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Parasitic Contamination of Commonly Consumed Fresh Fruits Sold At Tiko and Limbe Municipality Markets, South West Region of Cameroon

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ABSTRACT

The disease transmission potential and safety of fruits sold in the cities of Limbe and Tiko, Cameroon, for consumption was assessed with respect to their contamination by ova and cysts of parasites. In sub-Saharan-Africa, protozoan parasites and soil transmitted helminths are the leading intestinal parasites causing significant morbidity and mortality. This explains the frequency and high prevalence of intestinal parasitic infections in this region affecting nearly all inhabitants at some point of their lives. Fresh fruits are one of the main components of a healthy diet. A total of 424 different fruit types were randomly collected with 210 samples in the rainy season and 214 samples in the dry season from both towns for parasitological examination using normal saline sedimentation and zinc sulphate flotation techniques. The results showed that out of the 424 samples examined, 97 were contaminated with parasites giving an overall contamination rate of 22.9%. The rainy season had a higher contamination rate 29.5% than the dry season 16.4%. Among the fruits, mangoes, plum, pear, pawpaw were the most frequently contaminated and apples and watermelon were the least contaminated. The protozoans detected include E. histolytica/E. dispar (29.4%) being the most frequent, followed by E. coli (11.8%), Giardia lamblia, (6.1%) and Isospora belli (2.4%) being the least. The helminthic parasites detected included Ascaris lumbricoides (12.4%) being the most prevalent while the others were E. vermicularis (8.8%), T. trichuria(5.9%), Trichostrongyloides specie(5.9%), Taenia specie, F. buski, Ancylostoma specie, F. hepatica and O. sinensis 2.9% each. Fruits are still a means by which parasites are transmitted in the population considering the vast variety detected on different fruits and the high contamination rate. Therefore, emphasis should be put on educating the population about the importance of washing their fruits thoroughly and more than once before consumption. Vendors should also be educated on the proper means to display fruits for purchase by consumers and limit the touching of fruits by different consumers before purchase.

Keywords
Fruits, Helminths, Protozoa, Contamination rate.

Introduction
Intestinal parasitic infections are among the global public health problems that result in serious health and economic issues in many developing and developed countries [1]. Worldwide, about 3.5 billion and over 450 million people are affected and ill with parasitic infections, respectively [2,3]. In Sub-Saharan Africa, protozoan parasites and soil transmitted helminths are the leading intestinal parasites causing significant morbidity and mortality, thus the frequency of intestinal parasitic infections in this region is extremely high, affecting nearly all inhabitants at some point during their lives [2,4]. Food borne diarrheal diseases caused by intestinal protozoan parasites are major food-borne public health problem across the world and are a major cause of morbidity imposing an annual socio-economic burden on health services and individuals [5].

The route of transmission of most intestinal parasites is the fecal-oral route mostly through consumption of contaminated food and water or during direct hand-to-mouth contact [2,6]. Food items which are usually consumed raw, like fruits and vegetables, are potential sources of infection. Fruits and vegetables are considered vehicles that easily transmit parasites into individuals, especially when eaten raw or without peeling [1,2,7]. Studies have shown that *Ascaris lumbricoides*, *Cryptosporidium spp*., *Entamoeba histolytica*, *Enterobius vermicularis*, *Fasciola spp*., *Giardia intestinalis*, hookworm, *Hymenolepis spp*., *Taenia spp*., *Trichuris trichiura*, and *Toxocara spp*., can infect humans who consume contaminated, or improperly washed fruits [2,8-10]. Studies conducted on various fruits and vegetable samples in Cameroon showed the main parasitic contaminants to be *Balantidium coli*, *Entamoeba spp*, hookworm, *Strongyloides stercoralis*, *E. histolytica*, *Ascaris lumbricoides*, and *Trichuris trichuria* [11,12].

Fruits are of great importance for an adequate and balanced human diet owing to the role they play in preventing many health-related problems such as obesity, cancer, diabetes, and cardiac diseases [12]. Vegetables and fruits provide important nutrients to humans, including various essential vitamins and minerals [13]. The consumption of raw vegetables and fruits appears to be a quick, easy, and healthy source of nutrition. However, these fresh vegetables and fruits can be an important source of some food-borne pathogenic microorganisms, if they are contaminated [5]. The contamination of raw vegetables and fruits with human pathogenic parasites are now a global public health threat, despite the health benefits of these foods in non-pharmacological prophylaxes against diseases [5].

