environmental pollution.

The implementation of control measures in fresh produce preharvest and postharvest, as well as an adequate sanitary hygienic level of the producer, handler, and consumer, will be crucial to minimize the food transmission of protozoa and helminths. To control parasites at the time of cultivation and harvest, irrigation with properly treated water, monitoring the health and hygiene of agricultural workers, improving agricultural sanitation, and restricting access of livestock and other animals to crops and surface water bodies (building adequate drinking troughs) are needed. Additionally, proper construction and maintenance of septic tanks is important to prevent contamination by overflow (Tefera et al., 2018).

Unsafe agricultural practices are used very commonly by small farmers mainly in developing countries. To mitigate this problem, it is necessary to use treated water for irrigation, washing fresh produce, washing hands, and equipment. Good hygienic practices by farm workers involved in the cultivation, harvesting, and handling of produce are another important means of reducing the likelihood of contamination in endemic regions (Trelis et al., 2022), to ensure the safety of products from Latin American countries, and are not excluded from international markets, when implementing import restrictions from endemic countries, as suggested (Barlaam et al., 2021).

The recommendation is to impart hygienic practices through health education targeting farmers, traders, and consumers (Tefera et al., 2018; Trelis et al., 2022; Falcone et al., 2023). If programs are executed to guarantee sanitary control in the farms and the objectives of food security are achieved in production, exports would increase, translating to an increase in the economic income of the producing countries.

Conclusion

This study has highlighted significant parasitic contamination (63.4%), so much in fruits (48.4%), vegetables (67.8%), and leafy greens (76.9%), underscoring the potential health risks associated with the consumption of these products in their raw form without adequate hygiene practices. It illustrates how such products can become vehicles for the transmission of enteroparasites to both humans and animals, regardless of whether the area is endemic or non-endemic, where these items are distributed. Consequently, this research underscores the imperative for stringent hygienic protocols in the cultivation and harvesting phases. Effective mitigation strategies include the treatment of soil, manure, and irrigation water utilised in the agricultural process, alongside the enforcement of thorough disinfection practices prior to consumption.

Data availability

Underlying data

Figshare: Parasitic contamination of fruits, vegetables and leafy greens harvested in an Andean agricultural area, https://doi.org/10.6084/m9.figshare.22313335.v2 (González-Ramírez *et al.*, 2023).

This project contains the following underlying data:

- Data parasites fruits vegetables Ecuador.xlsx

Acknowledgements

The authors give thanks to Universidad Nacional de Chimborazo by the for approval the Project (Diagnóstico de factores de riesgo asociados a enteroparasitosis, en población de 4 a 99 años, procedentes de la parroquia San Andrés, Guano, Chimborazo-Ecuador, periodo 2021-2023). Thanks to all farmers for their collaboration in providing vegetables samples from their farms.

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Open Peer Review

Current Peer Review Status: ✔ ✔ ✔ ✔

Version 2

Reviewer Report 13 April 2024

https://doi.org/10.5256/f1000research.163126.r254884

✔ **Zulbey Rivero**
Chair of Parasitology, Universidad Tecnica de Manabi, Portoviejo, Manabí Province, Ecuador

I agree with the corrections made.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 12 March 2024

https://doi.org/10.5256/f1000research.163126.r254887

✔ **Alessandra Barlaam**
Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia, Italy

The article has been significantly improved, therefore, in my opinion it can be accepted for approved in the current form.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology; parasitic diseases; zoonoses; foodborne parasites; food safety;

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 30 January 2024

https://doi.org/10.5256/f1000research.145917.r228641

?

Zulbey Rivero
[1] Chair of Parasitology, Universidad Tecnica de Manabi, Portoviejo, Manabí Province, Ecuador
[2] Chair of Parasitology, Universidad Tecnica de Manabi, Portoviejo, Manabí Province, Ecuador

The article meets the objective proposed from the beginning and the introduction is brief but clear enough. The methodology is well described, but there is something that needs to be clarified and about which I am asking the authors in the comments placed as stickers in the PDF file. The most important findings are highlighted in the results, however it would be interesting if the authors could report which morphotypes of Blastocystis sp were detected in the samples evaluated. In addition, they must indicate if samples were found that showed contamination by more than one parasitic species in the vegetable or fruit analyzed. The discussion is fine, but depending on what you answer regarding polyparasitism and Blastocystis, you may have to add some of this to the discussion.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, Diagnostic Techniques

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Feb 2024

LUISA CAROLINA GONZALEZ

Revisor 4

We thank the reviewer for the importance has given to our research, the time spent correcting the manuscript, their knowledge, experience, and willingness to improve the article.

