# PRESENCE OF PARASITIC OVA, CYSTS AND LARVAE ON COMMON FRESH FRUITS AND VEGETABLES SOLD AT SOME MAJOR MARKETS IN IBADAN, OYO STATE, NIGERIA

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## Abstract

Several parasitic infections are associated with poor hygiene and environmental sanitation resulting in food and water containing ova, cysts or larva of these parasites. Therefore, a survey of fruits and vegetables sold at some major markets in Ibadan was carried out between January and June, 2011 to identify the ova, cyst and larva present on them. A total of 264 fruits and vegetables pieces were examined using standard wet mount procedure and Kinyoun acid fast stain to detect parasites. The percentage of fruits and vegetables with eggs, cysts or larvae of eight different species of parasites encountered were 38.09%, 20.38%, 18.56%, 8.33%, 6.42, 4.55%, 2.15% and 1.67% for *Ascaris lumbricoides* eggs, *Strongyloides stercoralis* larvae, hookworm eggs, Oocysts of *Cryptosporidium* , immature cyst of *Entamoeba histolytica/dispar*, *trichuris trichuira* eggs, *Taenia* species egg and oocyst of *Isospora* species respectively. Tomatoes had the highest percentage with parasites' ova, cysts or larva (73.33%) followed by *Irvingia* and carrots (66.7% each), mangoes (58.33 %), garden eggs (43.35%), cherries (33.33%), oranges (25.0%), cucumber (22.22%) and lime (12.5%). Considering the availability of different fruits at different seasons of the year and the present-day "back to nature" approach to healthy diet, it is pertinent to encourage proper washing of fresh and raw fruits and vegetables before they are sold or eaten, thereby preventing transmission either at point of sell or consumption of fruits and vegetables which form sources of infection via the oral route.

**Keywords:** fruits, vegetables, market survey, parasites, risk of transmission.

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## Introduction

Fruits are a major part of man's diet and in fact an important component of a healthy diet because they contain essential vitamins which are necessary for growth and body nourishment (Carey *et al* 1996). Raw fruits and vegetables are believed to be the principal source for the transmission of soil-transmitted helminthiasis (STH) when they are consumed without proper washing (Uneke, 2007). Intestinal parasitic infection is becoming an increasing concern because of the expanding number of individuals at risk of infection, particularly children, pregnant women, elderly and the immune-compromised persons (Erdogrul and Sener, 2005).

The extent of the occurrence of parasites' ova, cysts and larva on fruits and vegetables depends on several factors that include the handling of untreated wastewater, poor personal hygiene, poor sanitation, and the use of faecal matter as fertilizer on the farm (Amoah *et al* 2007). Faeces harbour helminth and protozoa eggs, cysts and larvae, according to Thaddeus *et al* (2005) and Amoah (2009) farmers do not allow the faeces to decompose very well before application on the farm. The feeding mechanisms and filthy breeding

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habits of synathropic insects like flies make them efficient vectors and transmitters of human enteric protozoan parasites (Thaddeus *et al*, 2005). Also, inadequate and unsafe disposal of human faeces can lead to the transmission of faeco-oral diseases. The resistance of parasites, eggs and cysts make them persistent in the environment for long periods of time. Soil-transmitted helminth infection is endemic in many areas of the world, principally in developing countries with poor environmental sanitation and personal hygiene (Naish *et al* 2004). Ibadan is a highly endemic city for Soil Transmitted Infections, STIs (Alli *et al* 2011). The mode of transmission is usually faeco-oral and there is high prevalence among people who live in areas with indiscriminate contamination of soil with human faeces, where sanitary facilities are inadequate. STI infection is mainly established by oral intake of infective eggs and, or cysts from the environment (Idowu and Rowland, 2006).

Oocysts of *Cryptosporidium* are widespread in the environment and can be found in lakes and streams. The resistant stages produced by *Cryptosporidium* oocysts are remarkably stable, and can survive for weeks and months in the environment. Transmission takes place by direct contact with infected cattle but also indirectly through drinking water and fruits (Ortega *et al* 1997). Cryptosporidiosis and isosporiosis are highly prevalent among people living with Acquired Immune-Deficiency Syndrome (AIDS).