The safety of fruits eaten raw is a great concern as they have been shown to harbor pathogenic parasites with poor hygienic practices in the production and postharvest system, contributing to contamination [1,11-12]. Use of human and animal excreta as natural fertilizer and untreated waste water for irrigation during cultivation are the main contributing factors in the preharvesting phase. In developing countries, majority local farmers use untreated human or animal dung as fertilizer and polluted or untreated water for irrigation which contributes to increased transmission of pathogenic parasites [11,12,14]. A lot of fruits are consumed raw to retain heat labile nutrients and the safety of these fruits eaten raw is of great public health importance as they have been shown to harbor and transmit pathogenic parasites [14-16]. Data on parasitic contamination of fruits in Cameroon is scarce despite the fact that it has witnessed an increase in fruit cultivation, selling, and consumption. Considering the important role of fruits, there is an urgent need for routine investigation of parasitic contaminants of fruits sold in Cameroon. Therefore, this study was aimed at identifying the parasitic contaminants of fruits sold in Tiko and Limbe municipalities and the type of fruits highly contaminated to highlight their potential in parasite transmission and provide data that could guide policy makers to improve food safety and safeguard public health.

Methods

Study area and Study Design

The study was conducted in two towns in Fako division, Cameroon; Tiko and Limbe municipalities. Tiko is 21 km from Limbe habouring indigenes and people from other places. It is a low land area by topography with little undulation and has a temperature range of 35–37°C. Limbe is situated along the Atlantic coast of West Africa. It is bordered in the north by Buea, east by Tiko, west by Idenau, and south by the Atlantic Ocean. Limbe have recently experienced an expansion in hotel and restaurant businesses as this town has been hosting many national and international events. Limbe and Tiko have warm equatorial climatic conditions with humidity as high as 80% [11].

This study was a cross-sectional study which lasted from April to July 2019/(rainy season) and January to April 2020/(Dry season) carried out on fruit samples purchased from randomly selected vendors in our local markets and along the streets of Tiko and Limbe Municipalities.

Sample collection and Processing

In each market, samples were collected under normal purchase conditions from randomly selected sellers. Different types of fruits that are frequently consumed in the municipality were sampled to check for intestinal parasites. Fruits that were screened for the presence of parasites were Orange (*Citrus sinensis*), Mango (*Mangifera indica*), Pawpaw (*Arica papaya*), Pineapple (*Ananas cosmosus*) Water melon (*citrullus lanatus*), Apple (*Aberia cattra*), Banana (*dusa accuminata*), Avogado (*Persea americana*) Garden egg (*Solanum melongena*), and plum. The fruits were collected in sterile plastic bags and buckets, sterilized with 10% chlorine water, sealed, labelled with a unique number and its date of collection, transported in a refrigerated chamber, and analyzed in the MAFLEKUMEN and Laboratory for parasitology of CRM at IMPM.

Examination for Parasitic contamination

Sedimentation method was carried out as described by Bekele and Shumbej [17]. Each sample (fruit) surface was washed separately in 500 mL of normal saline for detaching diagnostic stages of the parasites such as ova, larvae, cysts, oocysts of helminths and protozoan parasites commonly assumed to be associated with the contaminated fruit. After 30 minutes sedimentation of the washing solution, 15 mL of the sediment was collected and centrifuged at 3,000 revolutions per minute (rpm) for 5minutes, the supernatant was decanted leaving the sediment which was examined under a light microscope. For the identification of intestinal coccidian protozoan oocysts, a modified Ziehl-Neelsen staining technique was employed. Finally, the sediment was agitated gently by hand for each part to redistribute the parasitic stages, then used a dropper to drop the sediment on a glass slide and covered with a cover slip. The glass slide was examined under a light microscope using ×10 and ×40 objectives and the parasites were identified using the parasite identification key from Monica Cheesbrough.

The zinc sulphate floatation technique described by Judith et al. [12] was also used for concentrating the cysts and ova of the

parasites. The samples were washed in distilled water and the washings were centrifuged at 250 rpm for 1 minute, after which the supernatant was discarded using a Pasteur pipette. Two mL of the sediment was placed in a 15mL test tube which was filled with zinc sulphate solution to the brim and a cover slip was placed on top. The cover slip was removed after 20 min to allow enough time for the cysts and ova to float. To examine for the cyst and ova of parasites, a cover slip was placed face downwards on a slide and was examined under ×10 and ×40 objectives of a light microscope with a drop of iodine. The various cysts and eggs of the parasites were identified by their morphological characteristics such as the shape and size of the eggs using the guide from Centers for Disease Control and Prevention (DPDx) and Cheesebrough [18,19].

