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# Detection of Medically Important Parasites in Fruits and Vegetables Collected from Local Markets in Dire Dawa, Eastern Ethiopia

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*Objective:* The objective of this study was to determine the prevalence & determinants of medically important parasites in fruits & vegetables collected from local markets in Dire Dawa City, Eastern Ethiopia.

*Methods:* A cross-sectional study involving standardized parasitological techniques and structured questionnaire was used to collect the data from September 14 to October 29, 2015. Eight types of fruits and vegetables (lettuce, cabbage, carrot, tomato, green pepper, banana, orange, and spinach) were collected from nine conveniently selected local markets in Dire Dawa City. Equal numbers of samples (47 each, totally 376 samples) were randomly collected from the selected markets retail fruits and vegetables. The collected data were entered and analyzed using SPSS version 20. Descriptive statistics, bivariate and multivariate logistic regressions were used in the analysis.

*Keywords:* *medically important parasites, fruits and vegetables, local markets, dire dawa, ethiopia.*

*GJMR-K Classification:* *NLMC Code: QX 45*


*Strictly as per the compliance and regulations of:*

# Detection of Medically Important Parasites in Fruits and Vegetables Collected from Local Markets in Dire Dawa, Eastern Ethiopia

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*Results:* Out of the total 376 samples of fruits and vegetables examined, 178 (47.3%) were positive for medically important parasites. The highest rate of parasitic contamination was detected from lettuce, 29 (61.7%) and the least from orange, 12 (25.3%). The medically important Protozoans and Helminths identified were *Gardia lamblia,* 35 (9.3%), *Entamoeba histolytica,* 33 (8.8%), *Strongyloide* spp, 30 (8%), *Cryptosporidium* spp, 29 (7.7%), *Cyclospora* spp, 28 (7.4%), *Ascaris lumbricoides,* 24 (6.4%), *Isospora* spp, 16 (4.3%), *Trichuris trichiura,* 7 (1.9%) and *Hymenolepis* spp, 6 (1.6%). Significantly higher parasitic contamination rate was detected from fruits and vegetables which had not washed before display (AOR: 3.24; 95% CI: 1.54-6.80) and those displayed on a floor (AOR: 5.56; 95% CI: 3.26-9.49).

*Conclusions:* Almost half of fruits and vegetables sold in the local markets of the study area were being contaminated with medically important parasites which are probably potential sources for the transmission of intestinal parasites to humans. Therefore, health education on proper handling and washing of fruits and vegetables should be given to the venders and consumers.

*Keywords: medically important parasites, fruits and vegetables, local markets, dire dawa, ethiopia.*

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## I. Background

Consumption of fruits and vegetables is highly beneficial for maintenance of health and prevention of diseases since they form a major component of healthy diet [1]. Fruits and vegetables are valued mainly for their high carbohydrate, vitamins, minerals, and fiber contents. WHO recommended the intake of a minimum of 400g of fruits and vegetables per day for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries [2]. However, consumption of unwashed, raw and unhygienically prepared vegetables and fruits is considered a risk factor for human parasitic infections [3]. On the other hand, they can act as potential sources for the spread of various infectious parasitic diseases [4].

Intestinal parasites cause significant morbidity and mortality throughout the world, especially in tropical and sub-tropical countries [5]. Besides causing morbidity and mortality, infection with intestinal parasites has known to cause iron deficiency anemia, growth retardation in children and other physical and mental health problems [6, 7]. Globally, it is estimated that 3.5 billion people are affected, and that 450 million are sick from intestinal parasitic infections with an estimated 200,000 deaths annually [8].

Fruits and vegetables may get exposed to parasitic contaminants during pre-harvest (cultivation, irrigation, livestock manure etc.), post-harvest handling-storage, transportation, or while processing for consumption [9, 10]. It has been reported that the use of insufficiently treated wastewater to irrigate vegetables was responsible for the high rates of contamination with pathogenic parasites in many developing countries [10]. Contamination of soil with animal wastes and increased application of improperly composted manures to soil in which vegetables are grown also play a role in parasite contamination to fruits and vegetables [11]. Bad hygienic practice during production, transport,

processing and preparation by handlers including consumers also contribute in vegetable contaminations [12]. Other factors which affect the susceptibility of the public to food-borne diseases also play a role in increasing the number of infected cases. Because of ageing, malnutrition, HIV infection and other underlying medical conditions, highly susceptible persons are markedly increased. Changes in lifestyle and food consumption patterns such as the increase in the number of people eating meals prepared in restaurants, canteens and fast food outlets as well as from street food vendors who do not always respect food safety increase the risk of exposure to food borne infections [13].

