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Parasitic Contamination and Microbiological Quality of Commonly Consumed Fresh Vegetables Marketed in Debre Berhan Town, Ethiopia

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ABSTRACT: Parasitic and microbial contamination and the pattern of occurrence of the parasite species depends on weather conditions, socio-cultural conditions, sampling season, analyzed vegetable products, and other factors. Therefore, local assessment of vegetable contamination is crucial for targeted and effective interventions. A cross-sectional study was conducted from February to August 2022. A questionnaire was used to assess factors associated with parasite contamination of vegetables during the marketing period. The selected vegetables were purchased and processed for parasite and microbial analysis using standard methods. Finally, all data were summarized and analyzed using SPSS software version 25. A total of 180 vegetable samples were purchased from 180 vendors. This study identified a total of 129 parasites from 180 vegetable samples, with an overall contamination rate of (75; 41.7%). Both protozoa (41; 31.8%) and helminthes (88; 68.2%) were identified from vegetables. Contamination with more than one parasite species was (38; 21.1%). The kind of produce, finger nail status of vendors/sellers, the medium of the display, the type of market and not washed prior to display were significantly associated with parasite contamination. The results also showed that vegetable microbial load for total heterotrophic count, total coliform count, fecal coliform count, yeast count, and mold count was higher in the afternoon than in the morning. To decrease risks to public health, local health authorities and/or market inspectors should establish and implement strategies to reduce contamination such as encouraging specific display medium and washing of vegetables prior to display.

KEYWORDS: Parasite, microbial load, contamination, vegetable, risk factors, market

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Background

Consumption of unwashed, raw, or unhygienically processed vegetables is a potential source of the spread of various infectious diseases.^{1–6} Parasites and bacteria are one of the biggest public health problems worldwide, especially in tropical and subtropical countries. It is estimated that 3.5 billion people are affected worldwide, 450 million people are infected with food-borne parasites, and about 200 000 people die each year.⁷ Intestinal parasitic infections not only cause morbidity and mortality, but are also associated with iron deficiency anemia, infant growth retardation, and other physical and mental health problems.^{8–10}

Parasitic infective stages can contaminate vegetables from planting to consumption.¹¹ The most important factors in the pre-harvest stage are the use of human and animal manure as natural fertilizers and the irrigation of vegetables with untreated wastewater. Most local farmers in developing countries use untreated human and animal waste as fertilizer and polluted water for irrigation, contributing to increased transmission of intestinal parasites.¹² Hygienic standards for storage, transportation, and marketing conditions, catering and processing for consumption play an important role in the post-harvest stage.^{13,14}

Ethiopia is a country with many intestinal parasitic infections due to lack of clean water and sanitation.¹⁵ As a result, open defecation is expected to contaminate farmland with infectious intestinal parasites. In addition, natural fertilizers (human and animal waste) are widely used by the country's farmers, and irrigation water is often contaminated.¹⁵ All of these factors lead to infective stages of parasites contaminating vegetables, making these foods important vectors of transmission to humans.¹⁶ A recent study in Ethiopia found parasites in 25.1% to 57.8% of vegetable samples collected during the marketing phase.^{15–23} However, contamination and parasite species occurrence depend on weather conditions, sociocultural conditions, sampling season, analyzed vegetable products, and other factors.

The presence of bacteria in the soil can also affect food quality. This is most common in vegetables grown with contaminated irrigation water, as well as in human and animal feces, and grazing areas.²⁴ Pathogenic bacteria do not exist naturally in vegetables and raw foods.²⁵ However, consumption of vegetables is now generally considered a risk factor for the transmission of enteric pathogens.²⁶ Therefore, this study was designed to determine the parasitic and microbial contamination levels of commonly consumed marketed vegetables in Debre Berhan town, Ethiopia.



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Methods

Study design and area

A cross-sectional study was conducted from February to August 2022 in the town of Debre Berhan, 130 km northeast of Addis Ababa. The main economic sectors of the city and surrounding villages are horticulture, agro-industrial processing, urban agriculture and various service industries. Vegetables are readily available in the town's local markets, and most items are eaten raw. At local markets, consumers bought most of their vegetables directly from farmers, traders, or middlemen.