Ethical Consideration

An administrative authorization to carry out the work was obtained from the regional delegation of public health for the South West Region. Oral informed consent was gotten from the fruit vendors.

Data Analysis

The results obtained were checked, verified, recorded, and every week for the use of correct codes and consistency. The data was entered and analyzed using Microsoft Excel and SPSS version 18 and the results were presented with frequency tables and bar charts. Descriptive statistics like frequency and proportion were calculated to describe the characteristics and contamination status of fruits.

Results

Parasitic Contamination rate of fruits

A total of 424 fruit samples were collected with 210 samples in the rainy season and 214 samples in the dry season from both towns. From the total number of fruit samples collected, 97 fruit samples were contaminated with at least one type of parasite species, giving an overall contamination rate of 22.9%.

During the rainy season, 62 samples were identified as being contaminated with at least one type of parasite; the overall contamination rate was 29.54%. Contamination with multiple types of parasites was observed in all fruits in this season except one fruit. Among the fruits, mangoes were the most frequently contaminated, followed by plum, pineapple, orange, pawpaw, and lastly watermelons as seen in table 1 below.

Table 1: Parasitic contamination rate of fruits during the rainy season.

Type of Fruits Examined	Number Examined	Number Positive (%)	Types of Parasites Detected
Mango	35	27 (77.14)	3
Plum	35	19 (54.28)	2
Pineapple	35	07 (20.00)	2
Orange	35	05 (14.28)	2
Papaya	35	03 (8.57)	2
Water Melon	35	01 (0.47)	1
TOTAL:	210	62 (29.52)	_

The distribution of contamination rate of fruits during the rainy season are as shown in figure 1. Out of all the collected fruit samples watermelons had the least contamination rate 1/35 (0.47%) and just one type of parasite detected on it followed by pawpaw 3/35/(8.6%) and two types of parasite detected on it. Mangoes had the highest contamination rate of 27/35/(77.1%) in the rainy season and three different types of parasites detected on it followed by plums 19/35(54.3%), pineapples 7/35/(20%) and oranges 5/35/(14.3%) which all had two types of parasites detected on them.

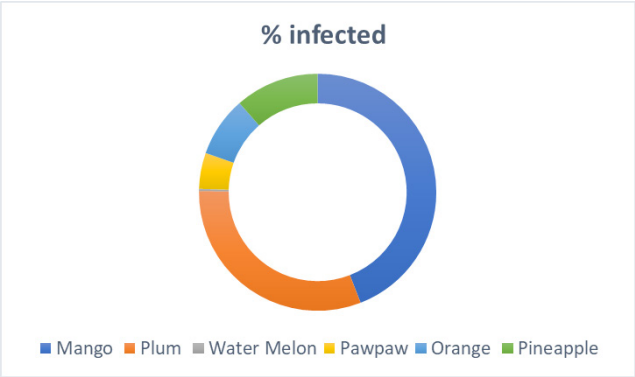

Figure 1: Contamination rate of fruits in the rainy season.

During the dry season, the results of the study showed that out of the 214 samples examined, 35 (16.4%) samples were identified to be contaminated with at least one type of parasite. Pear had the highest (27.3%) contamination rate, followed by pawpaw (26.7%), mangoes (21.1%), oranges (16.0%), bananas (15.4%), water melon (12.5), garden egg (12.0) pineapple (11.5) and finally apples (7.1) as seen in table 2 and figure 2 below.

Table 2: Distribution of fruits and their parasitic contamination rates during the dry season.

Kind of fruit	Num. examined	Num. infected	% infected
Pears	22	6	27.3
Pawpaw	15	4	26.7
Mangoes	19	4	21.1
Oranges	25	4	16.0
Bananas	52	8	15.4
Water melon	16	2	12.5
Garden egg	25	3	12.0
Pineapple	26	3	11.5
Apples	14	1	7.1
Total	214	35	16.4

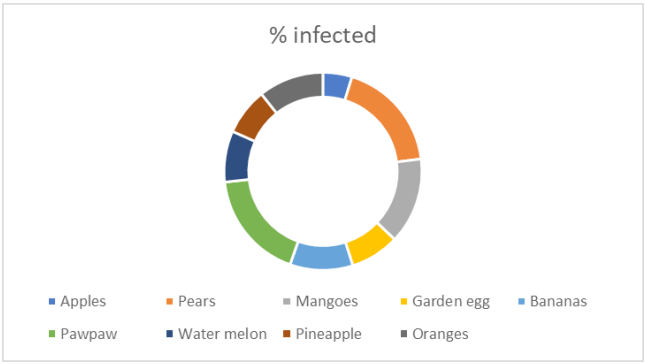

Figure 2: Contamination Rate of fruits in the dry season.