The risk of parasitic infections has been reported to be higher among the inhabitants of towns of developing countries like Ethiopia where there is a poor hygienic and sanitation practice [2, 5]. However, there is a little information available on the risks of parasitic infections associated with the consumption of fruits and vegetables in Ethiopia. As these parasites are highly resistant and able to withstand harsh conditions, assessing the sources of infectious agents and their level of contamination is paramount on the prevention and control of medically important parasitic diseases. Therefore, this study was aimed to determine the prevalence and determinants of medically important parasites in fruits & vegetables collected from local markets in Dire Dawa City, Eastern Ethiopia.

## II. Methods and Materials

*a) Study Design and Setting*

A cross-sectional study involving parasitological analysis and structured questionnaire was conducted to determine the level of parasitic contamination of fruits and vegetables sold in selected local markets in Dire Dawa City from September 14 to October 29, 2015.

*b) Sample Size Estimation*

Sample size was determined using a single population proportion formula with assumptions that the overall prevalence of medically important parasites in fruits and vegetables was 57.8% from previous study [14], confidence level 95% and degree of precision 5%. Accordingly, the calculated sample size was 376.

*c) Sampling Techniques and Sample Collection*

First, the study area (Dire Dawa City) was stratified by villages based on their proximity, and nine local markets were selected. Then, the total sample size was distributed proportionally to the size of fruits and vegetables retail in the villages after having sampling frame. Two trained data collectors were recruited for sample collection and interview. Eight types of fruits and vegetables (lettuce, cabbage, carrot, tomato, green pepper, banana, orange, and spinach) were purchased from the selected markets during data collection period. The samples were put in plastic bags, properly labeled, and transported to Microbiology Laboratory of Biology Department of Dire Dawa University for parasitological analysis. Equal number of samples (47 each, totally 376 samples) were randomly collected from the selected markets retail fruits and vegetables. In addition, the fruits and vegetables venders were interviewed regarding their educational status and service factors.

*d) Sample Preparation and Parasitological Examination Procedures*

A portion (200g) of each fruits and vegetables was washed separately in 500ml of normal saline (0.85% NaCl) for detaching the stages (ova, larvae, cysts, and oocysts) of parasites commonly assumed to be associated with contamination.

The washing solution was then allowed to stand on the bench for overnight to allow proper sedimentation. After discarding the supernatant with a Pasteur pipette, 15 ml of the sediment was transferred to a centrifuge tube using a sieve so as to remove undesirable matters. For concentrating the parasitic stages, the tube was centrifuged at 3000 rpm for five minutes [15]. After centrifugation, the supernatant was decanted carefully without shaking. Then, the sediment was agitated gently by hand for redistributing the parasitic stages. Finally, the 100µl sediment was transferred to a clean glass slide covered with cover glass, and examined under a light microscope using ×10 and ×40 objectives.

Modified Zeihl-Neelsen staining technique was used for identification of oocysts of *Cryptosporidium*, *Isospora* and *Cyclospora* species [16]. In this method, a thin smear was prepared directly from the sediment and allowed to air dry. Then, the slides were fixed with methanol for 5 minutes and were stained with carbol fuschin for 30 minutes. Next, the slides were washed with tap water and decolorized with acid alcohol (1ml Hcl and 99 ml of 96% ethanol) for 1-3 minutes. After washing the slides with tap water, they were counterstained with methylene blue for 1 minute. Finally, the slides were washed in tap water and allowed to air dry. The slides, then, were observed under light microscope with x1000 magnification. Each slide was observed for 10 minutes to decide whether it was negative or positive.