Comment:

The article meets the objective proposed from the beginning and the introduction is brief but clear enough. The methodology is well described, but there is something that needs to be clarified and about which I am asking the authors in the comments placed as stickers in the PDF file. The most important findings are highlighted in the results; however it would be interesting if the authors could report which morphotypes of *Blastocystis* sp were detected in the samples evaluated. In addition, they must indicate if samples were found that showed contamination by more than one parasitic species in the vegetable or fruit analyzed. The discussion is fine, but depending on what you answer regarding polyparasitism and *Blastocystis*, you may have to add some of this to the discussion.

Response

Observations made in the text

Investigation design

A field study, cross-sectional, observational and descriptive, was carried out during 1 month of rain / 7 months of drought. The snowball sampling technique was applied, whereby a grower helped locate the nearest farm and so on. All types of products found were included in the sampling (1,416 samples in total); the inclusion criteria were that all agricultural products must come from San Andrés fields and those not cultivated in the community were excluded.

A separatory funnel was not used; it has been a translation error

The reviewer is right. We apologize for the writing mistake. The allusion to a funnel has been deleted (see line 6 paragraph 8)

It would be interesting if the results indicated whether, in some samples, more than one parasitic species was obtained from the studied vegetables, lettuce, or fruit. Something akin to polyparasitism in humans.

This is a very important observation of the reviewer as most samples already presented polyparasitism. We have now described this in our results as well as in the discussion section (please see lines ???? in the new version of the manuscript).

Given that *Blastocystis* was the primary microorganism found, if the authors have the information, it would be important to indicate which morphotype was most frequently observed.
The morphotype of *Blastocystis* identified in the samples is now indicated in the result section (please, see lines 9 and 10)

I assume that leafy vegetables were combined to enable pairwise comparisons. If that is the case, it should be noted that, for statistical reasons, sample groups were combined
The reviewer is right. We have now indicate that some groups were combined for statistical purpose.

Competing Interests: No competing interests were disclosed.

Reviewer Report 24 January 2024

https://doi.org/10.5256/f1000research.145917.r228648

?

Alessandra Barlaam
[1] Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia, Italy
[2] Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia, Italy

The aim of the study was to assess the occurrence of the parasitic contamination of fruits, vegetables and leafy greens grown in Ecuador, one of the most important exporters of fresh vegetables. In total 63.4% of the samples were found positive for a variety of parasites which highlights the need to improve and look into the management of the products from farm to fork.

Abstract:
In the Abstract, Methods: “Each sample were washed” should be replaced with “Each sample was washed”.
Abstract, Results: The English language should be revised as the paragraph is not as clear as it should be. In addition, some repetitions occur (the most contaminated) and the use of , and ; is confusing.
Abstract, Conclusion: Delete “From these crops” given that it is a general concept regardless of the obtained results.

Introduction:
Given the variety of fresh produce included in the study and the number of parasites detected, the introduction is too concise and should be enriched. In addition, the importance of leafy greens as

vehicles of foodborne parasites is emphasized whereas the role vegetables and fruits, including berries (also analyzed in this study and for which relevant and recent publications are available) is completely overlooked.

When discussing import of fresh produce from endemic countries and the spread of parasites to nonendemic ones the authors fail to include relevant and recent bibliography on the subject. There are, in fact, recent studies that show how fresh produce imported from countries endemic for certain parasites have been found contaminated in the importing country. These articles have been completely overlooked and should be included. I recommend:

- Barlaam *et al.* (2021[1]);
- Temesgen *et al.* (2022[2]);
- Barlaam *et al.* (2022[3]).

Methods:
The data are quite old (the samples were collected four years ago) and so is the methodology used for processing the fresh produce. The detection is also not quite in step with the times.
Specifically, in the present study, the occurrence of parasites in fresh produce was investigated by microscopy although it is not clear how the genera and species were verified. Nowadays, with molecular tools available it would be auspicable to use them not only to verify the microscopy results but also to gather more information on the detected parasites (pathogenicity, zoonotic relevance etc.). I would like to ask the authors whether the samples were not tested molecularly because of lack of equipment/resources or for different reasons.