Fruits have nutritive values and are desirable, readily available by the road-sides and at major market places and as such, there is a great tendency to eat these fruits unwashed or improperly washed. Also the handling of fruits and vegetables in market places allows for the transfer of parasites' infective stages to these fruits which are exposed for sight attraction, and even cut into pieces for affordability.

This study was therefore carried out to determine the extent of risk of parasites' species transmission via fruits and vegetables.

## Materials and methods

### Survey area

Ibadan, capital of Oyo State in south-western Nigeria is a major center of commerce in the state. The city covers a land area of 240 km$^2$ and has an estimated human population of 3,139.500 as at 2003. Ibadan lies within the secondary rainforest and savanna vegetation zone, with a mainly tropical wet and dry climate, a lengthy wet season with an estimated annual rainfall of about 1,250 mm (UNCHS and UNEP, 1997) and relatively constant temperatures throughout the year. The wet season runs from March through October, with a lull in precipitation in August. There is low level of sanitation which is characterized by the presence of massive refuse dump sites at market places. Ibadan has various markets which include Alesinloye, Sabo, Bodija, Mokola, Gbagi, Oje, Beere, Bode, Oja'ba, Oke Ado, Monatan and Moniya markets. Fruits and vegetables are brought in by the farmers and traders from the growing areas into these markets.

### Collection of fruits and vegetable samples

A total of 264 fruits and vegetable pieces were bought from Oje, Sabo, Beere, and Bodija markets in Ibadan where fruits and vegetables are deposited in large quantities on daily basis (Figure 1). The fruits and vegetables bought included: tomatoes (*Lycopersicum esculentum*), mango (*Mangifera indica*), Wild mango (*Irvingia gabonensis*), lime (*Citrus aurantifolia*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*), orange (*Citrus sinensis*), cherry (*Chrysophyllum albidum*) and garden egg (*Solanum incanum*). The survey was carried out between the months of January and June, 2011. Each set of fruits was packaged into different sterile polythene bags, and transported to the Parasitology Laboratory, Department of Zoology, University of Ibadan for examination.

### Laboratory examination of fruit samples

Each fruit was washed with 100 ml of distilled water into separate containers and the resultant liquids were allowed to stand for 20 minutes in appropriately labelled specimen bottles. The supernatants were discarded and the sediments centrifuged for 5 minutes at 2,000 rpm.

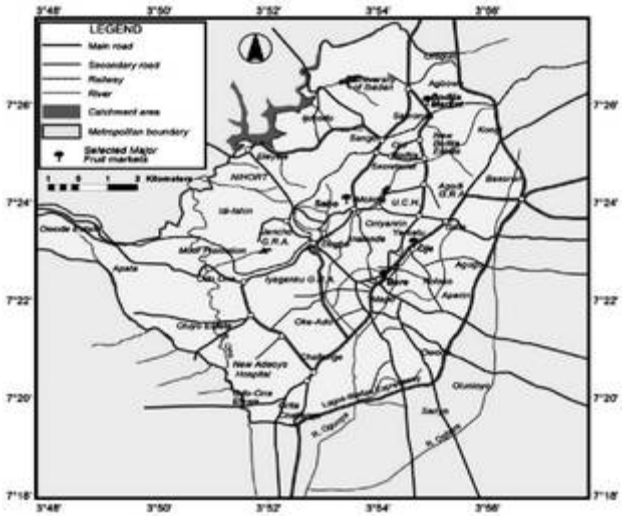


**Figure 1:** Map of Ibadan showing selected major markets involved in the fruit survey.

Wet smear preparation

The sediments were examined in drops under the light microscope.

Kinyoun acid-fast staining procedures

The slides were allowed to air dry and then fixed in 10% methanol. The fixed slides were dipped in Kinyoun stain for 12 minutes and rinsed in tap-water. Each slide was decolourized in 4% sulphuric acid for 60 seconds and washed in tap-water. The slides were finally stained in malachite green for 5 minutes and after that, it was rinsed in tap-water and allowed to dry.

Parasite identification and count

A drop of oil immersion was placed on each slide and examined microscopically. Identification was carried out using standard guide as described by Arora and Arora (2005).