## III. Statistical Analysis

The data collected from the questionnaire and the results of the laboratory investigations were cleaned and entered into a computer and statistical analysis was performed using SPSS for windows version 20. Descriptive statistics such as frequency for categorical variables and percentage (prevalence) of fruits and vegetables with different stage of parasites were determined by dividing the total number of positive samples with the total sample size. Bivariate and

multivariate logistic regressions were used to observe the effects of independent variables on the outcome variable while simultaneously controlling for other potential confounding factors. Those variables that emerged from the bivariate analysis as appearing to be statistically significant predictors of status of parasitic contamination at a cut-off point 0.05 were then used as independent variables in multivariate logistic regression. Variables which showed association in multivariate analysis were considered as final predictors of the status of parasitic contamination. The strength of association between different exposure variables and the outcome variable was measured through adjusted odds ratios.

## IV. Results

*a) General characteristics of the examined fruits and vegetables and venders*

A total of 376 fruits and vegetables samples were used in this study. Majority of the venders were females (93.4%) and few of them had no formal education (18.4%). Majority of the fruits and vegetables (85.1%) were not washed before display. About one fourth of the samples were displayed on floor/ground, and 79% samples were collected from open market category (Table 1).

*Table 1:* General information of the examined fruits and vegetables and venders, Dire Dawa, Ethiopia, October to November 2015

| Variables (N=376) | Categories | Frequency | % |
|---|---|---|---|
| Sex of venders | Male | 25 | 6.6 |
| | Female | 251 | 93.4 |
| Educational level of venders | No formal education | 69 | 18.4 |
| | Formal education | 307 | 81.6 |
| Washed before display | Yes | 56 | 14.9 |
| | No | 320 | 85.1 |
| Type of water used for washing (For those washed) | Waste water | 15 | 26.8 |
| | Clean water | 41 | 73.2 |
| Means of display | On floor/ground | 95 | 25.3 |
| | On table/shelf | 281 | 74.7 |
| Market category | Grocery | 79 | 21.0 |
| | Open market | 297 | 79.0 |

*b) Prevalence of Medically Important Parasites in fruits and vegetables*

Out of 376 fruits and vegetable samples examined microscopically, 178 (47.3%) were positive for at least one type of medically important parasites. From 47 samples examined for each items of fruits and vegetables, the highest level of parasitic contamination was detected from lettuce, 29 (61.7%) followed by carrot, 27 (57.4%) and cabbage, 26 (55.3%) and the least was from orange, 12 (25.5%) (Table 2).

*Table 2:* Frequency distribution of parasitic contaminations among fruits and vegetables sold in local markets, Dire Dawa, Ethiopia, September - October 2015

| Types of Fruits & Vegetables | Status | | |
|---|---|---|---|
| | Positive (at least one parasite) | Negative | Total |
| Lettuce | 29 (61.7%) | 18 (38.3%) | 47 |
| Carrot | 27 (57.4%) | 20 (42.6%) | 47 |
| Cabbage | 26 (55.3%) | 21(44.7%) | 47 |
| Spinach | 25 (53.2%) | 22 (46.8%) | 47 |
| Tomato | 23 (48.9%) | 24 (51.1%) | 47 |
| Banana | 19 (40.4%) | 28 (59.6%) | 47 |
| Green paper | 17 (36.2%) | 30 (63.8%) | 47 |
| Orange | 12 (25.5%) | 35 (74.5%) | 47 |
| Total | 178 (47.3%) | 198 (52.7%) | 376 |

*c) Types of Parasites Detected in Fruits and Vegetables*

The types and stages of parasites detected in the samples were oocysts of *Cryptosporidium* spp., *Giardia lamblia* cysts, *E. histolytica/dispar* cysts, larvae of *Strongyloide* spp., oocysts of *Cyclospora* spp., *Ascaris lumbricoides* eggs, oocyst of *Isospora* spp., *Hymenolepis nana* eggs and *Trichuris trichuria* eggs. The most prevalent parasite detected in the fruits and vegetables samples was *G. lamblia*, 35 (9.31%) followed by *E. histolytica/dispar,* 33 (8.78%) and *Cryptosporidium* spp., 29 (7.71%) and the least prevalent parasite was *Hymenolepis nana,* 6 (1.60%) (Table 3).

