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Study on parasitic contamination of common edible fruits and vegetables sold in local markets of Tiruchirappalli, South India

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ABSTRACT

Fresh fruits and vegetables provide lot of essential supplements to the human health. Intestinal parasitic infections are acquired in different ways including consumption of contaminated fruits, vegetables and water. While eating unclean, raw or undercooked fruits and vegetables, the transmission of intestinal parasitic infections is propagated. A study aimed at determining the prevalence and predictors of parasitic contamination of fruits and vegetables collected from local markets in Tiruchirapalli, Tamilnadu was conducted between July and August 2019. Out of the 360 samples examined, parasitic contamination was observed in 19.72% of fruits and vegetables and according to parasite type it was 22.22%. *S. stercoralis* was the most frequent contaminant among the parasites observed as well as among the markets where the samples were collected. Tomatoes, carrot and beetroot were the most frequently contaminated fruits and vegetables and Potato and beetroot were contaminated with diverse types of parasite. Thus, this study recommends for random sampling of the marketing fruits and vegetables for parasitic determination and proper and routine health education should be given to the vendors and order to aware about infectious diseases spreading through their products and practice hygienic methods to clean the surface and environmental sanitation.

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1. Introduction

Intestinal parasitic infections are widely disseminated all over the world and pose hazard to the public health and cause various complications. Infections with medically important parasites are closely linked with circumstances such as poverty, overcrowding, unsafe water, lack of sanitation and hygiene, use of contaminated water for washing the fruits and vegetables.^{1,2}

Fruits and vegetables are vital for a healthy human body, as they pose a major constituent of human diet in every family.³ They are the important source of water, essential nutrients, vitamins, minerals, proteins and fibers etc. Consuming fresh fruits and vegetables frequently diminishes the risk of health-related conditions such as stroke, cardiovascular diseases, and it gives protection

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against certain types of cancers and serious diseases.^{4,5}

All over the world, vegetables are eaten raw or cooked lightly to preserve flavor and this practice may very often lead to the food-borne parasitic infections in humans.^{6,7} Fruits and vegetables become a major source of human infections like enteric bacterial, viral and parasitic pathogens by means of contamination of fruits and vegetables during production, collection of the produces, transport, or during processing of fruits and vegetables.^{4,5,8} Moreover, the main sources of contamination often occur from soil which is contaminated, through human and animal excreta, consumption of contaminated water, etc. ^{5,9,10}

Microbial contamination of fruits and vegetables may also occur when fresh fruits and vegetables are rinsed, washed and sprinkled with contaminated water and untreated waste water which pose hazardous effect to the human. Night soil may be used as fertilizer, which contributes vastly to colonize on of fruits and vegetables.^{5,11}

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The consumption of uncleaned raw fruits and vegetables plays a significant epidemiological role in the transmission of various parasitic food borne diseases in many countries. Many outbursts of parasitic infections in humans have been stated due to consumption of raw fruits and vegetables. Preceding studies have shown that many types of fruits and vegetables bought at markets in different areas from many developing countries were contaminated with helminthic eggs as well as protozoan cysts.⁶

To the best of our knowledge, it was found that there is no published document about the level of parasitological contamination of common edible fruits and vegetables sold in local markets of Tiruchirappalli. Hence, this study is carried out to assess the parasitic contamination of fresh fruits and vegetables sold in markets of Tiruchirappalli.

2. Materials and Methods

This is a cross-sectional study conducted in a tertiary care teaching hospital at South India and the samples were collected from six different markets nearby the study location (not exceeding 30kms radius). A total of 360 samples of fruits and vegetables were included and were collected between July and August 2019. This study was conducted after getting approval from institutional ethical committee (Ref: No. 636/ TSRMMCH&RC/ ME-1/ 2019 - IEC No: 011 dated 17.07.2019) and verbal consent was obtained from the vendors. After explaining the objectives of the study to the participants, the details (as per the proforma) were collected from all the recruited 45 vendors in a semi structured questionnaire form for collecting data on factors associated with parasitic contamination of fruits and vegetables such as washed before display or not; freshly collected or stored more than a day; source of water used for washing fruits and vegetables; educational status of the vendors; type of market were recorded.