Data analysis

The data was entered into the SPSS Spread Sheet and two-way analysis of variance (ANOVA) was used to determine significant variation in prevalence of the parasites in relation to the fruits and vegetable types and markets.

## Results

Parasitic ova, cysts or larva encountered

Six different species of parasites' ova, cysts or larva were found; wet mount preparations showed occurrence of *Ascaris lumbricoides*, *Strongyloides stercoralis*, hookworm, *Entamoeba histolytica*, *Trichuris trichiura* and *Taenia* species while Kinyoun acid fast procedure confirmed the occurrence of *Cryptosporidium* species and *Isospora* species (Plate 1).

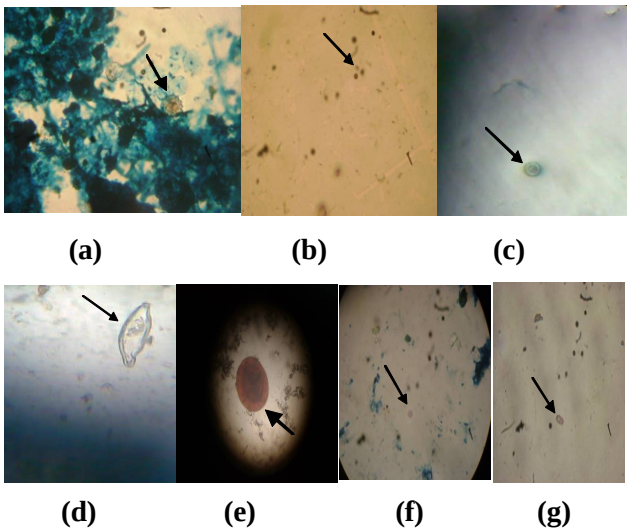


**Plate 1:** Parasite species found on fruits and vegetables from markets in Ibadan, January-June 2011.

**(a)** *Ascaris lumbricoides* ova x 40; **(b)** hookworm ova x 40; **(c)** *Entamoeba histolytica* cyst x 40; **(d)** *Trichuris trichiura* egg x 30; **(e)** *Taenia* species egg x 40; **(f)** *Cryptosporidium* oocyst x 100; **(g)** *Isospora* species x 100.

Overall frequencies of parasite on fruits and vegetables

Intestinal parasite eggs or cysts were found on 48.48% of fruits and vegetables (128/264; 95% confidence interval (C I), 39.98 ± 26.91) examined. The percentage of fruits and vegetables with parasitic stages by market class showed that Sabo Market had the highest frequency of intestinal parasites (63.33%) followed by Oje Market (48.35%) and Beere Market (48.33%) while the least, 43.37% was from Bodija Market. The *chi*-square test showed significant differences in number of fruits and vegetables with parasitic stages by market class. ($\chi^2_{7.82} = 81.14$).

Specific parasitic stages occurrence on fruits and vegetables

The frequency of parasite species occurrence based on the wet mount examination is shown in Figure 2, with *A. lumbricoides* occurring most with a percentage of 38.09. *S. stercoralis* had 20.38% followed by 18.56%, 6.42%, and 2.15% for hookworm, *E. histolytica* and *T. trichura*, respectively. The least was 1.68% for *Taenia* species.

Stained preparation

The Kinyoun acid fast staining technique showed a total of 12 *Cryptosporidia* oocysts in tomato, mango and garden egg fruits while a total of 2 *Isospora* oocyst were found in tomatoes and cucumber.

Fruits and vegetables specific prevalence

In relation to staining technique, 8.33% tomatoes, 2.78% of mangoes, 3.33% of garden eggs and 2.22% of cucumber were contaminated with *Cryptosporidium* and, or *Isospora* oocyst (Table 1). The most contaminated vegetable was tomatoes followed by carrots, garden eggs, and cucumber with percentages of 73.33%, 66.7%, 43.35%, and 22.22% respectively. Wild mangoes had the highest proportion of fruits with parasitic ova, cysts or larva (66.7%) among the fruits followed by 58.35% in mangoes, 33.33% in cherries, 25% in oranges and the least was 12.5% for lime.