*Table 3:* Types of medically important parasites detected in fruits and vegetables, Dire Dawa, Ethiopia, September – October 2015

| Type of parasites | | Types of Fruits and Vegetables | | | | | | | | Total (N = 376) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lettuce | Cabbage | Spinach | Carrot | Tomato | Banana | G/Pepper | Orange | |
| *Cryptosporidium* spp | + | 6(12.8%) | 6(12.8%) | 5(10.6%) | 5(10.6%) | 1(2.1%) | 0 | 4(8.5%) | 2(4.3%) | 29(7.7%) |
| | - | 41(87.2%) | 41(87.2%) | 42(89.4%) | 42(89.4%) | 46(97.9%) | 47(100%) | 43(91.5%) | 45(95.7%) | 347(92.3%) |
| *G. lamblia* | + | 11(23.4%) | 5(14.3%) | 4(11.4%) | 5(14.3%) | 3(8.6%) | 2(5.7%) | 1(2.9%) | 4(11.4%) | 35(9.3%) |
| | - | 36(76.6%) | 42(12.3%) | 43(12.6%) | 42(12.3%) | 44(12.9%) | 45(13.2%) | 46(13.5%) | 43(12.6%) | 341(90.7%) |
| *E.histolytica/dispar* | + | 4(12.1%) | 6(12.8%) | 3(9.1%) | 4(12.1%) | 4(12.1%) | 6(12.8%) | 2(6.1%) | 4(12.1%) | 33(8.8%) |
| | - | 43(12.5%) | 41(87.2%) | 44(12.8%) | 43(12.5%) | 43(12.5%) | 41(87.2%) | 45(13.1%) | 43(12.5%) | 343(91.2%) |
| *Strongyloide* spp | + | 3(10.0%) | 4(13.3%) | 5(16.7%) | 5(16.7%) | 2(6.7%) | 6(20.0%) | 4(13.3%) | 1(3.3%) | 30(8.0%) |
| | - | 44(12.7%) | 43(12.4%) | 42(12.1%) | 42(12.1%) | 45(13.0%) | 41(11.8%) | 43(12.4%) | 46(13.3%) | 346(92.0%) |
| *Cyclospora* spp | + | 5(17.9%) | 2(7.1%) | 4(14.3%) | 3(10.7%) | 4(14.3%) | 5(17.9%) | 4(14.3%) | 1(3.6%) | 28(7.4%) |
| | - | 42(12.1%) | 45(12.9%) | 43(12.4%) | 44(12.6%) | 43(12.4%) | 42(12.1%) | 43(12.4%) | 46(13.2%) | 348(92.6%) |
| *A. lumbricoides* | + | 2(8.3%) | 5(20.8%) | 5(20.8%) | 5(20.8%) | 4(16.7%) | 1(4.2%) | 2(8.3%) | 0 | 24(6.4%) |
| | - | 45(12.8%) | 42(11.9%) | 42(11.9%) | 42(11.9%) | 43(12.2%) | 46(13.1%) | 45(12.8%) | 47(13.4%) | 352(93.6%) |
| *Isospora* spp | + | 4(25.0%) | 1(6.2%) | 2(12.5%) | 5(31.2%) | 1(6.2%) | 0 | 0 | 3(18.8%) | 16(4.3%) |
| | - | 43(11.9%) | 46(12.8%) | 45(12.5%) | 42(11.7%) | 46(12.8%) | 47(13.1%) | 47(13.1%) | 44(12.2%) | 360(95.7%) |
| *Hymenolepis* spp | + | 1(16.7%) | 2(33.3%) | 0 | 0 | 2(33.3%) | 0 | 1(16.7%) | 0 | 6(1.6%) |
| | - | 46(12.4%) | 45(12.2%) | 47(12.7%) | 47(12.7%) | 45(12.2%) | 47(12.7%) | 46(12.4%) | 47(12.7%) | 370(98.4%) |
| *T. trichuria* | + | 1(14.3%) | 2(28.6%) | 0 | 0 | 2(28.6%) | 2(28.6%) | 0 | 0 | 7(1.9%) |
| | - | 46(12.5%) | 45(12.2%) | 47(12.7%) | 47(12.7%) | 45(12.2%) | 45(12.2%) | 47(12.7%) | 47(12.7%) | 369(98.1%) |

*d) Bivaraite Analysis*

Crude analysis of variables on binary logistic regression showed that types of fruits and vegetables, washing status of fruits and vegetables and means of display were significantly associated with parasitic contamination at $p<0.2$. On the other hand, sex and educational status of fruits and vegetable venders did not show statistically significant association with parasitic contamination of fruits and vegetables in the bivariate analysis (Table 4).