Among the 360 samples, nine different types of fruits including apple, banana, grapes, guava, mango, orange, sapota, cucumber and tomato and eight different vegetables like potato, carrot, beetroot, spinach, cabbage, radish, brinjal and ladies finger were collected from the above six markets on different days. Equal numbers of samples were collected from these selected markets. The collected whole fruits and vegetables were put into plastic bags, properly labelled with a unique number and date of collection, and brought to the Department of Microbiology for parasitological analysis.

Nearly 200 grams of each fruits or vegetables were soaked (for 15 minutes) in physiological saline, followed by vigorous shaking with the help of a mechanical shaker for of 15minutes. After overnight sedimentation in physiological saline, the sediment was transferred into a centrifuge tube using a sieve, to remove undesirable matter. For concentrating the parasitic stages such as ova, larvae, cysts and oocysts, the centrifuge tube was spun at 3000rpm for five minutes. After centrifugation, the supernatant was decanted carefully without shaking the tube and the sediment was agitated gently by hand to rearrange the parasitic stages. Finally, the sediment was examined by saline and iodine mount under a light microscope using low and high power objectives. Modified Ziehl-Neelsen stained smears were prepared, and examined for the presence of coccidian oocysts such as *Cryptosporidium* sp, *Isospora belli* and *Cyclospora cayetanensis* etc. The data were analyzed by performing descriptive statistics. Chisquare test was used to evaluate the significance of factors associated with the parasitic contamination.

3. Results

All the 360 fruits and vegetables included in this study were subjected to determine the presence of parasites. While performing microbiological examinations, the notable parasitological contaminations were microscopically determined and recorded (Figure 1)

The determination of the parasitic contamination according to fruits and vegetables collected from the study area are visceral and found to be positive with the proportion of 9 and 27% respectively. The surface of banana, sapota and mango are not having any parasitic colonization. The detailed analysis is shown in Table 1.

Table 1: The proportion of parasitic contamination according to fruits and vegetables

Product	Number examined	Number positive
Fruits (n=144)		
Tomato	30	8 (26.6)
Cucumber	15	1 (6.6)
Apple	15	1 (6.6)
Orange	16	1 (6.6)
Guava	15	1 (6.6)
Grapes	15	1 (6.6)
Banana	13	0
Sapota	15	0
Mango	10	0
Vegetable (n=216)		
Carrot	30	16 (53.3)
Beetroot	30	15 (50)
Potato	30	8 (26.6)
Ladies finger	30	6 (20)
Radish	30	5 (16.6)
Brinjal	30	3 (10)
Cabbage	22	3 (13.6)
Spinach	14	2 (14.2)

[Figure in parenthesis denoted percentage]

When the results of parasitic contamination in relation to vegetables (n=216; 60%) and fruits (n=144; 40%) were analyzed, it was observed that a total of 71 (19.72%) were positive for at least one parasite. Among the positive samples, 58 (26.85%) were vegetables and 13 (9.02%)

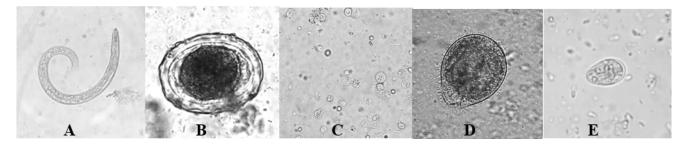


Fig. 1: Parasitic contaminations of fruits and vegetables: [A - Larva of *Strongyloides stercoralis;* B - Egg of *Ascaris lumbricoides;* C - Cyst of *Entamoeba histolytica;* D - Trophozoites of *Balantidium coli* and E - Cyst of *Giardia lamblia*]

were fruits. This gives an overall proportion of parasitic contamination of 19.72% according to the fruit and vegetable types.

Results of parasitic contamination by type of fruits and vegetables showed that among vegetables, carrot (16) and beetroot (15) had the highest parasitic contamination. Among the fruits, tomato (8) had the highest contamination and cucumber, apple, orange, grapes, and guava had the least contamination of each one.

Among the 13 contaminated fruit samples, 10 (77%) were contaminated with one parasite species and 3 (23%) were contaminated with two parasitic species whereas 52 (90%) and 6 (10%) of vegetables (n=58) were contaminated with one parasite and two parasitic species respectively (Figure 2).