Tables:
In all the tables there is a line that refers to “Protozoa”. I don’t understand why given that the protozoa identified are listed individually. Please clarify.
Abbraviations should be written in full at the bottom of the table (IC with a * that links the abbreviation and the extended form).

Discussions:
The discussions are well organized and the key concepts regarding parasitic contamination of fresh produce are covered.
First paragraph: See comment in the Introduction section about the risk that these products represent for people living in non-endemic areas. Please cite relevant literature.
When discussing contaminated berry products and the surface of such products is discussed, the following paper should be taken into consideration: Tefera *et al.* (2018[4]).
The paragraph starting with “Food-borne transmission of protozoan parasites…” needs to be amended in order to clarify two different concepts: foodborne transmission of protozoan parasites and detection of foodborne parasites into fresh produce. When listing the cases in which parasitic contamination of fresh produce occurred, the authors do not use the most recent bibliography available. They include, in fact, older articles, but they overlook more recent publications on the subject (among others, Barlaam *et al.*, 2021; Barlaam *et al.*, 2022; Temesgen *et al.*, 2022; Marques *et al.* (2020[5]); Faria *et al.* (2023[6]). Please update.

Conclusion:
It may start from three paragraph above (“In these tropical countries,..”) since they are very general concluding paragraphs.

References:
In some parts of the manuscript as stated in the previous comments the references are rather dated. For many subjects the authors write about, in fact, they cite articles that are not among the most relevant and recent on the subject. I recommend doing another bibliographic research and going through the references again.

English:
The use of the English language is generally good, however, some misspellings, inaccuracies and errors in the sentence structure have been spotted throughout the text and a further revision is highly recommended.

Taking everything into account this study has some limitations and the manuscript has some flaws, however, these data shed light on an important matter which is parasitic contamination of fresh produce in developing Countries which play a key role in our economy as exporters. This means that such issue is not limited to the Country in topic but potentially threatening for the rest of the world. For this reason, I think that it's important to share data as limited as they may be on the subject and raise awareness on the issue. Therefore, in my opinion the manuscript can be indexed after the points raised above are clarified and a thorough revision of the manuscript is made according to the revisions above.

References
1. Barlaam A, Temesgen TT, Tysnes KR, Rinaldi L, et al.: Contamination of fresh produce sold on the Italian market with Cyclospora cayetanensis and Echinococcus multilocularis.*Food Microbiol*. 2021; **98**: 103792 PubMed Abstract | Publisher Full Text
2. Temesgen TT, Stigum VM, Robertson LJ: Surveillance of berries sold on the Norwegian market for parasite contamination using molecular methods.*Food Microbiol*. 2022; **104**: 103980 PubMed Abstract | Publisher Full Text
3. Barlaam A, Sannella AR, Ferrari N, Temesgen TT, et al.: Ready-to-eat salads and berry fruits purchased in Italy contaminated by Cryptosporidium spp., Giardia duodenalis, and Entamoeba histolytica.*Int J Food Microbiol*. 2022; **370**: 109634 PubMed Abstract | Publisher Full Text
4. Tefera T, Tysnes KR, Utaaker KS, Robertson LJ: Parasite contamination of berries: Risk, occurrence, and approaches for mitigation.*Food Waterborne Parasitol*. 2018; **10**: 23-38 PubMed Abstract | Publisher Full Text
5. Marques CS, Sousa S, Castro A, da Costa JMC: Detection of Toxoplasma gondii oocysts in fresh vegetables and berry fruits.*Parasit Vectors*. 2020; **13** (1): 180 PubMed Abstract | Publisher Full Text
6. Faria CP, Pereira A, Almeida D, Pinto M, et al.: Molecular investigation of ready-to-eat salads for Giardia duodenalis and Cryptosporidium spp. in Portugal.*Food Waterborne Parasitol*. 2023; **30**: e00190 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology; parasitic diseases; zoonoses; foodborne parasites; food safety;

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Feb 2024

LUISA CAROLINA GONZALEZ

Revisor 3

We thank the reviewer for the importance has given to our research, the time spent correcting the manuscript, and their knowledge, experience, and willingness to improve the article.

Comment 1:
Abstract:
In the Abstract, Methods: “Each sample were washed” should be replaced with “Each sample was washed”.