*Table 4:* Bivariate analysis on binary logistic regression of factors associated with parasitic contamination of fruits and vegetables sold in local markets, Dire Dawa, Ethiopia, September - October 2015

| Variables | | Status of fruits and vegetables | | COR (95% CI) |
|---|---|---|---|---|
| | | Positive | Negative | |
| Sex of vender | Male | 15 | 10 | 1.73 (0.76, 3.96) |
| | Female | 163 | 188 | Ref |
| Educational level of vender | No formal education | 34 | 35 | 1.10 (0.65, 1.85) |
| | Having formal education | 144 | 163 | Ref |
| Types of fruit & vegetables | Lettuce | 29 | 18 | 1.30 (0.57, 2.96) |
| | Cabbage | 26 | 21 | 1.42 (0.62, 3.22) |
| | Spinach | 25 | 22 | 1.19 (0.52, 2.72) |
| | Carrot | 27 | 20 | 1.68 (0.74, 3.82) |
| | Tomato | 23 | 24 | 2.37 (1.04, 5.43)* |
| | Banana | 19 | 28 | 2.84 (1.23, 6.56)* |
| | Green paper | 17 | 30 | 4.70 (1.95, 11.34)* |
| | Orange | 12 | 35 | Ref |
| Washed before display | Yes | 15 | 41 | Ref |
| | No | 163 | 157 | 2.84 (1.51, 5.33)* |
| Means of display | On floor/ground | 73 | 22 | 5.56 (3.24, 9.49)* |
| | On table/shelf | 105 | 176 | Ref |

**Statistically significant at p value of 0.2*

*e) Multivariate Analysis*

A multivariate analysis involving all associated variables in the bivariate analysis was performed to identify independent predictors of parasitic contamination status of fruits and vegetables. Consequently, two variables were showed statistically significant association with parasitic contamination status of fruits and vegetables after adjusting for other variables. Thus, washing status and means of display of fruits and vegetables showed statistically significant association with parasitic contamination status at the p-value $< 0.05$. Fruits and vegetables not washed before display were 2.95 times more likely to be contaminated with medically important parasites compared to fruits and vegetables washed before display (AOR=2.95, 95% CI: 1.49, 5.84). Additionally, fruits and vegetables displayed on floor/ground were 5.21 folds more likely to be contaminated with medically important parasites compared to fruits and vegetables displayed on table/shelf (AOR=5.21, 95% CI: 2.99, 9.08) (Table 5).

*Table 5:* Multivariate analysis showing the final predictors of contamination of fruit and vegetables, Dire Dawa, Ethiopia, September - October 2015

| Variables | | Status | | COR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|---|
| | | Positive | Negative | | |
| Types of fruits & vegetables | Lettuce | 29 | 18 | 1.30(0.57, 2.96) | 1.47(0.60, 3.59) |
| | Cabbage | 26 | 21 | 1.42(0.62, 3.22) | 1.28(0.53, 3.09) |
| | Spinach | 25 | 22 | 1.19(0.52, 2.72) | 1.08(0.44, 2.62) |
| | Carrot | 27 | 20 | 1.68(0.74, 3.82) | 1.81(0.74, 4.42) |
| | Tomato | 23 | 24 | 2.37(1.04, 5.43) | 2.11(0.87, 5.14) |
| | Banana | 19 | 28 | 2.84(1.23, 6.56) | 2.82(0.91, 6.96) |
| | Green paper | 17 | 30 | 4.70(1.95, 11.34) | 4.71(0.98, 12.10) |
| | Orange | 12 | 35 | Ref | Ref |
| Washed before display | Yes | 15 | 41 | Ref | Ref |
| | No | 163 | 157 | 2.84(1.51, 5.33) | 2.95(1.49, 5.84)* |
| Means of display | On floor/ground | 73 | 22 | 5.56(3.26, 9.49) | 5.21(2.99, 9.08)* |
| | On table/shelf | 105 | 176 | Ref | Ref |

**Statistically significant at p value of 0.05*

## V. Discussion

Isolation of medically important intestinal parasites from fruits and vegetables suggested that fruits and vegetables are the possible sources of transmission of food borne diseases in humans. Their presence in those fruits and vegetables not only associated to the favorable climatic conditions for the survival and dissemination of the parasites but also due to the unsanitary conditions and ineffective hygienic practices that facilitate their transmissions [17, 18].