Data and sample collection

A pretested structured closed-ended questionnaire was used to collect data on socio-demographic characteristics, hygiene practices and sanitary conditions of the market. The questionnaire was administered by personal interview and supplemented with direct observation. The selection of vegetables for the current study was supported by data from observational studies at local markets. Six types of vegetables were purchased at the local market: lettuce, cabbage, spinach, carrots, tomatoes, and green peppers. An equal number of samples (30 each, 180 total) were randomly collected from 180 vendors. One type of vegetable sample was purchased from one vendor. Fresh vegetables were purchased from supermarkets and open markets and placed in sterile stomacher bags, appropriately labeled, and transported to the Debre Berhan University Parasitology and Microbiology Laboratory.

Sample processing

Approximately 200 g of each vegetable sample was minced with a sterile knife and cutting board, rinsed by soaking in a beaker containing 500 ml of saline (0.85% NaCl) for 20 minutes, followed by agitation on a shaker for 5 minutes for adequate washing. The samples were then removed from the beaker and the wash solution was transferred to separate test tubes for bacteriological and parasitological analysis. The wash solution used for parasitological analysis was incubated for 1 hour to allow the parasite stages to settle properly. The top physiological saline solution was then discarded carefully without shaking. Finally, the remaining sediment mixture was centrifuged (using gallenkamp angle head centrifuge cat.no cfb 700 0100 hz50) at 2000g for 5 minutes.¹² Then the final sediment was examined for parasite stages.²⁷

Laboratory analysis

Parasite detection. The sediment was mixed and stained and unstained smears were examined for parasites. For unstained smears, a drop of sediment was placed on a fresh clean slide and a coverslip was placed carefully to avoid air bubbles and flooding. The specimens were then examined under a light microscope using (100×) and (400×) magnification.²⁸ Stained

smears were prepared as per the unstained smears but Lugol's iodine solution was added. The zinc sulfate flotation method (specific gravity 1.18) was also used to detect helminth eggs and protozoan cysts.²⁹ Parasite stages were identified based on morphological details described by WHO.³⁰

Yeast and mold counts. Ten-fold serial dilutions of the samples were made in saline and approximately 0.1 ml of the solution was plated on sabouraud dextrose agar. Plates were then incubated at the appropriate temperature and yeast and mold were counted separately. Colonies on duplicate plates containing 30 to 300 colonies were counted and the final bacterial count was expressed in CFU/g.

Total heterotrophic plate count. Ten-fold serial dilutions of the samples were made in saline and 0.1 ml of the solution was spread on nutrient agar plates. Plates were then incubated at 37°C for 48 hours. Colonies on duplicate plates of 30 to 300 colonies were counted and the final bacterial count was expressed in CFU/g.

Total and fecal coliform count. Both were enumerated by multiple tube fermentation tests as described by APHA³¹ by using a set of test tubes containing Durham tubes and MacConkey broth. The first set of 3 tubes will have 10 ml of sterile double strength broth and the second and the third sets will have 10 ml of single strength broth. A concentration of 10, 1, and 0.1 ml quantities of samples were added in the 3 sets of tubes respectively and incubated for 24 to 48 hours at 37°C for total coliform count and for 24 hours at 44.5°C in water bath for fecal coliform count. Tubes showing production of gas and lactose fermentation was taken as positive reaction. Finally, the bacterial load was estimated using the Most Probable Number (MPN) table and the dilution factor. The final bacterial counts were reported as MPN/g.