The results indicate that fruits are more contaminated with parasites in the rainy season (29.54%) than in the dry season (16.4%).

Parasitic Contaminants of Medical Importance Detected on Sampled Fruits

Both protozoa and helminth parasites were detected as contaminants of fruits in the study area and during both seasons. The protozoans detected include *E. histolytica* being the most frequent, followed by *Giardia lamblia*, *E. coli*, and *Isospora belli* being the least. The helminthic parasites detected included Ascaris lumbricoides being the most prevalent while the others were *E. vermicularis, T. trichuria, Trichostrongyloides specie, Taenia specie, F. buski, Ancylostoma specie, F. hepatica* and *O. sinensis*.

During the rainy season (April to July 2019), the parasites detected were *Ascaris lumbricoides* ova, *Toxocara species* ova as helminths, and *Giardia intestinalis* cysts, *Isospora belli* oocysts as protozoans. Ascaris (10%) had the highest prevalence followed by Toxocara species (8.1%) and G. intestinalis (6.2%) while *Isospora belli* had the lowest prevalence as seen in table 3 below.

Table 3: Parasites detected in the rainy season.

Detected Parasites	Frequency	Prevalence
Ascaris lumbricoides	21	10.0%
Toxocara spp	17	8.1%
Giardia intestinalis	13	6.2%
Isospera belli	05	2.4%

The distribution of parasites detected in the rainy season found *Ascaris lumbricoides* [21] to be the most frequently detected parasite followed by *Toxocara species*, *Giardia intestinalis* and *Isospora belli* which had frequencies of 17, 13 and 5 respectively.

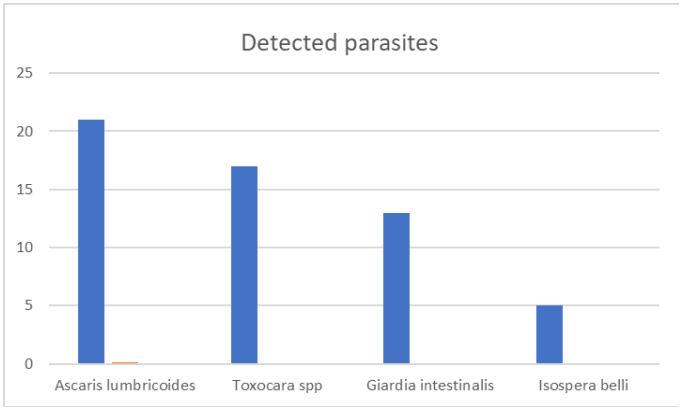

Figure 3: Frequency of Detected parasites in the rainy season.

During the dry season (Jan to April 2020), *E. histolytica* cysts had the highest prevalence (29.4%), being detected in mangoes, garden eggs, pawpaw, pear, water melon and pineapple followed by *A. lumbricoides* eggs (14.7%) detected in pear, mangoes, water melon and apples. *E. coli* followed with 11.8% detected in pineapple, banana, and garden eggs followed by *E. vermicularis* (8.8%) detected in watermelon, pawpaw and banana. *T. trichuria* ova detected in garden eggs and pear. *Trichostrongyloides* eggs detected in pawpaw and pear, *G. lamblia* cysts detected from pawpaw, oranges, had the same prevalence of 5.9%. The least of the prevalence (2.9%) was observed in the following; *Ancylostoma species* larvae detected in pears, hookworm ova detected in pears, *F. hepatica* ova detected in pears, *O. sinensis* ova detected in bananas, *F. buski* eggs detected in pear and finally *Tenia egg* detected in banana as shown in table 4 and figure 4 below. There was no intestinal coccidian observed.

Table 4: Frequency Distribution of Parasites Identified and fruits contaminated during the dry season.