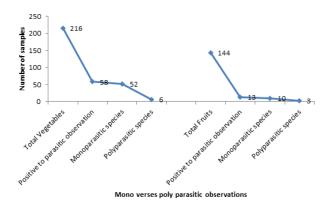


Fig. 2: Determination of mono and poly parasitic observations

The species and stages of parasites detected in the order of frequency were *S. stercoralis* larva (n=34; 9.4%); Cysts of *G. lamblia* (n=22; 6.11%); Cysts of *E. histolytica* (n=13; 3.6%); Trophozoites of *B. coli* (n=10; 2.7%); and Eggs of *A. lumbricoides* (n=1; 0.27%). *S. stercoralis* larva was the most frequently detected parasite followed by Cysts of *G. lamblia* and Cysts of *E. histolytica*. The least common parasite observed was egg of *A. lumbricoides* (0.27%). Proportion of parasitic contamination of 22.22% according to the parasite type was observed (Figure 3). The distribution of parasites in total of both mono and poly numbers were impregnated in the figure thereby the data related to fruits and vegetables were depicted in Figures 4 and 5 respectively.

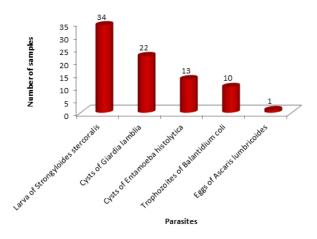


Fig. 3: Proportion of parasitic contamination according to parasite type

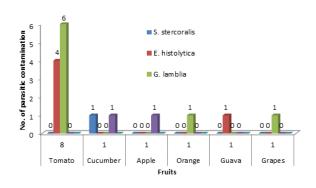


Fig. 4: Distribution of parasitic types identified in fruits

Among the nine fruit and eight vegetable types included in the study, *S. stercoralis* larva (n=34) was detected in one fruit namely cucumber (1/34) and five vegetables

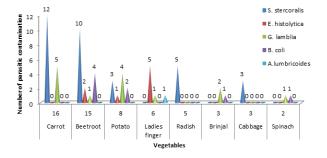


Fig. 5: Distribution of parasitic types identified in vegetables

namely carrot (12/34), potato (3/34), radish (5/34), beetroot (10/34), cabbage (3/34). The cysts of *G. lamblia* (n=22) was abundantly detected in samples of tomato (6/22), orange (1/22), and grapes (1/22), carrot (5/22), potato (4/22), brinjal (2/22), beetroot (1/22), spinach (1/22), ladies finger (1/22). The cysts of *E. histolytica* (n=13) was detected in tomato (4/13), Guava (1/13), potato (1/13), beetroot (2/13), ladies finger (5/13). Trophozoites of *B. coli* (n=10) was detected in cucumber (1/10), and apple (1/10), potato (2/10), brinjal (1/10), beetroot (4/10), spinach (1/10). The egg of *A. lumbricoides* (n=1) was detected in only one vegetable, ladies finger (1/1).

When the results were analyzed in relation to parasites and fruits and vegetables types, it was found that *S. stercoralis* larva was most common among carrots (12/34) followed by beetroot (10/34), and radish (5/34). As observed, cyst of *G. lamblia* was the parasite that contaminated many types of fruits (3/9) and vegetables (6/8). Potato and beetroot showed multi parasitic contamination (4/5).

The samples were collected from six different local markets in Tiruchirappalli. While analyzing the results in relation to the proportion of parasitic contamination of both fruits and vegetables according to markets, it was found that samples collected from market 1 (30.8%) had high contamination rate followed by samples collected from market 2 (20%); market 3 (19.8%); market 4 (18.3%) and market 5 (15.6%). No samples were found to be contaminated from market 6. The proportion of contamination was significantly different among samples collected from the different markets and the data were compared and depicted in Table 2.

When the parasite types were analyzed in relation to market, it was noticed that *S. stercoralis* was the most common parasite found in market 1 (n=12), market 2 (n=8), and market 4 (n=7). On the contrary, *G. lamblia* was the most common in market 5 (n=8) and *B. coli* in market 4 (n=6). It was also observed that *S. stercoralis* and *E. histolytica* were prevalent among 5 out of 6 markets included in this study whereas *G. lamblia* and *B. coli* among 4 out of 6 markets. The type of parasite prevalent according

 Table 2: Proportion of parasitic contamination of both fruits and vegetables according to markets

Market Location	Number of fruits and vegetables screened	Number contaminated	Percentage (%)
Market 1	52	16	30.8
Market 2	50	10	20
Market 3	81	16	19.8
Market 4	93	17	18.3
Market 5	77	12	15.6
Market 6	7	0	0
Total	360	71	19.7

to markets is depicted in Table 3.