Response 1:
We have done the change

Comment 2:
Abstract, Results: The English language should be revised as the paragraph is not as clear as it should be. In addition, some repetitions occur (the most contaminated) and the use of, and; is confusing.

Response 2:

The English in the Abstract and Result sections has been revised and improved.

Comment 3:
Abstract, Conclusion: Delete “From these crops” given that it is a general concept regardless of the obtained results.

Response 3:
The phrase has been deleted

Comment 4:
Given the variety of fresh produce included in the study and the number of parasites detected, the introduction is too concise and should be enriched. In addition, the importance of leafy greens as vehicles of foodborne parasites is emphasized whereas the role vegetables and fruits, including berries (also analyzed in this study and for which relevant and recent publications are available) is completely overlooked.

Response 4:
Thank you very much for these observations. The introduction has been enriched as suggested and the role of vegetables and fruits as parasitic foodborne carriers is now highlighted (see paragraphs 4, 5, and 6 in the new paper version).

Comment 5:
When discussing the import of fresh produce from endemic countries and the spread of parasites to nonendemic ones the authors fail to include relevant and recent bibliography on the subject. There are, in fact, recent studies that show how fresh produce imported from countries endemic for certain parasites have been found contaminated in the importing country. These articles have been completely overlooked and should be included. I recommend:

- Barlaam et al. (20211);
- Temesgen et al. (20222);
- Barlaam et al. (20223).

Response 5:
They have been included as suggested (see paragraph 2 new version of paper).

Comment 6:
Methods:
The data are quite old (the samples were collected four years ago) and so is the methodology used for processing the fresh produce. The detection is also not quite in step with the times. Specifically, in the present study, the occurrence of parasites in fresh produce was investigated by microscopy although it is not clear how the genera and species were verified. Nowadays, with molecular tools available it would be auspicable to use them not only to verify the microscopy results but also to gather more information on the detected parasites (pathogenicity, zoonotic relevance etc.). I would like to ask the authors whether the samples were not tested molecularly because of lack of equipment/resources

or for different reasons.

Response 6:

6.1. The data are quite old (the samples were collected four years ago)

The samples were collected a few years ago; however, the hygienic and sanitary practices in the area persist, and the prevalence of infection within the human populations remains constant (98.2%). No educational interventions have been implemented to enhance crop management hygiene. Additionally, indigenous communities do not readily alter their ancestral customs, suggesting that significant changes in these results are unlikely.

6.2. The methodology used for processing the fresh produce are quite old

The washing and sedimentation technique used in our study (Rivero de Rodríguez et al., 1998) has been widely used, even today (Al Nahhas et al., 2020; Devera et al., 2021; Falcone et al., 2023; Lucas et al., 2023; Yahia et al., 2023; Asfaw et al., 2023; El Safadi et al., 2023). These parasitological techniques are very useful in low-resource countries, such as our case in Ecuador, since the addition of coagulant or flocculant reagents would increase the overall cost of the process.

6.3. The detection is also not quite in step with the times

Optical microscopy remains the most used method in laboratories for the diagnosis of coproparasitoscopic. Its limitation has to do with the expertise of the laboratory technician to identify parasitic species. We have extensive experience in this type of diagnosis. On the other hand, we have no other diagnostic option due to the economic and logistical limitations of the area.

6.4. The occurrence of parasites in fresh produce was investigated by microscopy although it is not clear how the genera and species were verified.

The identification of parasitic elements (eggs, larvae, cysts, oocysts) was conducted by an expert in parasitic microscopy, boasting 33 years of experience, utilizing the dimensions of the forms (using an ocular micrometer) and morphological characteristics (WHO, 2019). Given the wide variety of human, animal, and free-living parasites recoverable from plant matter, coupled with the limitations of microscopy for precise species identification, we have reported them generically (e.g., Strongylida, Ascaris spp.) without specifying species.

6.5. Nowadays, with molecular tools available it would be auspicable to use them not only to verify the microscopy results but also to gather more information on the detected parasites (pathogenicity, zoonotic relevance etc.).

Undoubtedly, employing molecular methods (PCR) would yield more precise results and enable molecular epidemiology, contributing to addressing the issue of parasitic contamination. However, as previously mentioned, the application of these techniques was

unfeasible in our study, due to budget constraints. The parasitological findings from this study have unveiled significant insights into the environmental health of crops in the Ecuadorian Andean region.

6.6. I would like to ask the authors whether the samples were not tested molecularly because of lack of equipment/resources or for different reasons.