Detected Parasites	Fruits contaminated
Protozoans	
E. histolytica	Mangoes, garden egg, pawpaw, pear, watermelon & pineapple
G. lamblia	Pawpaw & oranges
E. coli	Pineapple, banana, garden egg
Helminths	
Ascaris lumbricoides	Pear, mangoes, watermelon & apples
E. vermicularis	Water melon, pawpaw & banana
T. trichuria	Garden eggs & pear
Trichostrongyloides spp	Pawpaw & pear
Hookworm	pear
F. hepatica	pear
F. buski	pear
O. sinensis	banana
Taenia spp	Banana

The distribution of parasites on contaminated fruits during the dry season as shown in figure 4 showed *Entamoeba histolytica* to be the most prevalent parasite with a prevalence of 29.4% followed by *Ascaris, Entamoeba coli*, and *E. vermicularis* which had a prevalence of 14.7%, 11.8% and 8.8% respectively. *T. trichuria, Trichostrongyloides* and *G. lamblia* had the same prevalence of 5.9%. The least of the prevalence (2.9%) was observed in *Ancylostoma species*, *F. hepatica, O. sinensis, F. buski*, hookworm, and *Taenia species*.

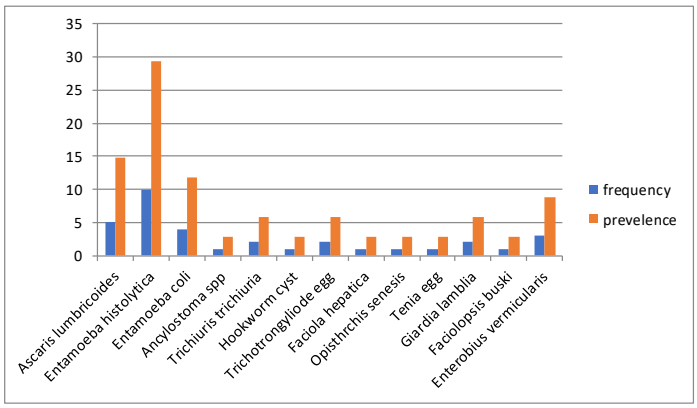

Figure 4: Frequency Distribution of Parasites Identified during the dry season.

Discussion

The present study for the first time, attempted to assess the level of contamination and prevalence of different intestinal parasites in various fruits during different seasons sold in the local markets of

Limbe and Tiko town, South west Region, Cameroon. This study revealed a high contamination rate and prevalence of parasites on commonly consumed fruits. Contamination was higher in the rainy season than in the dry season. This finding is of great public health significance to help curb the transmission of intestinal parasites in our population by educating the population on the presence of intestinal parasites on fruits which can cause them morbidity since most of these fruits are consumed raw. The presence of parasites on fruits could be as a result of contamination from fruit vendors and display positions. The fruits for sale are displayed on bare ground and in open shades for easy viewing by customers which makes the fruits easily contaminated by the infective stage of parasites found in the environment. There is also poor handling of the fruits by fruit vendors and their hygienic conditions are deplorable such as open defecation or urination due to lack of public toilets around sales points which makes the possibility of contaminating the fruits very high.

The overall parasitic contamination rate in this study is 22.9% which is higher than that of Judith et al (2018) in Buea, Cameroon, who reported 13.12% [12], Dauda et al. (2014) in Kaduna, Nigeria who reported 14.0% [23] in a similar study and lower than that of Gboeloh and Sounyo (2021) in Port harcourt, Nigeria who reported 40.3% [22]. This difference could be explained due to variations in geographical location, origin of the fruit samples, types of fruits sampled and the seasons during which the samples were collected. The type and number of fruits sampled in this study could also be responsible for the variation in the contamination rate. For example, in the study from Buea, fruits were sampled only in the rainy season where as in this study fruits were sampled from both the rainy and dry seasons. Another reason for the possible decrease in contamination of fruits in Buea unlike Limbe and Tiko could be the presence of many health educational schools and health non-governmental organisations in Buea which execute health and sanitation programs to reduce the transmission of parasites in the Buea community. The contamination rate of fruits was in agreement with the findings in a study performed by Alemu et al. (2020) in Bahir Dar city of Northwest Ethiopia who reported a contamination rate of fruits alone at 25.6% [2] but was lower than similar findings in Tarcha town of Southwest Ethiopia (39%), Dire Dawa (47.3%) and Arba Minch (54.4%) [4,10,17]. This could be due to Southwest Ethiopia being highly endemic for intestinal parasites with high prevalence of intestinal parasites in that locality.