Quality assurance

Data quality was ensured by prior training of data collectors on study objectives and data collection procedures. Prior to actual work, reagents were verified to function properly and handled according to standards. The quality of prepared media was constantly monitored according to the CLSI standard.³² *Escherichia coli* (ATCC 25922) was used as a quality control organism for bacteriological analysis. All laboratory tests were performed according to standard operating procedures.³⁰

Data entry, management, and analysis

Microbial counts were calculated as colony forming units per gram (CFU/g) and most probable numbers per gram (MPN/g) were converted to log₁₀ values. Data obtained from questionnaire and laboratory procedure were summarized and analyzed by using SPSS version 25 software. A quantitative value (frequency and proportion) was presented using statistical

tables. A one-sample proportion test was also used to assess the significance of the prevalence of vegetable contamination. All explanatory variables associated with outcome variable with $P < .25$ were entered into multivariable logistic regression analysis. The microbial load of vegetables was compared by using "One Way ANOVA." The significant association was identified by AOR, 95% CI, and P -value.

Results

Socio-demographic and hygienic practice of vendors

From a total of 180 vendors, (116; 64.4%) were female and (64; 35.6%) were male. The majority of the sample (108; 60.0%) was obtained from the supermarket. The majority of vendors obtained their produce from middlemen (63; 35.0%), and farmers (60; 33.3%). One hundred thirteen (113; 62.8%) of the vendors' finger nails were trimmed, while (67; 37.2%) were untrimmed. Most vendors (52; 28.8%) had attended college level education. Almost all vendors, (123; 97.6%), wash their produce with pipe water and display it after washing (Table 1).

Overall parasitic contamination of vegetables

Protozoa (41 samples; 31.8%) and helminths (88; 68.2%) were identified as contaminants for vegetables. The cyst of *Giardia lamblia* (24 samples; 18.6%) was the most frequently identified protozoa. The eggs of *Ascaris lumbricoides* (33 samples; 25.6%) was the most frequently identified helminths, followed by *Strongyloides* (24; 18.6%). Other helminths such as hookworm (10; 7.8%), *H. nana* (10; 7.8%), *Taenia* spp. (8; 6.2%), *Trichuris trichiura* (2; 1.6%), and *Enterobius vermicularis* (1; 0.8%) were also identified (Table 2).

Distribution of parasite contamination in vegetables

Among examined vegetables, spinach was the most contaminated (31 samples; 24.0%) followed by cabbage (28 samples; 21.7%); tomato was found to be the least contaminated (14 samples; 10.9%). *Ascaris lumbricoides* was found to be the highest contamination in spinach (9; 27.3%), followed by *Strongyloides* (7; 29.2%). Also, both *Ascaris lumbricoides* and *Strongyloides* (6; 25%) were the major contaminants in cabbage (6 samples each). The results of the one-sample proportion test revealed that all of the parasite detection proportions were statistically significant (Table 3).

Poly-parasitic contamination of vegetables

Of the 180 samples examined, (75; 41.7%) were contaminated with at least one type of parasite. In addition, (37; 20.6%) and (23; 12.8%) vegetables were contaminated with 1 and 2 parasites, respectively. Contamination with multiple parasite species was also observed (Table 4).

Table 1. Socio-demographic characteristics and hygienic practice of vendors of vegetables, Debre Berhan Town, Ethiopia, 2022.

VARIABLES	FREQUENCY (N)	PERCENT (%)
Sex		
Male	64	35.6
Female	116	64.4
Educational level of vendors		
Unable to read and write	39	21.7
Primary education	44	24.4
Secondary education	45	25.0
College and above	52	28.8
Vendors finger nail status		
Trimmed	113	62.8
Untrimmed	67	37.2
Type of vegetable		
Lettuce	30	16.7
Cabbage	30	16.7
Spinach	30	16.7
Carrot	30	16.7
Tomato	30	16.7
Green pepper	30	16.7
Source of produce		
Farmers	60	33.3
Middle men	63	35.0
Merchant	57	31.7
Means of transportations		
By human	47	26.1
By cart	56	31.1
By car	77	42.8
Market type		
Supermarket	108	60.0
Open market	72	40.0
Washed before display		
Yes	126	70.0
No	54	30.0
Water source for washing		
Pipe water	123	97.6
Well water	1	0.8
River water	2	1.6

(Continued)

Table 1. (Continued)

VARIABLES	FREQUENCY (N)	PERCENT (%)
Means of display		
On the floor	64	35.6
In bucket	36	20.0
On the shelf	80	44.4
Sampling time		
Morning (8:30 a.m.)	90	50.0
Afternoon (2:30 p.m.)	90	50.0

Table 2. Prevalence of parasite in commonly consumed vegetables marketed in Debre Berhan Town, Ethiopia, 2022.