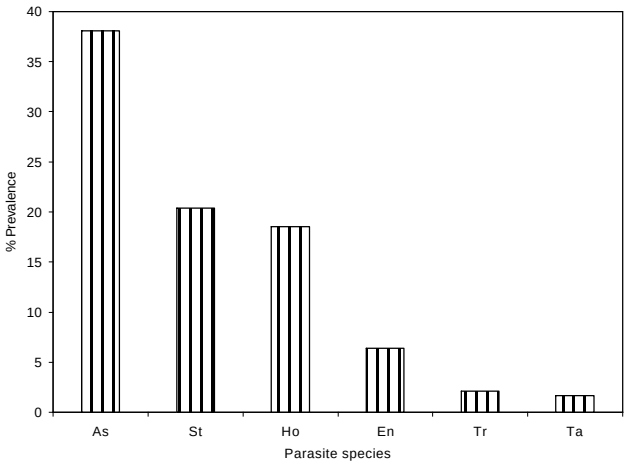


**Figure 2:** Percentage of parasite species observed, January-June, 2011.

As = *Ascaris lumbricoides*; St = *Strongyloides stercoralis*; Ho = Hookworm; En = *Entamoeba histolytica*; Tr = *Trichiuris trichiura*; Ta = *Taenia* species.

## Discussion

Transmission of pathogenic organisms from irrigated soil to crops, grazing animals and humans is not unusual in some parts of the world (Daryani *et al* 2008). These authors further suggested that poor sanitation, inadequate public hygiene and eating raw vegetables with or without peeling were probably reasons for high prevalence of intestinal parasites among the inhabitants of Ardabil City, Iran (Daryani, 2002) and even outbreaks of intestinal parasitic infections in developed and developing countries (Oterga *et al* 1997 and Mintz *et al* 1993). Menge and Arias (1996), detected *Cryptosporidium* oocysts in lettuce, parsley, cilantro and blackberries. Viable *Cryptosporidium* and *Isopora* oocysts combined were found on tomatoes which is the most frequently affected (8.33%) of all fruits and vegetables examined. The present study showed that fruits and vegetables purchased from the four major markets surveyed harboured intestinal parasite eggs and cysts which are usually voided with faeces by humans and animals. The source of acquisition of parasites' eggs, cysts or larva by the fruits and vegetables examined could be attributed to the usage of faecal matter as fertilizer (Uga *et al* 2009) and untreated waste-water for irrigation purposes (Amoah, 2009), a conditional practice in some vegetable and fruit-growing dry areas of Nigeria when there is shortage of water.

A higher percentage (48.48%) of intestinal parasites on fruits and vegetables was reported in this study than 26% reported by Uga *et al* (2009), in Hanoi, Vietnam, and 36% reported by Damen *et al* (2007) in Jos, Nigeria. The period of investigation could explain such differences in percentages. Uga *et al* (2009) did not observe seasonal variation in the prescence of ova, cysts and larva on fruits and vegetables between the rainy and dry season. The period of this study coincided with the late dry season and the early rainy season characterized with low amount of available rain-water, dried-up-wells, taps, ponds and streams. Expectedly, there will be an increased tendency to depend on available water sources, thus suggesting the possibility of a lower occurrence of parasites' ova, cysts and larvae on fruits and vegetables as the rains progress and safe water becomes available.

**Table 1:** Percentage of *Cryptosporidium* and *Isospora* species.

| Fruit Types | No. of fruits examined | No. of fruits with parasittic stages ( %) | | Overall percentage |
|---|---|---|---|---|
| Tomatoes | 60 | 5 | (8.33) | 1.89 |
| Mangoes | 36 | 1 | (2.78) | 0.38 |
| Garden eggs | 30 | 1 | (3.33) | 0.38 |
| Lime | 16 | 0 | (0.00) | 0.00 |
| *Irvingia* | 24 | 0 | (0.00) | 0.00 |
| Cherry | 30 | 0 | (0.00) | 0.00 |
| Cucumber | 45 | 1 | (2.22) | 0.38 |
| Carrot | 15 | 0 | (0.00) | 0.00 |
| Orange | 8 | 0 | (0.00) | 0.00 |
| Total | 264 | 8 | | 3.03 |

