PARASITIC CONTAMINATION IN FRUITS FROM EFEHI STREET, NEW BENIN MARKET, BENIN CITY, EDO STATE, NIGERIA

BY

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DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

FEBRUARY, 2025.

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A DISSERTATION PRESENTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR IN SCIENCE (B. Sc) DEGREE IN ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

FEBRUARY 2025

CERTIFICATION

This is to certify that this project work on **PARASITIC CONTAMINATION IN FRUITS FROM EFEHI STREET, NEW BENIN MARKET, BENIN CUTY, EDO STATE NIGERIA** was carried out by **OMASANJUWA ANNETTE EDE (LSC2006647)** in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.)Degree in the Department of Animal and Environmental Biology, Faculty of Life sciences, University of Benin, Benin City.

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Student

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DR.MRS. O. EDO-TAIWO DATE

Project Supervisor

_____ _____

PROFESSOR MIKE OMOIGBERALE. DATE

DEDICATION

I dedicate this work to God for the grace to come this far, this work is a reality today because of God's wisdom and provision.

ACKNOWLEDGEMENT

I wish to specially appreciate my mother and my sisters, Daphne and Temisan for their constant support throughout the course of this work. To my ever-active supervisor, Dr. Mrs O. Edo-Taiwo thank you for your selfless actions towards us through the course of this work.

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ABSTRACT

A parasite is a living organism that lives inside or on another living organism, the host upon which it relies on for the resources necessary for its survival. A parasite depends on the host for its growth and reproduction. This study was conducted to investigate the prevalence of parasitic contamination in fruits from Efehi Street, New Benin Market and how the location affects it. The fruits examined were Cucumber (*Cucumis sativus),* Carrot (*Daucus carota),* African pear (*Dacryodes edulis),* and Garden egg (*Solanum aethiopicum).* The overall prevalence of parasitic contamination recorded in this study was 91.7%. Parasitic contamination was highest in Cucumber *(Cucumis sativus*) with Overall prevalence of 96.7% while the least contaminated was African Pear with prevalence of 86.7%. The parasites recovered from the fruits were *Ascaris lumbricoides*, *Strongyloides stercoralis, Trichuris trichiura, Enterobius vermicularis, Ancylostoma duodenale, Trichostrongylus* sp*., Taenia* sp*., Heterophyes heterophyes, Entamoeba* sp*., Toxoplasma gondii, Giardia intestinalis, Balantidium coli*, Unidentified nematode larvae and unidentified nematode egg. *Ascaris lumbricoides* was the most prevalent parasite and it was recovered from 60% of the samples. Most fruit samples got contaminated due to the unhygienic means of storage and transportation. The parasitic contamination of the fruits which were placed on the bare floor at the markets most seemed very much affected by this. Insufficient washing before consumption will aid in the transmission of these parasites to living organisms. Food-borne parasitic infections can be reduced by storing the fruits under proper sanitary conditions and ensuring proper washing of fruits with salt and water before consumption. Inculcating the practice of better hygiene by farmers, vendors and consumers will yield great results in the reduction of the transmission of these parasites

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Fruits are a major part of man's diet and an important component of a healthy diet because they contain essential vitamins which are necessary for growth and body nourishment (Carey *et al.,* 1996). Fruits have low fat, they also supply fiber, vitamins, minerals and health supporting phytochemicals. phytochemicals can help plants to survive, thrive, thwart competitors, predators and pathogens . Fruits contamination has gained interest from many researchers across the globe; this may be due to the increasing reports on foodborne illnesses linked with the consumption of fresh fruits contaminated with parasites (Akoma *et al.,* 2017). Parasitic helminthes affect more than 1.5 billion of the world's population and are considered as one of the main public health problems in developing countries, especially in tropical and subtropical regions (Flammer *et al.,* 2020). Eggs and larvae stages of helminthes may contaminate vegetables during pre-harvest or post-harvest Procedures (Luz *et al.,* 2017). The risk of several chronic diseases has decreased due to the regular consumption of wide variety of fruits, as they contain fibers and micronutrients including different minerals and vitamins (Liu, 2013). By infecting human beings, parasites can cause several health hazards, which can lead to significant complications such as malnutrition, severe anaemia, intestinal problems, delayed growth, lack of some vitamins, and so on (Bastien *et al.,* 2017). According to the World Health Organization (WHO), more than 1.4 million people have various types of intestinal parasites, which represent 24% of the world population with some kind of parasite (Ahmed, 2023).. Parasitic infections account for 25% of the 60 million deaths in the world (Arora *et al.,* 2008). Over 300 species of parasitic worms and over 70 species of protozoan

parasites in humans are acquired from food, raw fruits, water and animals (Alli *et al.,* 2011). There are several ways in which fruits can be contaminated; some of these become contaminated while still attached to the plant on the field due to untreated manure. Others become contaminated during harvesting, transportation, processing, distribution and marketing or even at home before consumption (Malann and Utitofon, 2016).

1.1 WHAT ARE PARASITES

Parasites are organisms which derive sustenance from their host while still causing it harm. Parasites cannot survive for long without their host. Parasitism is a type of consumer–resource interaction (Geltz, 2011). Parasites can also be defined as an organism living in or on another living organism obtaining from it part or its entire organic nutrient, and commonly exhibiting some degree of adaptive structural modification - such an organism that causes some degree of real damage to its host. Endoparasites include those parasites that are confined within the host's body (Bush *et al.,* 2001). They include the more familiar animal parasites such as protozoans, digeneans, cestodes, nematodes, and acanthocephalans. Many bacteria and all viruses are also endoparasitic. Parasites typically confined to the exterior of the host's body are called ectoparasites. Most parasitic arthropods and most monogeneans are ectoparasitic (Bush et al., 2001). The ingestion of parasites by mouth is one of the commonest methods of transmission of parasites which can get to the intestines by ingestion of food or water contaminated with faeces containing the infective stages of the parasites or ingestion of improperly washed fruits (Ocjei and kolkhatkor, 2008).

1.2 TYPES OF PARASITES

Parasites can basically be divided into protozoa, helminths and ectoparasites.

1.2.1 Protozoa

Protozoa is a large group of single-celled eukaryotes; they are motile and belong to the Kingdom Protista. They can survive as parasites or independently. Flagellates, amoeba, ciliates, and apicomplexa are the major medically important protozoans; they are responsible for some parasitic diseases such as amoebiasis and toxoplasmosis. Contamination of fruits with pathogenic protozoa could be due to fertilization with fresh animal manure, irrigation with polluted water, by infected food handlers and even in some regions because of the use of night soil (Nasser, 2022). Protozoans can be classified into the following according to modes of transmission:

1. **Enteric protozoans:** These parasites are majorly transmitted by faecal-oral route, contaminated food and water, they include: *Entamoeba*, *Giardia*, *Toxoplasma*, *Cyclospora*, *Balantidium*, *Microsporidia*, and *Cryptosporidium*.

2. **Sexually transmitted protozoans:** They are basically transmitted through sexual contact. An example is *Trichomonas*.

3. **Protozoans transmitted through Arthropods:** *Babesia*, *Plasmodium*, *Leishmania*, *Trypanosoma*.

4. **Other modes of transmission:** *Toxoplasma*, *Naegleria*, *Acanthamoeba*.

1.2.2 Helminthes

Helminthes is a general term which means worm, helminthes are worms. The helminthes are invertebrates and they possess elongated, flat or round bodies which can be in different forms. The different body forms of Platyhelminthes can be observed in Digenea where the schistosome

body form, amphistome body form, holostome body form and distome body forms are quite different from one another. There are two major phyla of helminthes known as Platyhelminthes and Nematoda. Platyhelminthes also called flatworms, which include the flukes (e.g. schistosomes) and tapeworms while the other phylum is Nematoda is also known as roundworms that include soil-transmitted helminthes and the filarial worms that cause lymphatic filariasis and onchocerciasis.

1.2.3 Ectoparasites

Ectoparasites are a taxonomically diverse group of organisms that infest the skin of human beings and other animals. Ectoparasitic arthropods and nematodes are similar in that an individual organism can produce skin lesions that are large enough to see with the unaided eye (Pollack *et al.,* 2017). Most ectoparasites are hematophagous, but a few feed on living skin cells and tissue. Some spend their entire life on one host, while others move from host to host as they develop and many simply alight on the host in order to feed. The definition of 'ectoparasite' is extended to include those parasites that burrow into the epidermis as well as those that remain on the surface. The vast majority of ectoparasites are members of the phylum Arthropoda. Humans are the preferred or only hosts of some ectoparasites, but the majority is less specific in their choice, or turn to humans only when their primary host is unavailable (Feldmeier, 2008).

1.3 PARASITES TRANSMITTED VIA FRUITS

Fruits and vegetables have held an importance place in dietary guidance because of their concentration of vitamins, especially Vitamins C and A; minerals (e.g. electrolytes) and more recently phytochemicals such as antioxidants (Joanne and Beate, 2012). Fruits are generally known to be a very important part of man's diet but what is not well understood is that

consuming fruits that are not properly cooked can lead to ingestion of parasites which are dangerous to human health. The cysts, ova and larvae of certain intestinal parasites of man can be recovered from fruits after proper examination and these parasites include: protozoa cysts, helminthes eggs and larvae.

1.3.1 Protozoan cysts found in fruits

Protozoan cysts are known to contaminate fruits; these cysts usually include those of *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Toxoplasma gondii*, *Cryptosporidium*, *Blastocystis* and *Cyclospora*. Protozoa typically have an active form called a trophozoite, and a dormant protective form called a cyst (Kamiya and Zundel, 2023). Although the protozoa that infect the human gastrointestinal tract are quite diverse, they all exhibit a similar life cycle and mode of transmission referred to as fecal-oral transmission (Fig. 1). The infection is acquired through the ingestion of food or water that has been contaminated with fecal matter. In most cases a specialized stage called the cyst initiates the infection. After ingestion the cyst converts into a trophozoite. The trophozoite is often motile and exhibits an active metabolism. Most importantly, the trophozoite is the replicative form of the parasite and leads to an expansion of the population. Some of the trophozoites convert back into cyst stages which are passed in the feces. The cyst stage is characterized by a cyst wall that makes the parasite more resilient to environmental elements such as desiccation. Many protozoan cysts can survive for months outside of the body if kept moist and cool. Furthermore, the cysts are highly infectious in that ingestion of only a few cysts can initiate an infection (Wiser, 2021).