Molecular techniques were not used by lack of equipment/resources as mentioned above.

Comment 7:
In all the tables there is a line that refers to "Protozoa". I don't understand why given that the protozoa identified are listed individually. Please clarify. Abbraviations should be written in full at the bottom of the table (IC with a * that links the abbreviation and the extended form).

Response 7:

In the protozoa section of the tables, we specify the frequency and percentage of contamination for fruits, vegetables, and leafy greens individually, rather than providing a cumulative count of protozoa. The same was done with the helminth section. This approach allows us to ascertain the prevalence of each category and facilitates meaningful comparisons between the two.

Comment 8:
Discussions:
The discussions are well organized and the key concepts regarding parasitic contamination of fresh produce are covered.

8.1. First paragraph: See comment in the Introduction section about the risk that these products represent for people living in non-endemic areas. Please cite relevant literature. When discussing contaminated berry products and the surface of such products is discussed, the following paper should be taken into consideration: Tefera et al. (2018).

Response 8.1:
Relevant literature, including the reference suggested, have been incorporated. Please, see lines 8 to Tefera et al., 2018; Marques et al., 2020; Barlaam et al., 2021, 2022; Faria et al., 2023, in the new version of the manuscript.

8.2. The paragraph starting with "Food-borne transmission of protozoan parasites..." needs to be amended in order to clarify two different concepts:
foodborne transmission of protozoan parasites and detection of foodborne parasites into fresh produce. When listing the cases in which parasitic contamination of fresh produce occurred, the authors do not use the most recent bibliography available. They include, in fact, older articles, but they overlook more recent publications on the subject (among others, Barlaam et al., 2021; Barlaam et al., 2022; Temesgen et al.,

2022; Marques et al. (2020); Faria et al. (2023). Please update.

Thank you for these observations. The paragraph has been modified in order to avoid confusion with the sense in which the term is used. In addition, the references suggested has been now included (Please, paragraph see 16 new version)

Comment 9:
Conclusion:
It may start from three paragraph above ("In these tropical countries,..") since they are very general concluding paragraphs.

Response 9:
We believe that with our results, we cannot conclude what occurs in other Latin American countries.

Competing Interests: No competing interests were disclosed.

Reviewer Report 24 January 2024

https://doi.org/10.5256/f1000research.145917.r228642

Papa Kofi Amissah-Reynolds
[1] Department of Biological Sciences Education, Faculty of Science Education,College of Agriculture Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana
[2] Department of Biological Sciences Education, Faculty of Science Education,College of Agriculture Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana

Generally, the manuscript is very good and relevant. However, the authors should consider the following:

1. The discussion can be revised as some aspects lack clarity and are difficult to read and/or understand.
2. The conclusion seems not to address the aim of the study. Comment on the occurrence of parasites in the conclusion.
3. Apart from using tables, can the authors consider other ways of presenting the results?

From the discussion:
Paragraph 3 (When constrasting ...)

How does access to the urban area influence parasitic contamination?

Paragraph 8 (Animal faeces ...)
Consider revising this paragraph. It is not easy to understand some aspects of this paragraph.

Paragraph 21 (Our results ...)
How does altitude influence the evolution of soil-transmitted helminths? Can you provide a reference for this?

Paragraph 23 (Food-borne ...)
Link the results from the developed countries you have stated to your work and explain any differences there may be.

Paragraph 24 (Information collected ...)
What were the inadequate handling practices and insanitary conditions at the market?
This article (Duedu *et al.*, 2014[1]) could be useful.

Paragraph 25 (In these tropical ...)
Which tropical countries are you making reference to?

Paragraphs 26 & 27
There are no references in these paragraphs. This article could be relevant to your work (Amissah-Reynolds *et al.*, 2020[2])

References

1. Duedu KO, Yarnie EA, Tetteh-Quarcoo PB, Attah SK, et al.: A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-aired markets in Accra, Ghana.*BMC Res Notes*. 2014; **7**: 836 PubMed Abstract | Publisher Full Text
2. Amissah-Reynolds P, Yar D, Gyamerah I, Apenteng O, et al.: Fresh Vegetables and Ready-to-eat Salads: Sources of Parasitic Zoonoses in Mampong-Ashanti, Ghana. *European Journal of Nutrition & Food Safety*. 2020. 47-55 Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Zoology, Parasitology, Zoonosis, One-Health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 25 Feb 2024

LUISA CAROLINA GONZALEZ

Revisor 2

We thank the reviewer for the importance has given to our research, the time spent correcting the manuscript, their knowledge, experience, and willingness to improve the article.