In this survey, percentage of the fruit and vegetables from the different markets harbouring parasites was considered in relation to the level of environmental and food hygiene status. Hence, Sabo Market would come as the most unhygienic market that favours the risk of transmission of intestinal parasites to buyers while Bodija Market was the least. This suggestion is however contrary to the report by Alli *et al* (2011) who indicated that Bodija Market had the highest percentage. These contrasting results are likely to be due to peculiarities of prevailing conditions; variety of produce and quantity, economical, physiological and ecological factors.

Among parasites identified in this study, *A. lumbricoides* ova were the most commonly encountered on fruits and vegetables. This is in agreement with previous reports (Uneke, 2007; Alli *et al* 2011; Abougrain *et al* 2009; Ekwunife and Akolisa, 2009). The major reason for this status of *Ascaris* may not be unconnected with the endemic nature of soil-transmitted helminth infections in most developing countries,

associated with poor sanitation, environmental and personal hygiene.

Cryptosporidiosis is now highly prevalent among persons with acquired immune deficiency syndrome (Paul and Gordon, 2002). This study revealed the occurrence of 12 viable *Cryptosporidium* oocysts and two *Isospora* oocysts on fruit and vegetable samples, which is a signal of high risk confronting this increasing category of susceptible individuals particularly when there is lack of self discipline with standard personal hygiene. A study carried out by Marcarisin *et al* (2010), indicated that neonatal mice developed cryptosporidiosis after ingestion of apples which were experimentally contaminated with *Cryptosporidium* oocyst.

Various levels of contamination relative to vegetable types have been reported by several authors (Daryani *et al* 2008; Uga *et al* 2009; Damen *et al* 2007 and Abougrain *et al* 2009). In contrast to report by Damen *et al* (2007) who attributed lowest occurrence of intestinal parasites on tomatoes to the smooth surface which reduces the rate of parasitic attachment; tomatoes had the highest percentage of parasites. Considering the nature of tomatoes; the high risk of spoilage and damage resulting from post-harvest handling, it is not unexpected that tomatoes were found to be the most contaminated with parasites. In spite of the rough surface, lime had the least percentage in this study. These observations show that other factors apart from skin surface texture might be in play to enhance or inhibit parasite occurrence on fruits and vegetables. Wells and Butterfield (1997) implied that there was likelihood; produce contamination with *Salmonella* depended on the presence of stem scar that is the corky area on top of tomato fruit that was attached to the stem which of course is not smooth and soft or translucent skin which are signs of spoilage and may not be visible. The contrasting rate of tomatoes harbouring intestinal parasites reported by Alli *et al* (2011) and our findings in this study might as well be explained by the varying stages of tomatoes' spoilage, water sources for irrigation, a practice of keeping produce fresh and cool, and the exposure of stem scar, potential preferred spots by intestinal parasites.

Furthermore, the indirect influence of mechanical injury reported by Saltveil (1977) to increase respiration rates of fruits and vegetables and with increasing degree of wounding, occurrence of intestinal parasites on fruits and vegetables cannot be ruled out, thus creating a variation pattern in fruits and vegetables acquiring parasites' cysts, eggs and larvae. According to Saltveil (1977), basically, parasitic eggs and cysts can survive for a week or two or even a month depending on duration of storage of night soil and faecal-polluted water available for use by farmers and dealers in fruits and vegetables, hence cysts and eggs viability and physiological maturation period also vary in relation to time interval between parasitic pollution of night soil and water sources and acquisition by fruits and vegetables. Hence, the risk of raw consumption will depend on how long produce can remain dangerous after acquiring parasites.

In conclusion, our findings are suggestive of parasitic stages adherence mainly with strong evidence of indication that consumers of raw fruits and vegetables are at a high risk of intestinal parasitic infection. The relevance of fruit and vegetables in the diet cannot be over emphasized and so is the need for adequate enlightenment programmes at primary health care level to help reduce transmission. Public health facilities should however consider legislative actions towards improvement of production, storage and marketing strategies for farm produce.

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