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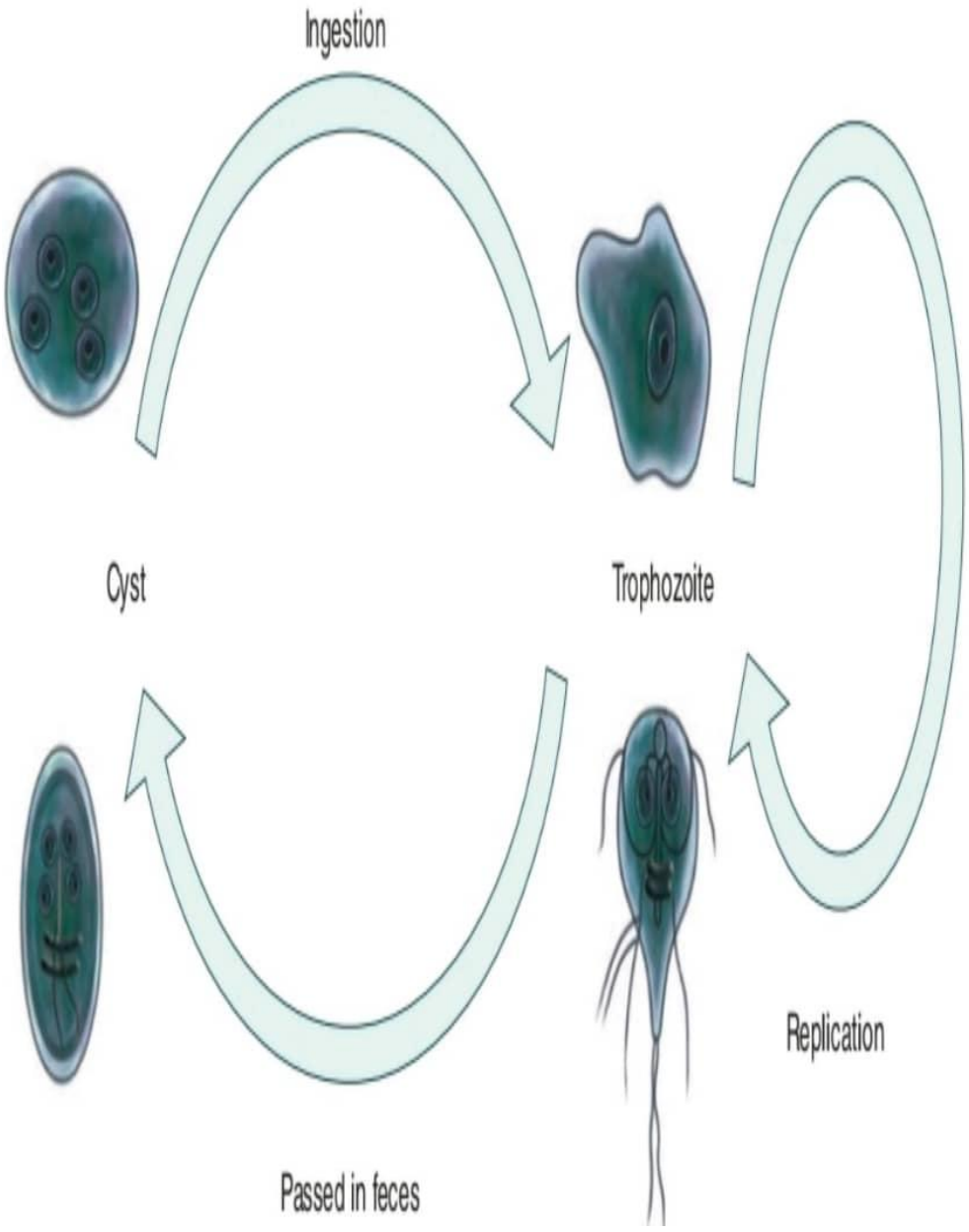

Fig. 1.2 protozoan life cycle showing the stages and morphology

Source: Wiser(2021).

1.3.2 Nematodes found in fruits

Nematodes eggs are also transmitted via ingestion of contaminated fruits, these fruits are usually contaminated with the eggs and larvae of the nematodes. Human intestinal nematode parasites include *Enterobius vermicularis, Ascaris lumbricoides, Strongyloides stercoralis* and *Ancylostoma duodenale.* The life cycle of parasitic nematodes is clinically important. Some nematode infections can be transmitted directly from infected to uninfected people; in others, the nematode eggs must undergo a process of maturation outside the host. In a third category, the parasites may spend a part of their life cycle in the soil before becoming infective to humans (Karanis *et al.,* 2007). The intestinal nematodes undergo a direct life cycle not involving intermediate host as shown in figure 2. In the simplest type of life cycle e.g *Enterobius vermicularis,* embryonated eggs are ingested by the host and hatch in the intestine where they reach maturity, only burying themselves temporarily in the mucus membrane. (Daminabo and Damen, 2020). Some directly developing species e.g *Ascaris lumbricoides,* after hatching in the intestine undergo tissue migration through the body returning to become established in the intestine. The simple type of life cycle represented by *Ascaris* may be varied in other species by the first-stage embryo hatching outside the body, developing to an infective larva, and re-infecting its host by skin penetration (Petri and Singh, 1999). In yet another type of life history, there may be an alternation of a parasitic generation with a free living one. The best known is those of various species of the genus *Strongyloides*, in which the free larvae may develop to free living adults if the external environment is suitable (Daminabo and Damen, 2020). In the intestine, the parasite grows and develops into adult male and female which lay eggs

after mating when matured. The adults attach to the mucosa of the host and inhabit the intestine for as 140 years as in Hookworm, 1-2 months as in *Enterobius vermicularis* and about 12 months for *Ascaris lumbricoides* (Procop, 2001). The severity of infections caused by worms which do not divide within the host is proportional to the number of infecting worms e.g *Ancylostoma duodenale* (Hookworms) which attach to the intestinal wall, consumes about 0.25 ml of host blood per day.

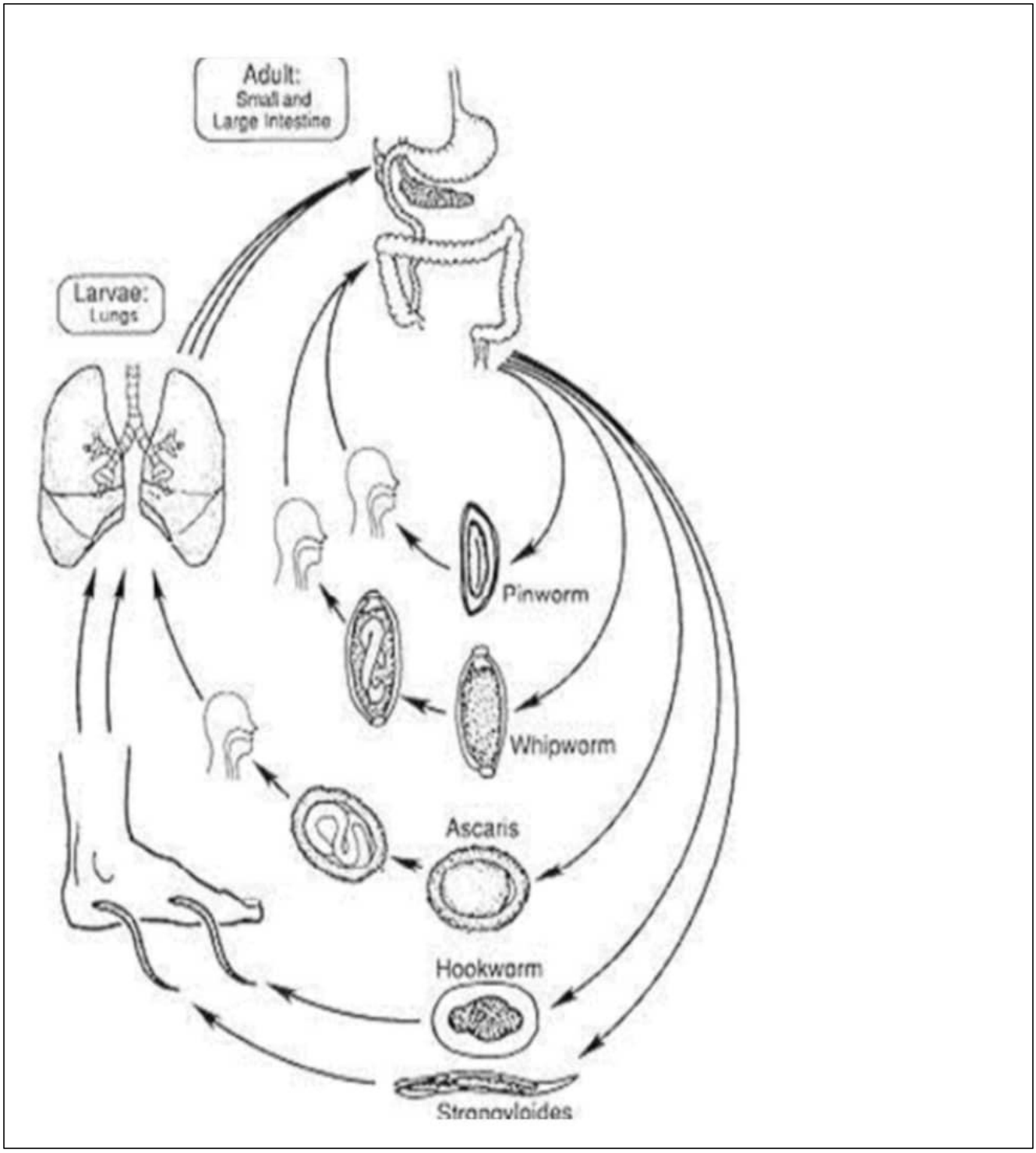

Fig. 1.3: Life cycle of intestinal nematodes

Source: Daminabo and Damen (2020)

1.3.3 Platyhelminthes found in fruits

Platyhelminthes is the other helminth group which consists of worms which are segmented. Major parasitic platyhelminthes that can be contaminated via fruits include flukes and tapeworms; *Fasciola gigantica, Taenia, Schistosoma mansoni, Clonorchis sinensis, Paragonimus westermani.* Platyhelminthes undergo an indirect life cycle which involves an intermediate host. A typical example of a platyhelminths life cycle can be observed in *Taenia* species. Contamination or autoinfection from taeniasis cases results in aberrant intermediate infection (cysticercosis)