Generally, the manuscript is very good and relevant. However, the authors should consider the following:

Comment 1:
1. The discussion can be revised as some aspects lack clarity and are difficult to read and/or understand.

Response 1:
The discussion has been revised, incorporating straightforward language and addressing all the reviewer's recommendations.

Comment 2: The conclusion seems not to address the aim of the study. Comment on the occurrence of parasites in the conclusion.

Response 2:
A comment regarding the occurrence of parasites in the samples examined has now been included in the conclusion section, as follows:

This study uncovers significant parasitic contamination in fruits (48.4%), vegetables (67.8%), and leafy greens (76.9%), from San Andrés a principal agricultural hub in the Ecuadorian Andes, attributed to poor hygiene practices in agriculture. The detection of multiple enteric parasites in these foods highlight the potential risk of transmitting infections if consumed without adequate sanitation. The local, national and international distribution of these foods, amplifies the risk of disseminating parasites to non-endemic regions, thereby increasing the likelihood of disease outbreaks as it was shown in studies on leafy greens and berries (Tefera et al., 2018; Marques et al., 2020; Barlaam et al., 2021, 2022; Faria et al., 2023).

When comparing the results of vegetable contamination from the San Andres capital, with an overall prevalence of 63.4%, (fruits 48.4% and vegetables 67.8%), was lower than the detected in provinces located at high altitudes and more indigenous populated with overall prevalence of 70.6% (fruits 67.1% and vegetables 73.6%) (González-Ramírez et al., 2022), this could be explained to the access of better methods of sanitation, cleaner restrooms with proper septic tanks and overall more preventive education and information on food handling (González et al., 2022).

Comment 3: Apart from using tables, can the authors consider other ways of presenting the results?

Response 3:
We value the recommendation to enhance the article's dynamism. While we acknowledge the appeal of diversifying presentation, we have opted for tables due to their capacity to encapsulate detailed information on parasitic contamination, which may not be effectively conveyed through figures alone.

Comment 4: From the discussion: Paragraph 3 (When constrasting ...)
How does access to the urban area influence parasitic contamination?

Response 4:
Urban area used to have access to better methods of sanitation, cleaner restrooms with proper septic tanks, drinking water, and overall more preventive education and information on food handling than rural areas. This could explain why central town of San Andres showed lower percent of parasitic contamination in their vegetable products when compared to the contamination rate determined in products from rural provinces located at high altitudes (63.4% vs 70.6%) (González-Ramírez et al., 2022).

Comment 5: Paragraph 8 (Animal faeces ...)
Consider revising this paragraph. It is not easy to understand some aspects of this paragraph.

Response 5:
The paragraph was revised as suggested and now it can be read as follows:

Animal feces are a nutrient-rich fertilizer for agricultural systems and offer a low-cost solution (Daniel et al., 2016). However, without prior treatment (composting, storage, chemical treatment, drying, fermentation), it is a vehicle for microorganisms (Amissah-Reynolds et al., 2020). This risk factor was identified in the agricultural practice of San Andrés. (González-Ramírez et al., 2021), suboptimal crop management practices, including open defecation near crops without handwashing by farmers due to a lack of portable toilets, irrigation of crops with contaminated water and persistent unsatisfactory sanitary conditions in the areas where they sell their products (González-Ramírez et al., 2021, 2022).

Comment 6: Paragraph 21 (Our results ...)
How does altitude influence the evolution of soil-transmitted helminths? Can you provide a reference for this?
Response 6:
Our results differ from those obtained in Brazil (Honório Santos et al., 2019), with prevalence of 70% in fruits: guava (90%), lemon and apple (70%) and grape (50%). The highest prevalence in this study was of the helminths *A. lumbricoides*, Ancylostomids, *Taenia* spp., and *E. vermicularis*, followed by *B. coli* and *E. coli*. These differences might be due to the high altitude of San Andrés (3,020–6,310 m above sea) could affect the evolution of soil-transmitted helminths due to the extreme environmental conditions such as low temperatures (0–19 °C), intense solar radiation and low rainfall (250 and 500 mm/year). In addition, these conditions affect the soil composition which is constituted by very thin layers of lithic materials of volcanic origin (González-Ramírez et al., 2022). Effect of altitude on helminths has been reported elsewhere (Chammartin et al., 2013)

Comment 7: Paragraph 23 (Food-borne ...)
Link the results from the developed countries you have stated to your work and explain any differences there may be.