ISOLATES	FREQUENCY (N)	PREVALENCE (%)
<i>Entamoeba histolytica/dispar</i>	17	13.0
<i>Giardia lamblia</i>	24	18.6
<i>Ascaris lumbricoides</i>	33	25.6
<i>Enterobius vermicularis</i>	1	0.8
<i>Trichuris trichiura</i>	2	1.6
<i>Strongyloides</i>	24	18.6
Hookworm	10	7.8
<i>Hymenolepis nana</i>	10	7.8
<i>Taenia</i> spp.	8	6.2
Total	129	100

Factors associated with parasite contamination in vegetables

Finger nail status of vendors/sellers was significantly associated with parasitic contamination. Consequently, fruits and vegetables purchased from person who have untrimmed finger nail status were most likely to be contaminated (AOR=1.414; 95% CI: 0.191-0895, $P=.025$) compared to those have trimmed fingernails status. And also, leafy vegetables were at higher risk of being contaminated compared to vegetables having smooth surface or non-leafy (AOR=3.423; 95% CI: 1.084-6.433, $P=.036$) (Table 5).

Microbial contamination level on vegetables

The highest mean count was the total heterotrophic count for all vegetables, followed by total coliform count. However, the lowest mean count was yeast and mold count. Fecal coliforms were also abundantly obtained from all vegetables. The mean

total heterotrophic count and total coliform count was found to be statistically different between different vegetable varieties ($P<.05$). Mean fecal coliform count, yeast count, and mold count did not show statistically significant differences between different vegetables ($P>.05$) (Table 6).

All the mean microbial counts in samples taken in the afternoon were higher than sample taken in the morning. For example, total heterotrophic counts were 5.79 ± 0.76 and 5.78 ± 0.76 for the afternoon and morning samples, respectively. All microbial counts in samples taken in the morning and afternoon were significantly different ($P<.05$) (Table 7).

Discussion

The isolation of medically important intestinal parasites from vegetables suggests that vegetables are potential sources of foodborne illness in humans. Their presence in the vegetables is associated not only with climatic conditions favorable to the existence and spread of parasites, but also with sanitary conditions and hygienic practices that facilitate their transmission.^{25,33} In this study, a total of 129 parasites were identified from vegetables, with an overall contamination rate of (75; 41.7%). This is consistent with results reported in southern Ethiopia^{15,22,34} and elsewhere.¹ On the other hand, it is low compared to some studies in Ethiopia²³ and elsewhere.^{8,35} Discrepancies between this study and previous studies may be due to differences related to geographic and environmental conditions, sample types, methods used, and socioeconomic status. Both protozoa (41; 31.8%) and helminthes (88; 68.2%) were identified as contaminants of vegetables. Similarly, various Ethiopian studies have identified both protozoa and helminthes.^{15-23,35} The occurrence of protozoa and helminthes in this study might be due to lack of clean water, diversity and density of the population, low levels of hygiene, and close contact with infected reservoir animals.

Ascaris lumbricoides (33; 25.6%) was the most common contaminant followed by *Strongyloides* (24; 18.6%). Similarly, previous studies conducted in Ethiopia^{23,35} and abroad^{1,8,11} have reported similar results. This may be due to the cosmopolitan nature of the *Ascaris lumbricoides*, the large number of eggs produced by female parasites, and the strong and resilient nature of the eggs, which allows them to survive in harsh environments. Eggs are known to survive in the absence of oxygen for 2 years at 5°C to 10°C and are immune to desiccation for up to 3 weeks.³⁶ In this study, all stages of *Strongyloides* (Free living adult worms, *Strongyloides* eggs, Rhabditiform larva, and infective filariform larva) were detected. The predominance of *Strongyloides* is similar to similar studies conducted elsewhere.^{12,28,37,38} The highest prevalence of *Strongyloides* might be due to the fact that the parasite has a free living state and does not require a host for its proliferation, in addition to its parasitic mode of life.¹²