1. Humans eat raw or undercooked cysticerci infected meat
2. Parasites grow to its adult form in the small intestine (taeniasis).
3. Eggs are deposited in the large intestine and released to the environment.
4. Cattle (or pigs in the case of *T. sagenata*) become infected by ingesting infective eggs in the environment, which can be found in fruits and vegetables
5. Oncospheres penetrate the intestine wall and travel to the musculature.
6. Oncospheres develop into cysticerci in muscle of the intermediate host (cysticercosis). (Ortiz *et al.,* 2024)

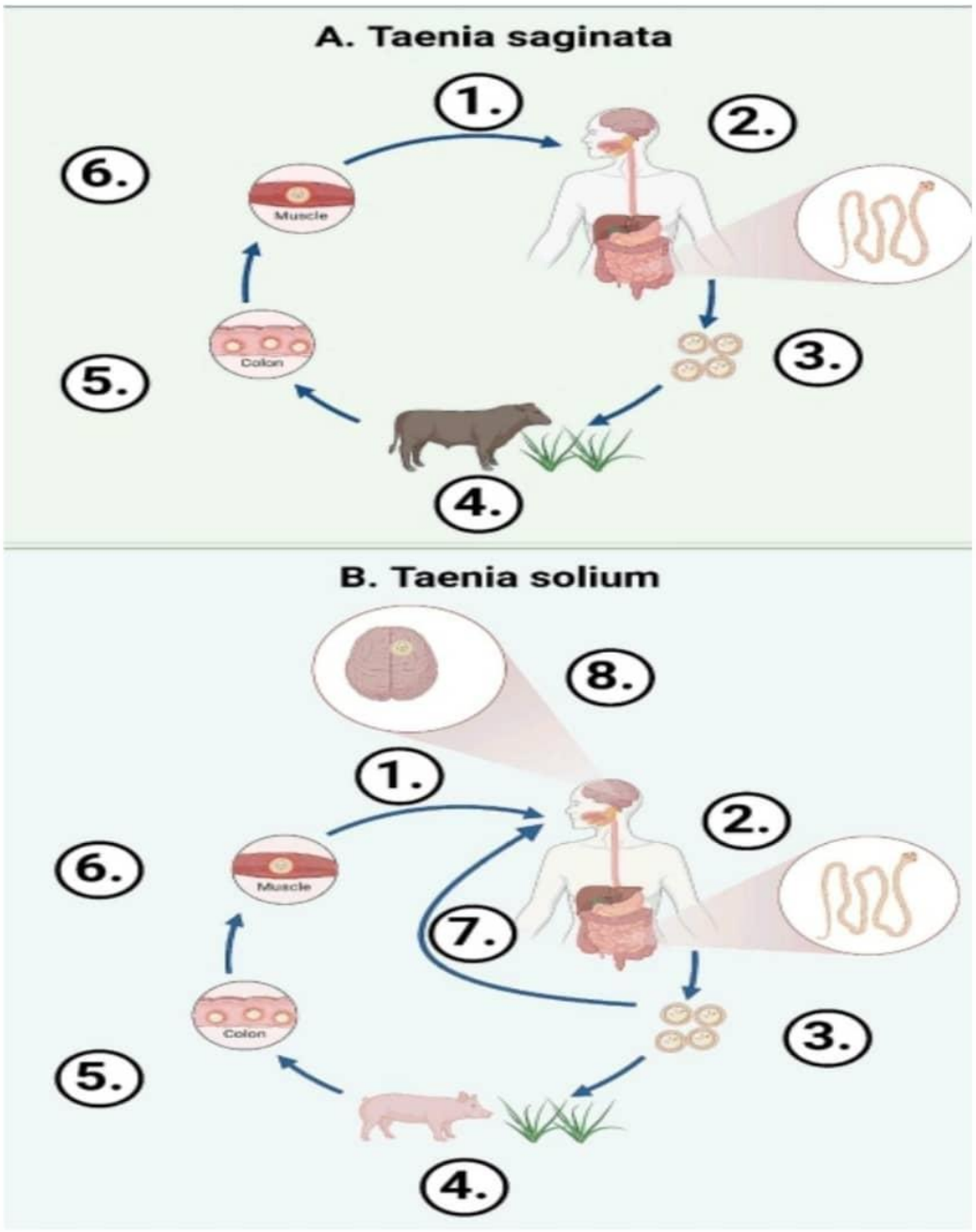

Fig 3: Life cycle of a typical food-borne platyhelminth

Source: Ortiz *et al., (*2024)

1.4 AIMS AND OBJECTIVES OF THE STUDY

This study is aimed at evaluating the activities of parasites in fruits sold in a major market, Efehi, New Benin market, Benin City. The objectives are to determine:

1. The rate of parasitic contamination of fruits obtained from Efehi Market.
2. The species of parasites in the samples
3. The prevalence of each specie of parasites in the sample
4. The stages of the parasites present in the samples
5. How the location which the fruits were purchased impacts the parasitic contamination of these fruits.

1.5 JUSTIFICATION OF THE STUDY

The rate of parasitic contamination is high and most of the fruits gotten from major markets in local areas have been observed to play a part in the transmission of parasites. The knowledge of this has led to my inquisitiveness concerning this topic and this made me choose a major market which supplies fruits to majority of fruit sellers in Benin City and it's environs, also considering that Efehi street in New Benin market hasn't gotten as much attention as it should get. This study intends role bridge the gap and she'd more light on the rate of parasitic contamination of the fruits from this market and the possible reasons behind it, in addition to this, possible solutions will be proffered.

CHAPTER TWO

2.0 LITERATURE REVIEW

Infections from contaminated fruits have been as issue in the world for a long time. Many scientists have considered this menace in the past and this has led to the conduction of different researches within and outside Nigeria.

Hassan et al. (2011) carried out a survey on the presence of parasitic ova, cysts and larva on common fresh fruits and vegetables sold at some major markets in Ibadan, Oyo State, Nigeria. A total of 264 fruits and vegetables pieces were examined using standard wet mount procedure and Kinyoun acid fast stain to detect parasites. 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 The percentage of fruits and vegetables with eggs, cysts or larvae of six 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%). Intestinal parasite eggs or cysts were found on 48.48% of fruits and vegetables (128/264; 95% confidence interval (C I), 39.98 Ñ 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.

Ohaeri and Unogu (2011) carried out a study on soil transmitted helminths of some common fruits and vegetables in Umuahia, Abia State Nigeria which involved the assessment of Soil transmitted helminths of some common fruits and vegetables sold in various markets in Umuahia using formal-ether concentration technique. High level of contaminations were found in both fruits (43.8%) and vegetables (77.5%) examined. Talinium triangulare recorded the highest rate of contamination (100%) while Persea americana (Avacado pear) was the least contaminated (20.0%). The helminths identified were *Ascaris lumbricoides,* hookworm, *Strongyloides stercoralis* and *Trichuris trichuria, Ascaris lumbricoides* was the most prevalent (80.0% and 80.6%) in fruits and vegetables respectively, while *Trichuris trichuria* was the least encountered. The study indicated a high rate of geohelminth contamination of fruits and vegetables in the study area.

Elom *et al.* (2012) carried out a study on The prevalence of geohelminths on edible fruits and vegetables cultivated in rural villages of Ebonyi State, Nigeria, a total of 250 samples (five per product from each market) were examined, sedimentation and floatation methods were used in the examination of the samples. 250g samples of each fruits and vegetables were washed in distilled water in a sterile beaker for the removal of the parasitic ova, larva or cysts. The suspension was strained through a sterile sieve to remove undesirable materials. The filtrate was centrifuged at 3000 rpm for 15 minutes and the supernatant was discarded into the disinfectant jar. The sediment was mixed up and a drop was applied on the centre of a clean grease-free microscope slide and a clean cover slip was placed gently to avoid air bubbles and over-flooding.

The preparation was examined under microscope for parasites using X10 and X40 objectives.101 (40.4%) of the two hundred and fifty (250) fruits and vegetables were positive for geohelminths microscopically, among the fruits, pineapple had the highest number of geohelminths, (72%) while Pawpaw had the lowest (12 %) and among the vegetables, water leaf had the highest number of geohelminths (64 %) while bitter leaf had the lowest number of geohelminths (28 %). Geohelminths identified were ova of *Ascaris lumbricoides* (54.5 %), *Strongyloides stercoralis* (6.9 %), ova of Hookworm (23.8 %), *Trichuris Trichiura* (8.9 %), *Enterobius vermicularis* (5.9 %). This study showed high level of fruits and vegetables contamination with geohelminths in the study area.

Hassan et al. (2013) carried out a survey on the presence of parasitic ova, cysts and larva on common fresh fruits and vegetables sold at some major markets in Ibadan, Oyo State, Nigeria. A total of 264 fruits and vegetables pieces were examined using standard wet mount procedure and Kinyoun acid fast stain to detect parasites. 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 The percentage of fruits and vegetables with eggs, cysts or larvae of six 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

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Bekele *et al.* (2014) conducted a study on parasitic contamination of raw vegetables and fruits collected from selected local markets in Arba Minch town, Southern Ethiopia. A total of 360 samples of 8 different types of fruits and vegetables were examined, the eight types of fruits and vegetables were *Persea americana* (Avocado)*, Lactuca serriola (*Lettuce*), Brassica oleracea (*Cabbage*), Daucus carota (*Carrot*), Lycopersicon esculentum (Tomato), Capsicum annuum*(Green pepper*), Musa paradisiaca (*Banana*), and Mangifera indica* (Mango) were purchased from four conveniently located local markets, namely, Sikela (1235 meters above sea level), Secha (1300 meters above sea level), Yetnebersh (1290meters above sea level), and Konso-Sefer (1275 meters above sea level) in Arba Minch town. An equal number of samples (45 each, total 360 samples) were collected from the markets. Each sample was placed in a separate plastic bag and labeled with a unique number and its date of collection, and brought to the Arba Minch Hospital Leishmaniasis Research Centre Laboratory for parasitological analysis. Approximately 200 grams of each vegetable or fruit was soaked (for 15 minutes) in one liter of physiological saline, followed by vigorous shaking with the aid of a mechanical shaker for 15 minutes. After overnight sedimentation in the washing solution, 15 milliliters of the sediment was transferred to a centrifuge tube using a sieve to remove undesirable matter. For concentrating the parasitic stages (ova, larvae, cysts, and oocysts), the tube was centrifuged at

3000 revolutions per minute for five minutes. After centrifugation, the supernatant was decanted carefully without shaking. Then, the sediment was agitated gently by hand to redistribute the parasitic stages. Finally, the sediment was examined under a light microscope using × 10 and × 40 objectives. Out of the 360 samples examined, 196 (54.4%) were contaminated with at least one type of parasite. The parasites detected included ova of *Ascaris lumbricoides, Toxocara spp., Hymenolepis nana,* and *H. diminuta; oocysts of Cyclospora, I. belli, and Cryptosporidium spp.; cysts of G. intestinalis and E. histolytica/dispar. A. lumbricoides* (20.83%) was the most frequently detected parasite, followed by *Toxocara*(15.83%), *Hymenolepis nana* (15.56%), *E. histolytica/dispar (14.44%), G. intestinalis (*10.0%), H. *diminuta (*7.78%*), Cyclospora Cryptosporidium* (4.72%*), and I. belli (3.06%).* It was also observed that decreased parasitic contamination was significantly associated with washing the products before displaying it for selling.