When all the samples were examined by modified Ziehl –Neelsen staining to detect cysts of coccidian parasites, it was found to be negative in all the 360 samples. In addition to the parasitological investigations, factors associated with contamination of fruits and vegetables were also assessed. These factors were evaluated by interviewing the vendors of fruits and vegetables in the markets of the study area. The educational status of the vendors was ascertained and the majority (51%) of the vendors had primary education, while 42% of the vendors had secondary education and 7% had no formal education. There was no significant association between education level of vendors and parasitic contamination rate of the produces they were selling (Table 4).

When the results of the factors associated with parasitic contamination of both fruits and vegetables were analyzed in relation to wholesale and retail vendor, it was statistically significantly associated with the retailer. In this study, out of 82% of the products were not washed before display showed 17% rate of contamination. On the contrary, only 31.8% were contaminated among the 18% of the produces that were washed before displaying for sale. Hence, the association between washing and parasitic contamination was statistically significant. It was also noticed that significant percentage of produces handled by bare foot walkers (25.4%) showed a higher rate of contamination. This study didn't show any correlation between the educational status, tobacco use, food habit of the vendor, means of display of fruits and vegetables, whether they were covered or not and kept in closed room or open area.

4. Discussion

The present study attempted to assess the level of contamination and prevalence of different parasites in various fruits and vegetables sold in selected local markets of Tiruchirappalli. In this study, out of total of 360 samples examined for parasitic contamination, 71(19.7%) samples were found to be contaminated. This is in discordance with

Parasite	Market 1	Market 2	Market 3	Market 4	Market 5	Market 6
Strongyloides stercoralis larva	12	8	6	7	1	0
Cysts of G. lamblia	3	0	7	4	8	0
Cysts of <i>E.</i> histolytica	1	2	4	3	3	0
Trophozoites of B. coli	1	0	2	6	1	0
Eggs of A. lumbricoides	0	0	0	0	1	0

Table 3: Type of parasite prevalent according to markets

 Table 4: Sociodemographic details of the vendors and their awareness scale

Variables	Number of fruits and vegetables collected	Parasite Positive (%)	P value	
Locality	5			
Urban	174	33 (19)		
Rural	102	26 (25.5)	0.113558	
Semi urban	84	12 (14.3)		
Food habit of the vendor				
Vegetarian	40	7 (17.5)	0.869805	
Non-vegetarian	320	65 (20)	0.809803	
Usage of footwears*				
Yes	120	10 (8.3)	0.000216	
No	240	61 (25.4)	0.000210	
Usage of Tobacco				
Smoker	42	9 (21.4)		
Non-smoker	293	59 (20.1)	0.808036	
Tobacco chewing	25	3 (12)		
Types of vendor*				
Wholesale	25	10 (40)	0.017269	
Retailer	335	61 (18.2)	0.017209	
Educational status of the ven	dor			
No formal	28	5 (17.9)		
Primary	183	39 (21.3)	0.61199	
Secondary	149	27 (18.1)		
Means of display of fruits and	d vegetables			
On the floor	238	50 (21)	0.473561	
On the table/ shop	122	21 (17.2)	0.475501	
Washed before display*				
Yes	66	21 (31.8)	0.010417	
No	294	50 (17)		
Covering of fruits and vegeta	bles			
Yes	7	2 (28.6)	0.908779	
No	353	69 (19.5)		

[*P value < 0.05; Chi-square test was used to determine the significance between the factors associated with the level of parasitic contamination]

the study at Lahore and Ethiopia which showed the parasitic contamination of 31.2% and 57.8% respectively.^{8,12} A study from Iran showed a lower prevalence of (3.5%) parasitic contamination of herbs.¹³