In this study, it was seen that the contamination rate was higher in the rainy season (29.5%) than in the dry season (16.4%). This is because the transmission of parasites and hence the prevalence are relatively low during the dry season [2]. This can be due to the lack of rains which cause floods that mix with contaminated soil hence decreasing the chances of transmission to fruits in the dry season unlike the rainy season. In this study it was also seen that the contamination rate of individual fruits between the two seasons was different. Mangoes and pineapples had a higher contamination rate in the rainy season than dry season while orange, watermelons and pawpaw had a higher contamination rate in the dry season than rainy season. These differences can be attributed to the number of samples collected per fruit per season for parasitological analysis and to the fruits being seasonal fruits which are more common in one season than another season hence more handling and contamination by vendors and consumers.

The highest contamination was found in Plums, Mangoes, Pear, Pawpaw, Pineapple, Bananas, and oranges, respectively, while the least contamination was found in watermelons and apples. Mangoes being amongst the most contaminated is similar to the study done by Judith et al. [12] where it was also found out that mangoes were the highest contaminated fruit. This can be due to excess handling and touching by many clients as well as vendors during harvesting, transportation, and sales since it's a tropical fruit loved by many individuals in the study area. The fruit type is also another reason for the high contamination because these fruits are small and portable and are easily touched or pushed to the floor during purchase. They are fruit types loved by majority of the population and are frequently purchased for consumption hence higher chances of contamination during handling and purchase. The variation in contamination between the products might be explained by the fact that some vectors such as flies can easily contaminate some fruits like mango and bananas. The other highly contaminated fruits are also frequently touched by the bare hands of clients during purchase which could contaminate them. Their manner of harvesting which causes the fruit to come in contact with the soil and their manner of display can also be responsible for the increased contamination of these fruits. The least contaminated fruits are not frequently touched due to watermelons being heavy and apples displayed in a manner which discourages touching by clients during sales. The smooth surface of water melon, and apples might reduce the rate of parasitic attachment, hence explaining the lower contamination rate observed in this study [12].

Both protozoa and helminth parasites were detected as contaminants of fruits in the study area and during both seasons, which is similar to the findings done by Judith et al. [12] and Alemu et al. [2] but different in the types of parasites identified. This difference can be attributed to the origin of the fruit, season, storage conditions and geographical location. It can also be attributed to the hygiene practices of farmers and sellers of the fruits with some sellers displaying their fruits in deplorable sanitary conditions in the different towns.

In this study, *A. lumbricoides* was the most frequently detected helminth while *E. histolytica* was the most frequently detected protozoa. This is in accordance with the study done in Buea, Kaduna, and Port Harcourt where both parasites were also the most frequently detected parasite except for hookworms which was the least in this study. This dominance might be associated with this parasite's ubiquitous distribution, the high number of eggs produced by the fecund female parasite, and cysts by the protozoan, which contributes to the parasite ubiquitous distribution, and the strong and resistant nature of the eggs/cysts that enables them to survive unfavourable conditions. Fifteen parasitic protozoans and helminths were detected similar to a study by Akoachere et al. (2018) on vegetables in the same community where ten different parasitic protozoans and helminths were detected in vegetables [11]. The condition in which fruits are

exposed to the public and the practice of touching by vendors as well as buyers is responsible for contamination of the fruits with parasites. In the present study, it was not possible to separately report only species of human medical importance as their eggs are difficult to distinguish. Moreover, factors associated with parasitic contamination in the supply chain of fruits were not assessed. All clients who purchase fruits from vendors should make it a habit to wash the fruits before eating or cooking or even saving in the home for later consumption.

Conclusion

The level of parasitic contamination of fruits in the study area is quite high with contamination rate higher in the rainy season than during the dry season. Mangoes, plums, and pears are more prone to parasitic contaminants than watermelons and apples. Fruits are still a means by which parasites are transmitted in the population considering the vast variety detected on different fruits and the high contamination rate. Therefore, emphasis should be put on educating the population about the importance of washing their fruits thoroughly and more than once before consumption. Vendors should also be educated on the proper means to display fruits for purchase by consumers and limit the touching of fruits by different consumers before purchase. The government should provide adequate resources for construction of toilets in the community to reduce the rate of open defecation and urination. This would have a direct and indirect effect on the rate at which fruits are contaminated by parasites. Large scale studies targeting fruits both at preharvest and postharvest phases using molecular detection methods are recommended to exhaustively identify sources of contamination and to detect pathogenic parasitic contaminants. Fruit vendors are also recommended to be tested on their potential of being a source of contamination of commonly consumed fruits.

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