Tefera *et al.* (2014) conducted a study on parasitic contamination of fruits and vegetables collected from four local markets of Jimma town in Ethiopia, they investigated 45 samples each from 8 types of fruits and vegetables including lettuce, cabbage, carrot, tomato, green pepper, banana, mango(360 samples in total). A portion of each fruit and vegetable was washed separately in 500ml of normal saline for detaching parasitic stages of helminths and protozoan parasites, after overnight sedimentation, 15ml of the sediment was transferred to a centrifuge tube using sieve to remove undesirable matter. The tube was centrifuged at 3000rpm for five minutes to concentrate the parasitic stages. After centrifugation, the supernatant was decanted carefully without shaking, then the sediment was agitated gently by hand, the sediment was examined under a microscope afterwards. 208 samples were identified to be contaminated with at least one type of parasite with an overall contamination rate of 57.8% .The highest prevalence

of intestinal parasites was recorded in salad (16.8%) followed by cabbage (14.9%), carrot (13.5%), lettuce (12%), green pepper (11.5%), banana (11.1%), and tomato and mango each 10.1. Endale *et al.* (2015) conducted a study on the detection of medically important parasites in fruits and vegetables collected from local markets in Dire Dawa, Eastern Ethiopia. 47 samples each of 8 types of fruits and vegetables (lettuce, cabbage, carrot, tomato, green pepper, banana, orange, and spinach) were randomly collected from nine conveniently selected local markets, 376 samples were collected in total. 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. 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. 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 *Giardia lamblia*, 35 (9.3%), *Entamoeba histolytica*, 33 (8.8%), *Strongyloide spp*, 30 (8%), *Cryptosporidium spp*, 29 (7.7%), *Cyclospora spp*, 28 (7.4%), A*scaris lumbricoides*, 24 (6.4%), *Isospora spp*, 16 (4.3%), T*richuris 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 and those displayed on a floor.

Luka *et al.* (2016) conducted a study on Prevalence of helminths on raw vegetables and hygienic practices among vegetable marketers in Maiduguri, Borno state, Nigeria. A total of 320 samples of spinach, cabbage, lettuce, carrot, green beans, green pepper, garden egg and tomatoes were collected. 40 samples per each vegetable type were collected in the study, while 10 samples per vegetable were collected from every market. One hundred questionnaires were administered to 100 vegetable marketers in order to evaluate hygienic practices during sourcing, transportation and retailing of vegetables. Collected vegetables were analysed using standard parasitological technique. In all, 29 (9.06%) vegetables were contaminated with one parasite or the other, consisting of *Strongyloides* egg 5 (1.56%), *Taenia* egg 4 (1.25%), Nematode larvae 17 (5.31%), *Ancylostoma* egg 1 (0.31%) and *Ascaris lumbricoides* egg 2 (0.62%). Based on the type of vegetable; cabbage and lettuce each had 5 (12.5%) parasites, while carrot and green beans had 2 (5.0%) and 17 (42.5%) respectively. Recovered parasites were significantly associated with the vegetable types. Custom/Gamboru Market recorded the highest contamination 9 (11.25%) parasites, followed by University/Bama Road 7 (8.75%), Baga Road/Monday Market 5 (6.25%), while Bulumkutu/Gomari had least contamination rate of 8 (10.0%). It was concluded that there was no statistically significant association between parasites isolation and the market location. Eighteen (18)(62.07%) of the contaminated vegetables were unwashed

Auta *et al.* (2017) performed a study on Parasitic Contamination of Common Fresh Fruits and Vegetables Sold in Markets within Dutsin-Ma Town, Katsina State, Nigeria, they investigated 18 samples each from 3 fruits(cucumber, orange and watermelon) and 3 vegetables (spinach, cabbage and roselle). 250g of each vegetable sample were weighed aseptically and hand washed

by shaking vigorously for 2 minutes in 500ml of normal saline (0.85% NaCl). Non- leaf vegetables and fruits were washed directly in 500ml of normal saline. The suspension was strained through sterile 0.4 mm standard sieve to remove undesirable materials. The filtrate was left to stand for 24 hours. 480 ml of the supernatant was decanted and kept aside while the sediments in the remaining 20ml were resuspended by votexing for 10 second. Aliquot 10ml was then centrifuged at 2000 rpm for 20 minutes. Roughly 90% of the supernatant was decanted after centrifugation and the deposits votexed for another 10 seconds. Two drops of the suspension were placed on clean microscope slide. A drop of lugols iodine was added, covered with a clean cover slip and observed under binocular compound light microscope at x10 and x40. A total of 45 (41.67%) samples were found to be positive for parasites during the study. *Ascaris lumbricoides* was the most common parasite found, with prevalence of 11 (10.19%), while *Strongyloides stercolaris, Schistosoma spp, Giardia lamblia and Entamoeba hartmani* were least, with 1 (0.93%) each. Results showed spinach (vegetables) having the highest parasitic contamination 15 (83.33%) each, while cucumber and watermelon (fruits) had the least contamination, 2 (11.11%)

Istifanus *et al.* (2017) conducted a study on Parasitic agents in fresh fruits and vegetables sold in open markets in Bauchi, Nigeria. A total of 776 samples comprising 182 samples of four different types of fruits, including banana (*Musa sapientum*), mango (*Mangifera indica*), orange (*Citrus sinensis*), and guava (*Psidium guajava*); and 594 samples of six different vegetable types, including cabbage *(Brassica oleraceae*), spinach (*Spinacia oleraceae*), carrot (*Daucas carota*), lettuce (*Lactuca sativa*), onion (*Allium cepa*), and tomato (*Lycopersicon esculentum*) were randomly obtained from different open markets in Bauchi metropolis. each sample of fruit and vegetable was thoroughly washed in distilled water. Subsequently, the contaminated water was

processed through simple brine floatation as well as formol-ether concentration techniques in accordance with the procedure outlined by Cheesbrough (2006). Samples were then examined for ova and cysts. In order to differentiate the intestinal nematodes, larvae were cultured and subsequently harvested using the Baerman's method.They identified the isolated parasitic ova and cysts on the basis of morphological characteristics. Their results showed 26 out of 182 fruit samples (14.3%), and 82 out of 594 vegetable samples (13.8%) were contaminated with parasitic ova and cysts of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, *Taenia spp*, *Giardia intestinalis*, *Entamoeba coli* and *Hymenolepis nana*. *Ascaris lumbricoides* was the most common parasitic contaminant in both fruits and vegetable samples with prevalence of 36.6% followed by *Taenia* (24.4%), *Ancylostoma duodenale* (18.3%), *Strongyloides stercoralis* (7.3%), *Entamoeba coli* (6.1%), *Giardia intestinalis* (6.1%) and *Hymenolepis nana* (1.2%).

Ikpeze *et al.* (2017) carried out a study on soil-transmitted helminth parasites contaminating edible raw vegetables and fruits sold at nkwo-edo market nnewi nigeria. 100g of each leafy vegetables ('Utazi' (*Gongronema latifolium),* Garden egg *(Solanum macrocarpon),* Bitter leaf *(Vernonia amigdalina) and f*resh fruits *(A*frican pear *(Dacryodes edulis),* Oil-palm *(Elaeis guineensis),* Carrots *(Daucus carota) and* Garden *egg (Solanium macrocarpon*) were weighed out and further examined for ova and larvae of soil transmitted helminths using sedimentation method.Equal weights (100mg) of each sample was taken into properly labeled sterile polythene bags and transported to the laboratory for parasitological analysis within 20 minutes of collection. Each sample was washed for the separation of any ova or larvae. Concentration of the eggs in the sediment was carried out by centrifugation. The sediment from each vegetable and fruit was put in different centrifuge tubes and centrifuged at 5000 rpm for 5

minutes. The supernatant was decanted and the sediment was stirred. A drop of each of the sediments was put on the center of clean grease-free glass slide and stained with Lugol's iodine after which a clean cover slip was placed gently to avoid air bubbles and over flooding. The preparation was examined under light microscopy using x10 and x40 objective lens. From the results, 36% of Garden egg, 24% of Bitter leaf, and 12% of 'Utazi' leafy vegetables, as well as 45% of oil palm, 35% of African pear, 20% of Carrots, and 10% of Garden egg fruits were contaminated with different soil-transmitted helminths. Generally, 24% and 27.5% of all vegetables and fruits sampled were respectively contaminated with soil transmitted helminths, comprising eggs of *Ascaris lumbricoides* (59.3%), larvae *of Strongyloides stercoralis (*22.2%*), eggs of hookworm species (*14.8%*), and ova of Trichuris trichiura* (3.7%).