The discordance between this study and others might be attributed to the variations in geological locations, climatic and environmental circumstances, soil type, rainfall, and alterations in the sample size, the techniques used to detect the parasite, poor handling method and socioeconomic grade. In the present study, *S. stercoralis* (9.4%) was the most common parasite contaminating vegetables followed by *G. lamblia* (6.1%) and *E. histolytica* (3.6%). In concordance with our study, a study from Southwest Ethiopia also reported high prevalence of *S. stercoralis*¹² and also from similar studies conducted elsewhere. 3,10,14,15 This may be due to the fact of free living state of the parasite and mostly for its proliferation it does not require a host, in adding to its parasitic mode of life³ and also the habit of walking with bare foot by the vendors. The present study didn't correlate

with the study from Alexandria, Lahore, southern Ethiopia which observed egg of *A. lumbricoides* as the most frequent parasite.^{6,8,16} And the finding is in contrast with what was reported by other investigators where *Cryptosporidium* spp, *E. histolytica / dispar*, and *Toxocara* spp were the predominant parasites detected.^{17–19}

The present study showed that *S. stercoralis* contamination was most common in carrots (12/34) followed by beetroot (10/34), radish (5/34). This may be due to the rough surface of the fruits and vegetables which helps in adhesion of the parasitic larvae and eggs to its surface when these produces are washed with contaminated water. This correlation may also be due to the fact that these vegetables growing very close to or in the ground may lead to higher risk of contamination with night soil fertilizer. Use of night soils and untreated wastewater is very common within low-income nations which may have led to the prevalence.

In this study, *G. lamblia* was the second most common parasite that affects many types of fruits and vegetables. It causes giardiasis which may be due to contamination of fruits and vegetables by infected faeces, washing with water contaminated by *Giardia*. This parasite lives within intestine and expelled from the body in faeces and its able to survive outside the body for considerable period of time during this period they may contaminate the fruits and vegetables.

The result of this study showed that fruits and vegetables from Siruganur market were highly contaminated compared to the samples collected from other markets. The parasitic contamination rate was significantly different among samples collected from the different markets. The detection of intestinal parasitic stages from these fruits and vegetables is an indicative of the faecal contamination from human or animal by the unhygienic status of the vendors and consumers.¹⁴

As presented in table 2, *G. lamblia* was the parasite that contaminated many types of vegetables (6/8) and fruits (3/9). Potato and beetroot were contaminated with the highest number of parasites (4/5). Contamination with several species was detected in all kinds of fruits and vegetables in this study, which possibly results in multiple parasitic infections in humans. It might also designate the persistence of intestinal parasitic infections in the area.¹⁴

Fruits and vegetables which were directly provided by farmers to whole sale vendors (n=25) were more prone for contamination (n=10; 40%), as compared to those supplied by retailers (n= 335) with the contamination rate of 18.2%. So, farmers must be educated on health risks of using waste water to irrigate fruits and vegetables. This study has the limitations that not to deal the effect of seasonal variation on the contamination of the fruits and vegetables as the study period was only two months and could not highlight the infectivity of the parasitic stages detected as viability study was not conducted. Further, this study recommends including all products from all the vendors from all the

markets for parasitic determination, regular training on personnel hygiene such as hand hygiene as well as general cleanliness will be given to all the vendors of Tiruchirappalli market and bacterial and fungal contamination of fruits and vegetables will also be assessed.

5. Conclusion

The present study reveals that parasitic contamination rate in fruits and vegetables sold at local markets of Tiruchirappalli is significantly considerable. S. stercoralis was the most frequently detected parasite followed by G. lamblia and E. histolytica. G. lamblia was the parasite that contaminated many types of fruits and vegetables. Potato and beetroot were contaminated with different types of parasites. S. stercoralis was the most frequent parasite found in 50% of the markets. Tomatoes, carrot and beetroot and were the most commonly contaminated fruits and vegetables. We recommend to the local public health Department to launch a system which will be useful for continuous monitoring of contamination of fruits and vegetables sold at local markets. The public health department should also advocate to the public to wash the fruits and vegetables adequately with clean water before consumption, so that many diseases can be prevented. Providing health education is important to the vendors for washing fruits and vegetables adequately with clean water and maintain hand hygiene while selling.

6. Institutional Ethical Committee Clearance

Ref: No. 636/ TSRMMCH&RC/ ME-1/ 2019 – IEC No: 011 dated 17.07.2019.

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8. Conflict of Interest

The authors declare no conflict of interest.

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