Cysts of *Giardia lamblia* 24 (18.6%) were the most commonly identified protozoa, followed by *Entamoeba histolytica/dispar* 17 (13.0%). These results are similar to Ethiopian

Table 3. Prevalence and distribution of parasitic contamination among commonly consumed vegetables marketed in Debre Berhan Town, Ethiopia, 2022.

PARASITE	LETTUCE, N (%)	CABBAGE, N (%)	SPINACH, N (%)	CARROT, N (%)	TOMATO, N (%)	GREEN PEPPER, N (%)	P-VALUE
<i>Entamoeba histolytica</i> (17)	6 (35.3)	2 (11.8)	4 (23.5)	0 (0.0)	5 (29.4)	0 (0.0)	.001*
<i>Giardia lamblia</i> (24)	6 (25.0)	4 (16.7)	5 (20.8)	1 (4.2)	5 (20.8)	3 (12.5)	
<i>Ascaris lumbricoides</i> (33)	4 (12.1)	6 (18.2)	9 (27.3)	7 (21.2)	0 (0.0)	7 (21.2)	
<i>Enterobius vermicularis</i> (1)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
<i>Trichuris trichiura</i> (2)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	
<i>Strongyloides</i> (24)	3 (12.5)	6 (25)	7 (29.2)	2 (8.3)	3 (12.5)	3 (12.5)	
Hookworm (10)	1 (10.0)	3 (30.0)	2 (20.0)	3 (30.0)	0 (0.0)	1 (10.0)	
<i>Hymenolepis nana</i> (10)	1 (10.0)	4 (40.0)	2 (20.0)	1 (10.0)	0 (0.0)	2 (20.0)	
<i>Taenia</i> spp. (8)	2 (25.0)	1 (12.5)	2 (25.0)	1 (12.5)	1 (12.5)	1 (12.5)	
Total (129)	23 (17.8)	28 (21.7)	31 (24.0)	15 (11.6)	14 (10.9)	18 (14.0)	

*Significantly associated value.

Table 4. Prevalence of polyparasitic contamination in commonly consumed vegetables marketed in Debre Berhan Town, Ethiopia, 2022.

TYPE OF VEGETABLE	SAMPLE SIZE	CONTAMINATED NUMBER (%)	ONE, N (%)	TWO, N (%)	THREE, N (%)	FOUR AND ABOVE, N (%)
Lettuce	30	15 (50.0)	9 (30.0)	4 (13.3)	2 (6.7)	0 (0.0)
Cabbage	30	15 (50.0)	6 (20.0)	6 (20.0)	2 (6.7)	1 (3.3)
Spinach	30	17 (56.7)	9 (30.0)	2 (6.7)	6 (20.0)	0 (0.0)
Carrot	30	10 (33.3)	6 (20.0)	3 (10)	1 (3.3)	0 (0.0)
Tomato	30	10 (33.3)	7 (23.3)	2 (6.7)	1 (3.3)	0 (0.0)
Green pepper	30	8 (26.7)	0 (0.0)	6 (20.0)	2 (6.7)	0 (0.0)
Total	180	75 (41.7)	37 (20.6)	23 (12.8)	14 (7.8)	1 (0.6)

studies such as Aksum,¹⁹ Dire Dawa,¹⁷ Nekemte,²⁰ and Tigray.²¹ However, it is higher than studies conducted elsewhere such as Arbaminch,¹⁸ Jimma,¹⁶ Wolkite and Butajira,²³ and Sudan.³⁹ The highest prevalence of cysts of *Giardia lamblia* and *Entamoeba histolytica/dispar* in this study might be due to the use of iodine wet mount that increases the sensitivity of stool microscopy.