Response 7:
When comparing findings from agricultural products from industrialized nations with our study in Ecuador, we obtained a higher prevalence and diversity of human and veterinary parasitic species. For example, in Italy (Barlaam et al. 2021, 2022), Spain (Trelis et al., 2022); Portugal (Marques et al., 2020), Norway (Temesgen et al., 2022) and Sweden (Ahlinder et al., 2022), coccidia were mainly identified. Notably, species prioritized in Europe such as *Echinococcus, T. gondii*, *Toxocara*, and *Fasciola* were not detected in our research (Bouwknegt et al., 2018). However, European studies used to be done on fruits and vegetables from supermarkets, which are pre-washed or disinfected prior to sale, in contrast to our agricultural products directly obtained from farmers' fields. This is a factor that likely influences the observed prevalence rates.

Comment 8: Paragraph 24 (Information collected ...)
What were the inadequate handling practices and insanitary conditions at the market?

Response 8: The clarification has been made.
suboptimal crop management practices, including open defecation near crops without handwashing by farmers due to a lack of portable toilets, irrigation of crops with contaminated water and persistent unsatisfactory sanitary conditions in the areas where they sell their products (González-Ramírez et al., 2021, 2022).

Comment 9:
Paragraph 25 (In these tropical ...)
Which tropical countries are you making reference to?

Response 9:
In Latin America there are the highest records of contamination in vegetables (Cazorla-

Perfetti et al., 2013; Rodríguez et al., 2015; Polo et al., 2016; Luz et al., 2017; Benites Salcedo et al., 2019; Bracho et al., 2022; González-Ramírez et al., 2022; Lucas et al., 2023; Falcone et al., 2023), being countries endemic for parasites, from there they spread to other countries nonendemic, through fresh vegetables. Developing countries have not been able to control their parasites due to low socioeconomic and hygienic levels, and the inability to offer adequate health and education infrastructure that can change people's habits and prevent environmental pollution.

Comment 10:
Paragraphs 26 & 27
There are no references in these paragraphs. This article could be relevant to your work (Amissah-Reynolds et al., 2020)

Response 10:
It has been included.
Animal feces are a nutrient-rich fertilizer for agricultural systems and offer a low-cost solution (Daniel et al., 2016). However, without prior treatment (composting, storage, chemical treatment, drying, fermentation), it is a vehicle for microorganisms (Amissah-Reynolds et al., 2020). This risk factor was identified in the agricultural practice of San Andrés. (González-Ramírez et al., 2021), suboptimal crop management practices, including open defecation near crops without handwashing by farmers due to a lack of portable toilets, irrigation of crops with contaminated water and persistent unsatisfactory sanitary conditions in the areas where they sell their products (González-Ramírez et al., 2021, 2022).

The place sampling was identified as critical nodes for contamination (Lucas et al., 2023), this is linked to suboptimal crop management practices, including open defecation, the absence of handwashing due to a lack of portable toilets in the fields; and the use of fresh animal excrement as fertilizer (Amissah-Reynolds et al., 2020). Farmers neglect to sanitize work tools like shovels, picks, rakes, and wheelbarrows, facilitating the transfer of parasites between different crops. Furthermore, unsatisfactory sanitary conditions persist in the areas where they sell their products (González-Ramírez et al., 2022).

Competing Interests: No competing interests were disclosed.

Reviewer Report 19 December 2023

https://doi.org/10.5256/f1000research.145917.r221529

Samar Al Nahhas
1

Department of Animal Biology, Faculty of Science, Damascus University, Damascus, Syria
[2] Department of Animal Biology, Faculty of Science, Damascus University, Damascus, Syria

This study addresses an important knowledge gap regarding the prevalence of enteric parasites on fruits, vegetables and leafy green in an agricultural area of the Ecuadorian Andes. The study also indicated the necessity of dealing with these materials, which are responsible for infection in humans and animals, by treating the soil as well as the water used in irrigation.

I have previously reviewed this manuscript and gave my decision to accept it.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Parasitology, Protozoa, molecular diagnosis, infectious diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.