Etewa *et al.* (2017), carried out a study on parasitic contamination of commonly consumed fresh vegetables and fruits in some rural areas of Sharkyia Governorate, Egypt. They examined 420 samples in total of lettuce, watercress, parsley, cucumber, carrots and strawberry from some rural areas of Sharkiya governorate's markets, the samples were washed and the solution resulted from washing was filtered and centrifuged to concentrate the parasitic stages, sediments and supernatants were microscopically examined using iodine and modified Ziehl–Neelsen stained smears, then the positive samples were soaked separately in acetic acid 5% and potassium permanganate for 15 and 30 minutes, then they were tested by 0.2% trypan blue stain to detect the parasites. Parasites were detected in 164 (39%) samples, *Giardia lamblia* cysts were the most prevalent parasite(12.6%) followed by *Cryptosporidium spp. cysts (7.6%), Entamoeba spp.* cysts *(6*.2%), *Blastocystis hominis* cysts *(3.8%), Hymenolepis nana* eggs *(2.8%), Ascaris lumbricoides* eggs *(*1.9%*), various helminths* larvae *(1*.6%), *Enterobius vermicularis* eggs *(*1.4%) *and*

Balantidium coli cysts (*0.9%).* The highest contaminated was watercress (55.7%) and the least contaminated was carrots (27.1%).

Ogunremi *et al.* (2017) carried out a sampling on Human Enteric Parasitic Pathogens in Fresh Fruits and Vegetables Consumed in Ile-Ife, Osun State, Nigeria. A total of 350 samples of fruits and vegetables were gotten from five markets in Ile-Ife, The fruits and vegetables used in this study were selected from five markets in the study area. They include apple *(Malus domestica),* tomatoes (*Lycopersicon esculentum)*, carrot (D*aucus carota)*, Cucumber *(Cucumis sativu*s) Waterleaf (T*alinum trangulare*), Spinach (A*maranthus spinosu*s), White jute (*Corchorus olitorius*), African spinanch (*Solanecio biafrae*), Fluted pumpkin (T*elfairia occidentalis)*, Cabbage *(Brassica oleracea*). the samples were analyzed using sedimentation and floatation techniques. A total of 244 (69.7%) of the fruits and vegetables sampled were positive for eggs, cysts or oocysts of parasites, among the fruits, tomatoes had the highest number 18 (50%) of parasites while the lowest 8 (25%) was on apples. Four of the vegetables (water leaf, spinach, white jute and African spinach) showed equal degree of parasitic contamination which was 100% while cabbage had the lowest 22 (64.7%). The overall prevalence of isolated parasites from 350 samples of fruits and vegetables investigated in this study was: ova of *Enterobius vermicularis* 64 (18.29%); ova of Ascaris lumbricoides 31 (8.86%); cysts of Entamoeba histolytica 18 (5.14%); cysts of *Giardia lamblia* 19 (5.42%); ova of hookworms 8 (2.29%); cysts of *Entamoeba coli* 27 (7.71%); oocysts of C. cayetenensis 17 (4.86%); cysts of *Balantidium coli* 29 (8.29%); ova of *Teania spp* 15 (4.29%).

Yusof *et al.* (2017) conducted a study to investigate the occurrence of intestinal parasitic contamination in select consumed local raw vegetables and fruits in Kuantan, Malaysia. One kilogram of the following locally consumed raw vegetables and fruits were collected randomly

from the Kuantan wet market during the monsoon season and the dry season *Centella asiatica* (pennywort or pegaga), *Ipomoea aquatica* (water spinach or kangkung), *Persicaria odorata* (Vietnamese coriander or daun kesum), *Averrhoa carambola* (carambola or belimbing), Syzygium samarangense (rose apple or jambu air) and Psidium guajava (guava or jambu batu). To successfully extract parasitic ova, larvae, and cysts from the random samples, each type of vegetable and fruit was rinsed and soaked with 100 mL of distilled water in a plastic container for 15 minutes, a sterile strainer was used to filter the suspension, and the filtrate was centrifuged at 1,500 rpm for 10 minutes afterwards. The supernatant was discarded, and the remaining sediment was transferred to glass slides for microscopic observation. A standard wet mount procedure and modified Ziehl-Neelsen staining were used for the detection of parasites. The examination of vegetables showed five different parasite species (*Entamoeba, Blastocystis spp, Diphyllobothrium, Strongyloides* and unidentified trematode fluke). The vegetable samples collected were positive for both helminthes and protozoa. However, the fruits samples were negative for parasitic contamination. Pegaga was the most contaminated leafy vegetable in this study, and *Strongyloides* was the parasite found most frequently.

Amawulu *et al.* (2019) carried out a study on fruits consumption pattern and its implication on parasite transmission in Yenagoa metropolis, Nigeria. 400 fruit samples were examined, Fruits used in this study were Tomato, Carrot, garden egg and Pepper. A total of 400 fruits were picked randomly from ten stands in Tombia junction open market. Each fruit were washed thoroughly in an equal volume of distilled water into a clean flask. The preparation was filtered through wet gauze into a clean one-liter conical flask to remove debris. The elution of eggs and cysts of parasites from the fruits was done using a concentration method. Each preparation were dispensed into clean centrifuge tubes and centrifuged at 1500 rpm for minutes. The supernatant

was discarded into a disinfectant jar and the sediment was mixed with a few drops of Lugol iodine. A drop was applied on the center of a clean grease-free slide, and covered with slip. Examination was done under the microscope for parasites using 10X and 40X objectives. 100% of the total respondents accepted that they were used to eating fruits, Apple accounted for (36%) of the most preferred fruit consumed. The percentages of people, who always wash their fruits before eating, wash fruits with water and salt before eating and those who do not wash their fruits before eating were 52%, 18% and 30% respectively. Out of the 40 fruits examined for parasites infestation, 20% were infested with parasites, 5 species of parasites were recovered from the fruits. The fruits in the order of parasites infestation were a carrot (51.22%), Tomato (36.6%), Garden egg (17.1%), pepper (0.0%). Parasites in the order of occurrence were *Entamoeba histolytica* (58.5%), *Ancylostoma duodenale* (14.6%), *Ascaris lumbricoides* (17.1%) *Trichuris trichura* (12.2%) and *Strongyloides stercoralis* (2.44%).

Bekele *et al.,* (2019) carried out a sampling on fruit and vegetable contamination with medically important helminths and protozoans in Tarcha town, Dawuro zone, South West Ethiopia. They used a total of 270 fresh vegetables and fruits samples, the samples were microscopically examined after washing with 500mL normal saline and 24-hr sedimentation of the washing solution followed by centrifuging at 2000rpms for 5 minutes, after centrifugation, the supernatant was decanted leaving the sedimen, the sediment was examined under a light microscope for protozoans and helminths worm afterwards. Of the 270 fresh vegetable and fruit samples, 115 (42.6%) were found positive for intestinal parasites. A high level of contamination in fresh vegetable and fruit samples was recorded in cabbage 71.1% while tomato was the least contaminated 24.4% . The identified medically important parasites were *Entamoeba histolytica/dispar, Giardia intestinalis, Ascaris lumbricoides, Hymenolepis nana, Toxocara spp,*

Hymenolepis diminuta, and Cystoisospora belli. The most predominant parasite encountered was *Ascaris lumbricoides* (16.7%) whereas *Cystoisospora belli*(2.6%) the least detected, all of the vegetables and fruits that were contaminated were with more than one parasite species. They concluded that the high contamination rate of fruits and vegetables consumed in Tarcha town indicates that fruits and vegetables may serve as sources for parasitic infections among the community. They suggested that washing of fruits and vegetables, health education on improving hygienic practices for vegetable handlers, and improvements in sanitation of the town and its surrounding may help in preventing parasitic contamination.

Usman *et al.* 2020 did a research on Prevalence of intestinal parasites on fruits and vegetables sold in Bauchi North, Bauchi State, Nigeria. The study was to determine the prevalence and type of parasites on fruits and vegetables sold in the area. Four different fruits and vegetables were purchased from the main markets of the three selected Local Governments (Katagum, Jama'are and Shira) once in a week. A total of 288 samples, comprising of 144 fruits (watermelons, oranges, pineapples and Bananas) and 144 vegetables (cabbages, lettuces, onions and tomatoes) were collected once in a week from September to November and examined for intestinal parasites using sedimentation method, each sample was washed using 20ml of distilled water in a plastic container. The washed water was strained using a sterile sieve to remove any unwanted materials. The filtrates were poured into a test tube and centrifuged at 3000rmp for five minutes. The supernatants were discarded, and the remaining sediments were mixed. A drop of sediment was placed on the center of a clean grease-free slide using dropper. One drop of iodine was added and a clean cover slip was placed gently to avoid air bubbles and over-flooding. The prepared slides were examined under compound microscope for identifications of parasites at magnification X10 and X40. The recovered ova and cysts were identified based on their

morphological characteristics Intestinal parasites were detected on 29 (20.1%) fruits and 17 (11.8%) vegetables. *Entamoeba histolytica* 37.9% and *Ascaris lumbricoides* 41.2% were the most prevalent parasites on the fruits and vegetables samples respectively. They concluded that there is prevalence of intestinal parasite on fruits and vegetables sold in the study area. Poor environmental sanitation and preservation techniques by vendors, farmers and consumers as well as type of fertilizer and water used were also discovered to play a key role in contamination of fruits and vegetables, the researchers recommended that Farmers and consumers should be educated on good hygiene farming and consumption of fruits and vegetables.

Morales- Figueroa *et al.* 2021 worked on a sampling on the Occurrence of intestinal parasites in fruits and vegetables from markets of Northwest Mexico. They collected a total of 400 fruit and vegetable samples from unregulated open-air markets and closed markets in Caborca region of Northwest Mexico; including melon, peach, asparagus, and grapes. Each piece of melon and peach were washed in 1L of 0.95% NaCl in the sterile sampling bags during sampling. For the asparagus and grapes, a number of spears and bunches were taken randomly to complete a weight of approximately 250g per product. All the bundles of asparagus and bunches of grapes were subjected to the washing process. Next, the wash water was filtered by a vacuum pump using Whatman filters (1 μm). Filtration was performed at a flow rate of 0.01 L/s. While still in the filter, part of the material was processed using the diagnostic techniques to detect the genus and species of parasitic infections. Another portion of the material was re-suspended and homogenized in 3 ml of sterile distilled water, transferred to 5ml Eppendorf tubes, and then stored at -20°C for Enzyme-Linked Immunosorbent Assay (ELISA) analyses to diagnose species of Cryptosporidium and Entamoeba. An overall prevalence (45%) of parasitic contamination was found in the 400 fruit and vegetable samples. *Endolimax nana* (27.5%) and

Entamoeba coli (17.5%) were the most common nonpathogenic parasites, while the most prevalent parasitic pathogens were *Cryptosporidium spp*.(11.7%), *Cyclospora spp*.,(11.0%), and *Blastocystis hominis* (9.2%). Asparagus (31%) and grapes (38.9%) had significantly higher percentages of overall and multiple parasitic contamination than melon (10.6%) and peaches (19.4%). The fresh produce from the open-air markets had significantly higher overall parasitic contamination (53.5%) than those of the closed establishments (36.5%). They concluded that there was a high overall prevalence of parasitic contamination was found in the fruits and vegetables analyzed in the Caborca region showing a serious public health concern.