In this study, all tested vegetables were contaminated with a variety of parasites. However, spinach (31; 24.0%) was the most contaminated, followed by cabbage (28; 21.7%). On the other hand, tomatoes (14; 10.9%) were found to be the least contaminated vegetables. This was similar to other previous studies in Ethiopia³⁷ and in Egypt.⁶ The difference in contamination of the various vegetables analyzed in this study is that spinach, cabbage and lettuce have large, bumpy surfaces that help parasites to easily attach to the surface, whereas the smooth surface of tomato might hinder the rate of parasitic attachment and contribute to the lower contamination rate. This could indicate

the possibility of high levels of contaminated vegetables, which can lead to many parasitic infections in humans.

The need to understand the factors that contribute to parasitic contamination of vegetables is paramount to improve efforts to prevent and control intestinal parasitosis as a medical and public health problem. In this study, vegetables purchased from people who had untrimmed finger nails were most likely to be contaminated compared with those who had trimmed finger nails. This could be because untrimmed nails accumulate dirt and pests and contaminate anything they touch. Leafy vegetables are also more at risk of contamination than vegetables with smooth surfaces. In addition, vegetables that are not washed prior to display are more likely to become contaminated than vegetables that are washed prior to display. In addition, vegetables displayed in the open market on the floor are more likely to be contaminated than those displayed on the shelf. These results are consistent with a study performed in Jimma¹⁶ and Dire Dawa.¹⁷

Table 5. Factors associated with parasite contamination in commonly consumed vegetables marketed in Debre Berhan Town, Ethiopia, 2022.

VARIABLES		POSITIVE, N (%)	COR (95% CI)	P-VALUE	AOR (95% CI)	P-VALUE
<i>Vendors figure nail status</i>						
Trimmed		36 (31.9)	1			
Untrimmed		39 (58.2)	1.334 (0.179-0.625)	.001*	1.414 (0.191-0895)	.025*
<i>Kinds of produce</i>						
Leafy	Lettuce	15 (50.0)	1.201 (1.006-1.431)	.023*	3.423 (1.084-6.433)	.036*
	Cabbage	15 (50.0)				
	Spinach	17 (56.7)				
Non-leafy	Carrot	10 (33.3)	1			
	Tomato	10 (33.3)				
	Green pepper	8 (26.7)				
<i>Market type</i>						
Supermarket		35 (32.4)	1			
Open market		40 (55.6)	1.388 (0.210-0.716)	.002*	1.152 (1.362-3.666)	.001*
<i>Washed before display</i>						
Yes		43 (34.1)	1			
No		32 (59.3)	1.338 (0.175-0.655)	.001*	1.658 (1.257-1.688)	.000*
<i>Means of display</i>						
On the floor		38 (59.4)	1.67 (1.059-2.318)	.025*	1.798 (1.724-4.465)	.006*
In bucket		17 (47.2)	1.1056 (0.572-1.951)	.862		
On the table/shelf		20 (25.0)	1			

*Significantly associated value.

Table 6. Microbial load in commonly consumed vegetables marketed in Debre Berhan Town, Ethiopia, 2022.

TYPE OF VEGETABLE	MICROBIAL LOAD IN LOG ₁₀ ±SD				
	TOTAL HETEROTROPHIC COUNT	TOTAL COLIFORM COUNT	FECAL COLIFORM COUNT	YEAST COUNT	MOLD COUNT
Lettuce	6.02 ± 0.68	5.68 ± 0.65	4.95 ± 0.70	3.78 ± 0.58	3.64 ± 0.67
Cabbage	6.03 ± 0.75	5.4 ± 0.68	4.74 ± 0.62	3.57 ± 0.46	3.23 ± 0.59
Spinach	6.26 ± 0.73	5.93 ± 0.73	4.89 ± 0.58	3.70 ± 0.56	3.40 ± 0.72
Carrot	4.78 ± 0.35	4.55 ± 0.65	4.00 ± 0.60	2.95 ± 0.49	1.85 ± 0.99
Tomato	5.68 ± 0.69	4.79 ± 0.55	4.31 ± 0.63	2.69 ± 0.42	1.09 ± 0.21
Green Pepper	5.38 ± 0.64	4.85 ± 0.67	4.63 ± 0.66	3.68 ± 0.56	1.77 ± 0.91
P-value	0.046*	0.032*	0.077	0.133	0.888

*Significantly associated value.