The overall prevalence of parasitic contamination of fruits and vegetables of this study was found to be 47.3%. The result of the current study is lower than the findings reported in studies conducted in Kenya, Nigeria and Jimma [5, 14, 19] and higher than what were reported by others [3, 17-23]. These inconsistencies in findings might be attributed to varying environmental conditions and sanitation and hygiene practices of the study areas.

In this study, lettuce, carrot and cabbage were the most highly contaminated items which accounted 61.7%, 57.4% and 55.3%, respectively and orange was the least contaminated item (25.5%). This variation of contamination level among the items might be due to the fact that cabbage, lettuce and carrot have uneven/rough surfaces which make the parasitic stages attach more easily to the surface of these vegetables. The smooth surface of green pepper, tomato and orange might reduce the rate of parasitic attachment; hence they had lower contamination rate. On top of this, strong adhesion or internalization of the parasites to such leafy vegetables overcomes the effects of washing.

In this study, nine types of medically important parasites were detected from the fruits and vegetables. These parasites include: oocysts of *Cryptosporidium* spp., *Cyclospora* spp. and *Isospora* spp., cysts of *Giardia lamblia* and *E. histolytica/dispar*, larvae of *Strongyloide* spp, and eggs of *Ascaris lumbricoides*, *Hymenolepis nana* and *T. trichuria*. Most of the parasites isolated in this study were also isolated in the study conducted in Jimma [14] and studies conducted in Nigeria and Egypt [3, 24].

In the current study, the most prevalent parasite isolated was *G. lamblia* (9.31%) followed by *E. histolytica/dispar* (8.78%) and *Cryptosporidium* spp (7.71%), and the least prevalent parasite isolated was *Hymenolepis nana* (1.60%). In the study conducted in Jimma, larvae of *Strongyloide* spp., Ova of *Toxocara* spp and oocysts of *Cryptosporidium* spp were the most frequently detected parasites [14]. The findings reported by other investigators include *Ascaris lumbricoides*; *Cryptosporidium* spp, *E. histolytica/dispar* and *Toxocara* spp were as the predominant parasites detected [3, 20, 21, 25]. The discrepancy between the current study and the other studies might be as a result of the variations in geographical locations, climatic and environmental conditions, the kind of sample and sample size examined and/or hygiene practices.

The need to understand factors contributing to parasitic contamination of fruits and vegetables is paramount for improving the efforts in the prevention and control of intestinal parasitosis as a medical and public health problem.

Findings from multivariate analysis revealed that washing status and means of display were found to be independent predictors of parasitic contamination of fruits and vegetables at the p-value $< 0.05$. Those fruits and vegetables not washed before display were almost three times more likely to be contaminated with medically important parasites compared to fruits and vegetables washed before display. In addition, fruits and vegetables displayed on floor/ground were around five folds more likely to be contaminated with medically important parasites compared to those displayed on tables/shelves. These findings are consistent with a study conducted in Jimma [14]. This might be due to the fact that food items which are displayed for sale on the floor are exposed to dusts and flies. It is well established fact that flies can act as vectors for a number of pathogenic microorganisms including parasites like *Cryptosporidium parvum* and *Toxoplasma gondii,* thereby transmitting different parasites to the fruits and vegetables displayed for sale.

As a limitation, this study is a cross-sectional study which did not address the effect of seasonal variability on the contamination rate of the fruits and vegetables.

## VI. Conclusion

In conclusion, results of the current study showed high level of contamination of fruits and vegetables with medically important intestinal parasites. Almost half of the fruits and vegetables sold in the local markets of the study area were being contaminated with medically important parasites which are a potential source for the transmission of intestinal parasites to humans. Significantly higher parasitic contamination rate was detected from fruits and vegetables which had not been washed before display and those displayed on a floor. These findings highlight the public health implication of fruits and vegetables where farmers, sellers and consumers are being at a high-risk of infection with intestinal parasites. Therefore, it is advisable to wash fruits and vegetables thoroughly before eating or using for salad preparation.

*Declarations*

*Ethics approval and consent to participate*

The study was approved by Dire Dawa University Ethical Committee Board. Written informed consent was obtained from all participants.

*Consent for Publication*

Not Applicable.

*Availability of data and materials:* All the questionnaire and laboratory results are available with the authors.

*Competing Interests:* The authors declare that they have no competing interests.

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