Apuu *et al.* 2022, carried out a study on prevalence of parasites of medical importance on fruits and Vegetables sold in markets of Makurdi and Otukpo, Benue State, Nigeria. Samples were obtained from 3 markets per location and a total of 9 fruits and vegetables including tomato (*Lycopersicum esculentum*), Garden egg (S*olanum aethiopicum*), Tiger nut (*Cyperus esculentus L.)* and six (6) vegetables including Fluted pumpkin (*Telfairia occidentalis)*, Green pepper (*Piper nigrum*), Green beans (*Phaseolus* v*ulgaris*) , Scent leaf (*Ocimum gratissimum*), Carrot (*Daucus carota*), and Amaratus (*Amarathtus hybridus*) were used for the study. Sediments obtained from washing the fruits and vegetables with normal saline were centrifuged and then viewed under the microscope using x4 and x10 objectives. Eggs, cysts, and larvae of parasites including *Ascaris lumbricoides* (37.60%), *Giardia lamblia* (10.86%), E*ntamoeba histolytica* (14.13%), *Fasciola hepatica* (10.86%) and *Strongyloides stercolaris* (22.82%) were found to be prevalent in this study. The highest contamination was observed in pumpkin 15(50.0%) while Green pepper and Amarathus 9(30.0%) had the lowest contamination. They concluded from this study that there was high contamination (40.4%) of fruits and vegetables sold in Makurdi and Otukpo markets in

Benue State, The study further revealed that consumption of raw fruits and vegetables is the probable source of transmission for intestinal parasites to man.

Gemechu e*t al.* 2022 did an assessment on Intestinal Parasites and Its Associated Factors among Fruits and Vegetables Collected from Local Markets of Bule Hora Town, Southeast Ethiopia. 391 samples of selected vegetables and fruits were collected in total. A portion (200 g) of each fruit and vegetable was washed separately in 500 ml of normal saline (0.85% NaCl). To allow for appropriate sedimentation, the washing solution was then let to stand on the bench for a whole night. Then, using a Pasteur pipette to collect the supernatant, 15 ml of the sediment was transferred to a centrifuge tube using a sieve to filter out unwanted components. The tube was centrifuged for five minutes at 3000 rpm to concentrate the parasitic stages. After centrifugation, the sediment was gently stirred by hand to redistribute the parasitic stages before the supernatant was decanted carefully without shaking. The 100µl sediment was then placed on a clean glass slide and covered with a cover slide before being examined using a light microscope with a 10× and 40× objective lens, the modifed Zeihl–Neelsen staining technique was utilized to identify coccidian protozoan oocysts such as *Cryptosporidium spp., Isospora belli, and Cyclospora cayetanensis.* As a result, a thin smear was made immediately from the silt and let to dry naturally. Each slide was then treated with methanol 5 minutes later and stained with carbol fuchsin 30 minutes later. After staining, sediment was gently stirred by hand to redistribute the parasitic stages before the supernatant was decanted carefully without shaking. The 100µl sediment was then placed on a clean glass slide and covered with a cover slide before being examined using a light microscope with a 10× and 40× objective lens, the modifed Zeihl–Neelsen staining technique was utilized to identify coccidian protozoan oocysts such as *Cryptosporidium spp., Isospora belli, and Cyclospora cayetanensis.* As a result, a thin smear was

made immediately from the silt and let to dry naturally. Each slide was then treated with methanol 5minutes later and stained with carbol fuchsin 30 minutes later. After staining, the slide was rinsed with tap water and decolored for 1–3 minutes with acid alcohol, the slides were then counterstained with methylene blue for one minute after being cleaned with tap water. The slide was then rinsed with tap water and let air dry. The slide was examined using 100× objectives on a light microscope afterwards. 142 samples were identified as being contaminated with at least one type of parasite; the overall contamination rate was 36.3%. Of the contaminated fruits and vegetables, 130 (91.5%) had single contamination and 12 (8.5%) had mixed contamination. Cabbage was the most contaminated product with 7% and avocado was the least contaminated with 2.8%.

Eberemu *et al. (*2023) carried out to determine the prevalence of parasitic contamination of vegetables sold in Dustin-Ma markets, Katsina State. A total of 270 vegetables including of lettuce, cabbage, spinach and sesame were obtained from three selected markets (Kasuwann Laraba, Kasuwann Aminu and Kasuwann Kadangaru), and examined for presence of parasitic eggs, cyst, larvae and worms by using sedimentation method. This study found that of 88.9% of the vegetables sold in Dustin-ma markets tested positive for the presence of parasite contamination. The distribution of specific parasites among positive samples revealed the presence of *Ascaris species* 65(24.1%), hookworm 39(14.4%), *Trichuris* species 16(5.9%), *Taenia* species 23(8.5%), *Hymenolepsis* species 6(2.2%), *Enterobius vermicularis* 25(9.3%), *Fasciola* species 13(4.8), *Toxocara* species 4(1.5%) and *Strongyloides* stercoralis 49(18.1%). The lettuce, cabbage, spinach and sesame samples were contaminated with helminthic parasites with 27.1%, 28.8%, 27.1% and 17.1% respectively. Vegetable samples from Kasuwann Laraba had the highest prevalence of 85(31.5%), and Kasuwan Aminu with a total prevalence of

78(28.9%), while Kasuwann Kadangaru had the lowest prevalence with a total prevalence of 77(28.5%) at $P < 0.05$.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 THE STUDY AREA

The study was carried out in Efehi Street which is a part of New Benin market located in Benin City, Edo State which is located within the tropical rainforest region of Nigeria. This study was carried out within May-November, 2024. The study area lies between latitude 6.350774°N and longitude 5.629703°E. Benin City has a human population of 3,233,366 based on the 2006 census (Nigerian bureau of statistics, 2011). The city's landscape is generally flat with reduced elevation variations. This region is characterized by dense vegetation with tropical rainforest and derived savanna being the dominant vegetation types. Benin City experiences a lengthy rainy season that lasts from April to October, the dry season is usually November to April.

3.2 SAMPLE COLLECTION

30 samples from each species of these four selected fruits; Cucumber, Carrots, African pear and Garden eggs were obtained from fruit sellers at Efehi street, New Benin market, Oredo local government area, Benin City, Edo State, Nigeria. The sampling was carried out in May-November This market was selected because it serves as a major market for fruits in Benin City, people from Benin city and other neighboring towns usually get fruits from Efehi market, the market serves as a major wholesale market for fruits, vegetables and others, many retailers purchase fruits from Efehi Street at New Benin market. This market serves as a base for many fruits and vegetables wholesalers in Benin City.

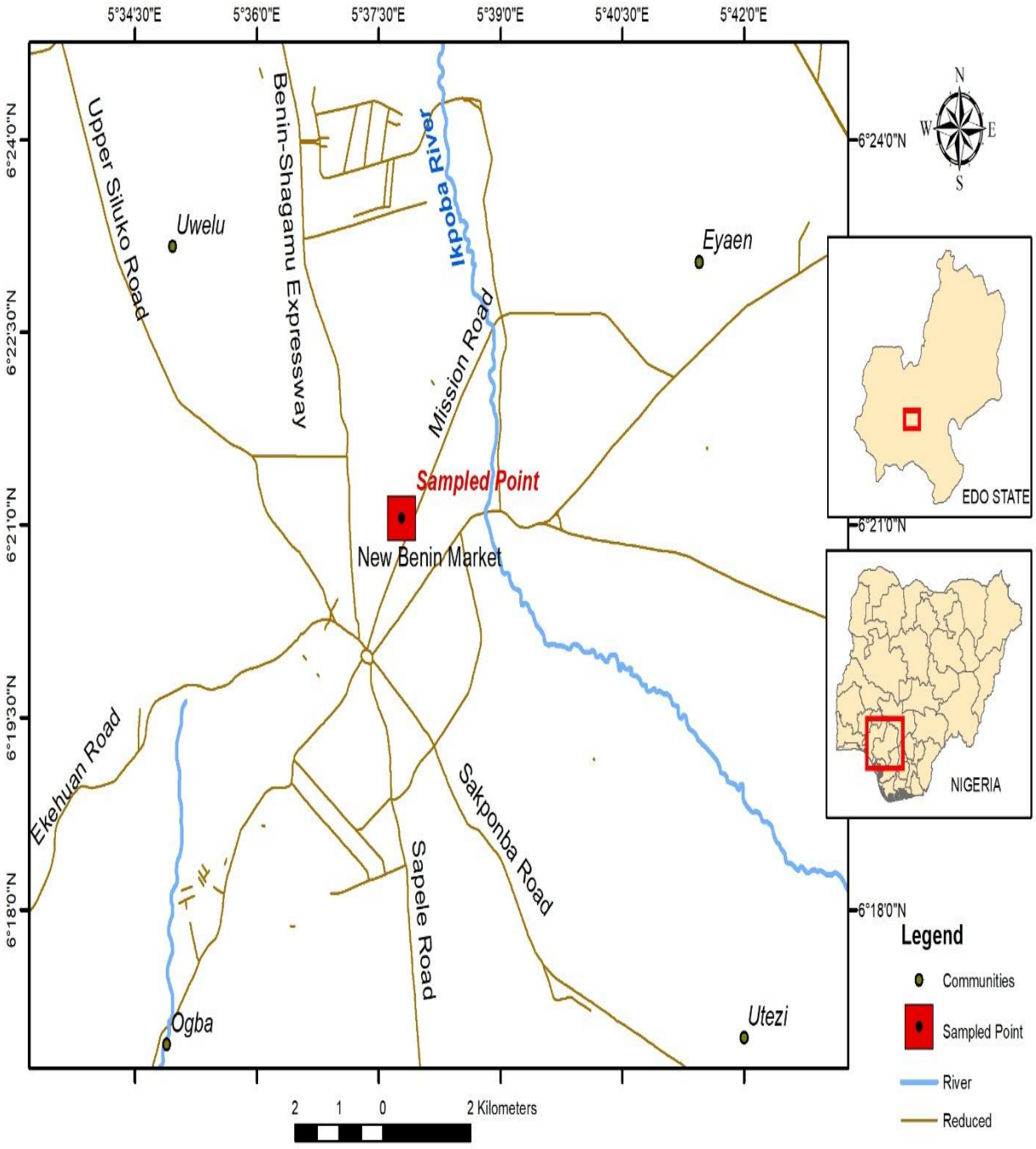

Figure 3.1 Maps of Nigeria, Edo State and Benin city showing the study area at Efehi street, New Benin market, Oredo L.G.A, Edo State, Nigeria

Plate 3.1 Sampled area, Efehi street at New Benin Market, Benin City, Edo State, Nigeria

3.3 TRANSPORTATION OF SAMPLES

The samples collected were placed in neat containers and tightly sealed. These containers were carefully arranged in a vehicle which moved them to the University of Benin where the preparation and analysis of the samples were carried out.