A large number of total heterotrophs, total coliforms, fecal coliforms, yeasts, and molds were observed in this study. It can come from various sources, such as from improper handling

practices in pre-harvest, harvest, and post-harvest activities. Evaluation of vegetable management in these marketing areas also showed various conditions such as: improper handling

Table 7. Comparison of microbial load by sampling time in vegetables marketed in Debre Berhan Town, Ethiopia.

MICROBIAL LOAD COUNT (LOG ₁₀ ±SD)	AVERAGE	MORNING	AFTERNOON	P-VALUE
Total heterotrophic count	5.86 ± 0.77	5.78 ± 0.76	5.79 ± 0.76	.013*
Total coliform count	5.47 ± 0.74	5.22 ± 0.71	5.63 ± 0.75	.002*
Fecal coliform count	4.7 ± 0.67	4.57 ± 0.66	4.79 ± 0.68	.000*
Yeast count	3.54 ± 0.55	3.27 ± 0.51	3.72 ± 0.57	.035*
Mold count	2.34 ± 0.36	2.28 ± 0.18	3.41 ± 0.49	.010*

*Significantly associated value.

and hygiene issues contribute to this high microbial count. Mean total heterotrophic counts and total coliform counts were found to be statistically different among different vegetable types ($P < .05$). Similar studies in Ethiopia^{22,40,41} also showed the presence of indicator organisms and pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp., and *Salmonella* spp.

The result showed that vegetable microbial counts of total heterotrophs, total coliforms, fecal coliforms, yeasts, and molds were higher in the afternoon than in the morning. This difference is also statistically significant ($P < .05$). According to this finding, the time of the day of collection had an effect on the microbial count of marketed vegetables, implying that the microbial count was lower in vegetables in the early time of the day and increased as the day progressed. This could be due to poor handling and hygiene issues in the marketing area. For example, in this study the market is crowded by vehicles emitting dust particles, vegetables were improperly transported over long distances and stored in the open, exposing them to various contaminants.

Conclusions and Recommendations

The results of this study demonstrate that contamination of fresh vegetables with parasites, bacteria and fungus is a public health risk. Similarly, some socio-demographic characteristics, as well as hygiene and safety conditions, were found to be significantly related to parasitic contamination of vegetables. According to this study, the microbiological count of vegetables sold in Debre Berhan town is influenced by time of the day. The microbial count of vegetables increases as the day advances, being lower in the morning and higher in the afternoon. Therefore, home consumers should go to the market in the mornings or early in the day to buy vegetables. In general, the results suggest that food safety practices in place by various actors along the food supply chain, from farming practices of farmers to handling practices of food retailers and distributors are generally poor. Food manufacturers, retailers, and distributors should minimize the risk of contamination of fresh vegetables with parasites, bacteria, and fungus to ensure acceptable

quality and safety in the production, transportation, storage, and sale of fresh vegetables. Food safety oversight of regulated parties must be ensured and safe food production and handling must be promoted throughout the entire food production chain.

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Author Contributions

TA and BY: Designed and write the project. TA, DS, TTS, and DG: Performed the experiment. TA, YEA, DS, and DG: Analyzed the data. BY: Supervise the laboratory activity. TA: Wrote the manuscript. All authors have read and approved the manuscripts.

Availability of Data and Materials

The dataset used and or analyzed for this study are available by the corresponding author upon reasonable request.

Consent for Publication

All authors are consented to the publication.

Ethics Approval and Consent to Participate

Ethical approval was obtained by Debre Brehan university Institutional Review Board [protocol number: IRB-003] and official permission was obtained from head department of Debre Brehan Town North Shoa Zonal Office. All participants were informed about the purpose of the study. Finally, written informed consent was obtained from each vegetables handlers and vendors.

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