3.4 PREPARATION OF SAMPLES

The samples were prepared using sedimentation and floatation method. The fruit samples were weighed individually and transferred to separate plastic containers, the containers were accurately labelled with masking tape and covered. 200ml of saline-water solution was added to each sample and washed thoroughly to extract the parasites. The containers were covered immediately after proper washing of the samples and kept carefully for a night. The next day, the samples were drained leaving behind the sediment which was transferred into centrifuge tubes using a funnel and surgical gauze to prevent the transfer of too much debris. The centrifuge tubes were carefully transferred into test tube racks and left to sit for another day. The samples in the centrifuge tubes were decanted and samples from the same initial sample were combined together in the same centrifuge tube. A solution of ZnSo4 and water was prepared by dissolving 330g of ZnSo4 in 670ml of water, the solution was added to the samples for proper preservation of parasite stages. The centrifuge tubes containing the samples were spun using a centrifuge at 1500rpm for 10 minutes for the parasites to float. Afterwards, the centrifuge tubes gently transferred to the racks awaiting analysis.

3.5 PARASITOLOGICAL ANALYSIS OF SAMPLES

A Pasteur pipette was used to collect drops of the sample and transferred to a microscope slide. A drop of lugol's iodine was added to the sample on the slide for staining. A cover slip was placed on the slide and properly placed in position on the microscope. The samples were examined under ×10 magnification and the parasite eggs, larvae and cysts were identified using parasitological guides including 'Atlas of parasitology '.

3.6 EQUIPMENT AND APPARATUS

Scale: This was used to weigh the salt and ZnSo4 used in the preparation of the samples, it was also used to weigh the fruits.

Surgical gauze: This was used to prevent debris while filtering

Microscope slide: This was used for microscopic analysis, the samples were placed on the microscope slide which was fixed into the microscope for viewing parasites

Microscope cover slip: This was used for covering the microscope slide and also compressing the parasites for clearer view of their parts for identification.

Microscope: This was used to view the samples, enlargement of the parasites for proper identification.

Centrifuge tubes: They were used for keeping the samples in a centrifuge.

Pasteur pipette: This was used for placing drops of the samples on the microscope slide.

Masking tape: This was used for labeling.

Test tube rack: The centrifuge tubes were arranged in the test tube rack for proper storage of samples.

Plastic collection box : This was used to transport the samples from the sampling site to the lab.

3.7 CHEMICALS AND REAGENTS USED

(NaCl: It was used in the preparation of saline for washing the fruits.

Zinc sulphate heptahydrate: It was also used in the preparation of the samples, it was used for preservation.

Lugol's Iodine: This was used for staining.

3.8 STATISTICAL ANALYSIS

Upon conclusion of the parasitological analysis, prevalence was calculated for all the parasite taxa noticed during the course of the analysis, this was calculated by dividing the percentage of contaminated fruits divided by the total number of fruits examined. Other mathematical analytical methods and graphs were also imployed in the statistical aspect of this work.

CHAPTER FOUR

4.0 RESULTS

4.1 OVERALL PREVALENCE OF PARASITIC CONTAMINATION IN FRUITS OBTAINED FROM EFEHI MARKET, EDO STATE, NIGERIA

A total of 120 samples of fresh fruits made up of 30 of each fruit were examined for parasite contamination. The fruit samples include cucumbers (*Cucumis sativus)*, Carrots (*Daucus carota),* African pear (*Dacryodes edulis)* and Garden eggs (*Solanum aethiopicum).* Of these, 110(91.67%) samples tested positive for at least one parasite. Table 4.1 and Figure 4.1 show the overall prevalence of parasite contamination in fruits from Efehi Street, New Benin market. Cucumber (*Cucumis sativus)* was the most contaminated fruit with a prevalence of 96.67%. This was followed by Garden egg (96.67%) and Carrot (90%). African pear (*Dacryodes edulis)* had the least prevalence (86.67) of parasitic contamination.

Plate 4.1: Carrots (*Daucus carota*) from Efehi Street, New Benin market, Benin city, Edo state, Nigeria

Plate 4.2: Cucumbers (*Cucumis sativus)* from Efehi Street, New Benin market, Benin city, Edo State, Nigeria

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Plate 4.3: Garden eggs (*Solanum aethiopicum*) from Efehi Street, New Benin Market, Benin City, Edo state, Nigeria

Plate 4.4: African pear (*Dacryodes edulis*) from Efehi Street, New Benin Market, Benin City, Edo State, Nigeria

Table 4.1 Overall prevalence of parasitic contamination in fruits obtained from Efehi Street New Benin Market, Benin City, Edo State, Nigeria

Fruit	Number of samples examined	Number of contaminated samples	Prevalence (%)
Cucumber (*Cucumis sativus)*	30	29	96.7
Carrot (*Daucus carota*)	30	27	90
African pear (*Dacryodes edulis*)	30	26	86.7
Garden egg (*Solanum aethiopicum*)	30	28	93.3
Total	**120**	**110**	**91.67**

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4.2 PREVALENCE OF CLASSES OF PARASITE CONTAMINATION IN FRUITS FROM EFEHI STREET, NEW BENIN MARKET, EDO STATE, NIGERIA

The parasites recovered from fruits examined in this study were grouped into 6 categories which are nematodes, cestodes, trematodes, protozoa, unidentified eggs and larvae .

The nematodes had the highest prevalence of 63.3%, trematodes and the unidentified larvae had prevalence of 17.5 each; while the protozoans and unidentified eggs had prevalence of 15.0% and 3.3% respectively.

Table 4.2 Prevalence of classes of parasites contamination in fruits from Efehi Street, New Benin Market, Edo State, Nigeria

PARASITE	NUMBER EXAMINED	NUMBER INFECTED	PREVALENCE (%)
Protozoa	120	18	15
Cestode	120	21	17.5
Trematode	120	16	13.3
Nematode	120	76	63.3
Unidentified eggs	120	04	3.3
Unidentified larvae	120	21	17.5

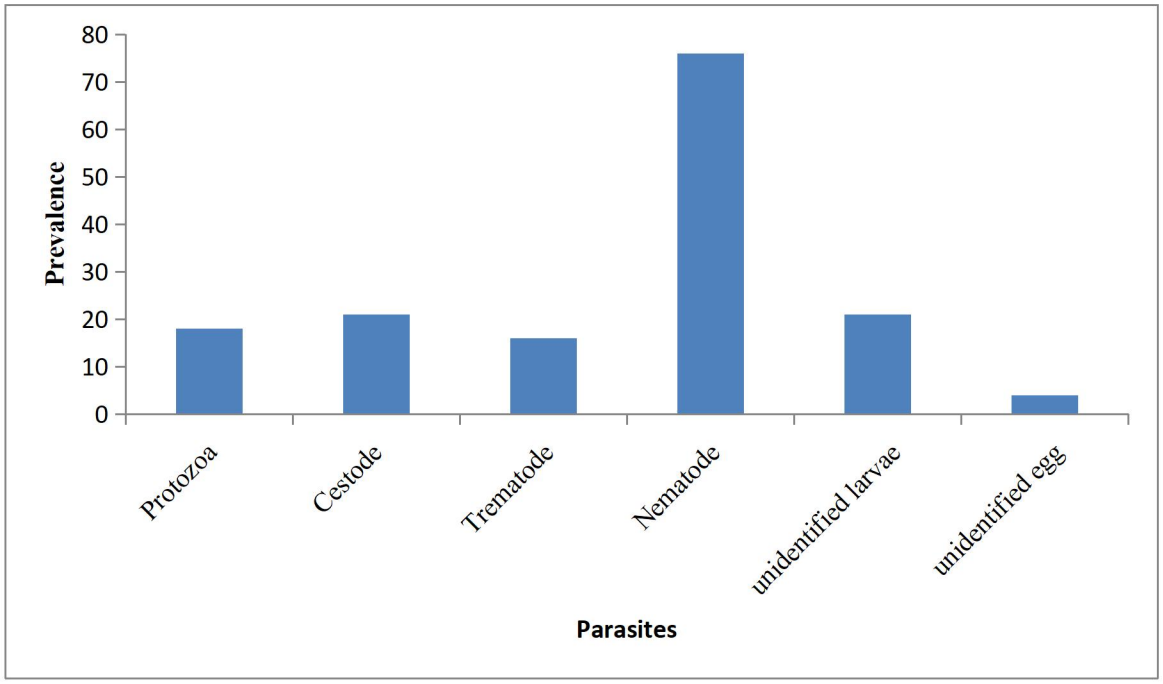

Fig 4.1 Overall prevalence of the parasite classes contamination in fruits from Efehi Street, New Benin Market, Edo state, Nigeria

4.3 PARASITES AND THEIR PREVALENCE OF CONTAMINATION IN FRUITS FROM EFEHI STREET, NEW BENIN MARKET, EDO STATE, NIGERIA.

Eggs and larvae of different parasites species were recovered from the fruits examined in this study. Among these are Protozoa: *Entamoeba* sp. **(plate 4.5)**, *Toxoplasma gondii* **(plate 4.6)**, *Giardia intestinalis* **(plate 4.8)** and *Balantidium coli* **(plate 4.7)**; Cestode: *Taenia* sp. **(plate 4.9)**; Trematode: *Paragonimus* sp. **(plate 4. 13)**, *Schistosoma* sp., *Fasciola* sp. **(plate 4.11, plate 4.12)** and *Heterophyes heterophyes* **(plate 4.10)**; Nematode: *Ascaris lumbricoides* **(plate 4.14)**, *Ancylostoma duodenale* **(plate 4.16)**, *Trichuris trichiura* **(plate 4.15)**, *Enterobius vermicularis* **(plate 4.17)**, *Strongyloides stercoralis* **(plate 4.19)** and *Trichostrongylus* sp **(plate 4.18)**.; Unidentified nematode eggs and larvae **(plate 4.20- plate 4.25)**. Amongst the Protozoans, *Entamoeba* sp. had the highest prevalence of contamination (13.6%), which was followed by *Toxoplasma gondii*, *Giardia intestinalis* and *Balantidium coli* which had a prevalence of 0.9% each. The only creators recovered during the course of this study was *Taenia* sp. with a prevalence of 36.6%. The trematodes recovered in descending order of prevalence are *Fasciola* sp. (5.5%), *Schistosoma* sp (4.5), *Paragonimus* sp. (1.8%) and *Heterophyes heterophyes* (0.9%). Nematode which had the highest level of prevalence in this study included these species *Ascaris lumbricoides* (60%), *Ancylostoma duodenale* (31.8%), *Trichuris trichiura* (4.5%), *Enterobius vermicularis* (13.6%), *Strongyloides stercoralis* (18.2) and *Trichostrongylus* sp. (0.9%)

PROTOZOA

Plate 4.5: *Entamoeba sp*. cyst and trophozoite

Plate 4.6: Sporulated *Toxoplasma gondii* oocyst

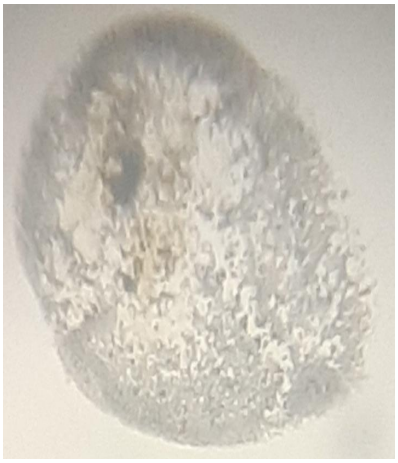

Plate 4.7: *Balantidium coli* cyst

Plate 4.8: *Giardia intestinalis*

CESTODE

Plate 4.9: *Taenia sp.* (Tapeworm) egg; Scale bar, 0.01mm

TREMATODE

Plate 4.10: *Heterophyes heterophyes* egg; scale bar, 0.01mm

Plate 4.11: *Fasciola sp.* egg; Scale bar, 0.01mm

Plate 4.12: *Fasciola sp.* egg; Scale bar, 0.01mm

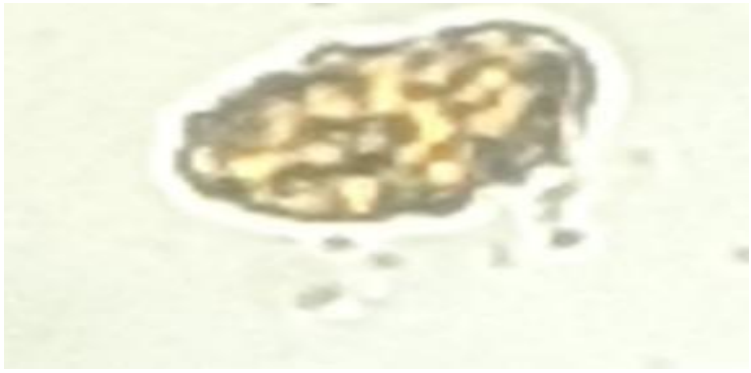

Plate 4.13: *Paragonimus sp.* egg; Scale bar, 0.01mm

NEMATODE

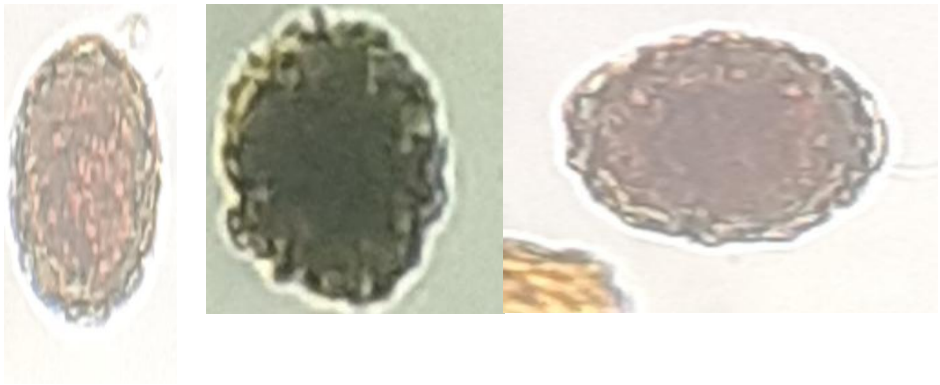

Plate 4.14: ***Ascaris lumbricoides*****; scale bar, 0.01mm**

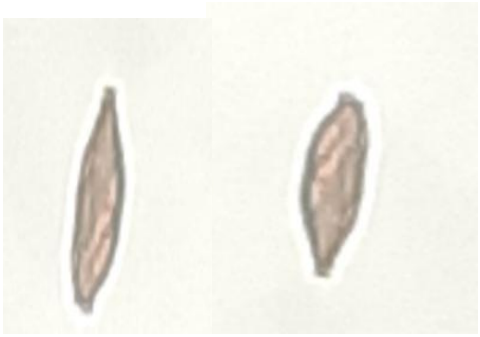

Plate 4.15 : ***Trichuris trichiura*** **(whipworm) egg; scale bar: 0.01mm**

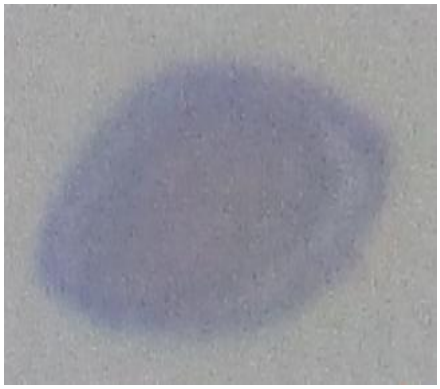

Plate 4.16: ***Ancylostoma duodenale*** **(Hookworm) egg; scale bar: 0.01mm**

Plate 4.17: *Enterobius vermicularis* (Pinworm egg); Scale bar: 0.01mm

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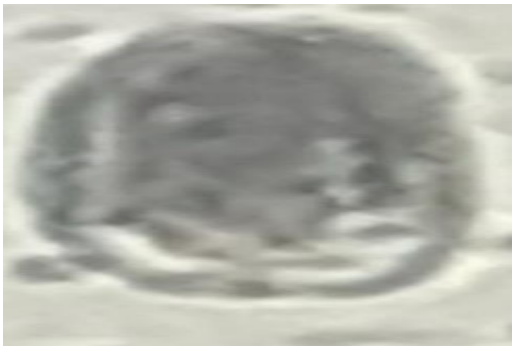

Plate 4.18: *Trichostrongylus sp.* Egg; Scale bar: 0.01mm

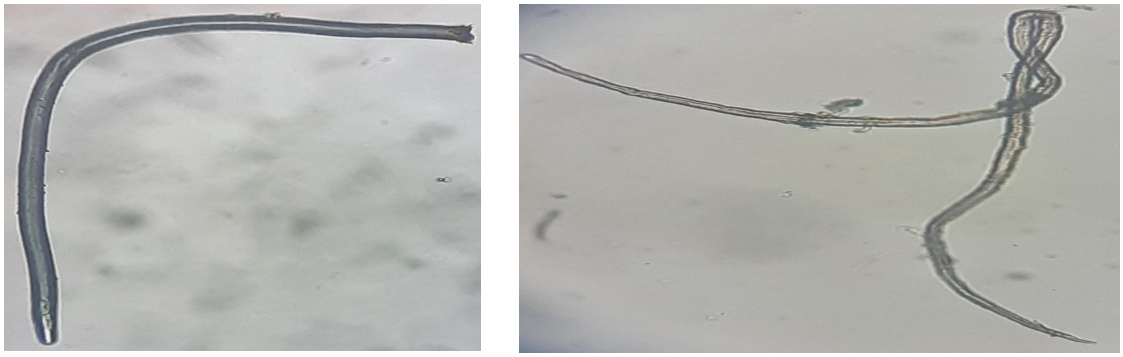

Plate 4.19: *Strongyloides stercoralis* larvae ; Scale bar: 0.01mm

UNIDENTIFIED NEMATODE OVA AND LARVAE

Plate 4.20: Unidentified larvae from Garden egg

Plate 4.21: Unidentified larvae from Cucumber

Plate 4.22: Unidentified larvae from Carrot

Plate 4.23: Unidentified larvae from African pear

Plate 4.24: Unidentified ova from African pear

Plate 4.25: Unidentified ova from Garden egg

Table 4.3: Prevalence of parasites in fruits from Efehi Street in New Benin market, Benin City, Edo State, Nigeria

Parasite	Fruit	Number examined	Number infected	Prevalence (%)	Overall prevalence (%)	Mean intensity
Protozoa						
Entamoeba sp.	Cucumber	30	—	—	**13.6**	—
	Carrot	30	05	16.7		**0.19**
	Pear	3	07	23.3		**0.27**
	Garden egg	30	03	10		**0.11**
Toxoplasma gondii	Cucumber	30	—	—		—
	Carrot	30	—	—	**0.9**	—
	Pear	30	01	.3		**3.3**
	Garden egg	30	—	—		—
Giardia intestinalis	Cucumber	30	—	—	**0.9**	—
	Carrot	30	01	3 3		**0.04**
	Pear	30	—	—		—
	Garden egg	30	—	—		—
Balantidium coli	Cucumber	30	—	—	**0.9**	—
	Carrot	30	01	3.3		**3.3**
	Pear	30	—	—		—
	Garden egg	30	01	3.3		**0.04**
Cestode					**19.1**	
***Taenia* sp.**	Cucumber	30	11	36.6		**0.34**
	Carrot	30	05	16.7		**0.19**
	Pear	30	03	10		**0.12**
	Garden egg	30	02	6.7		**0.07**
Trematode						
***Paragonimus* sp.**	Cucumber	30	—	—	**1.8**	—
	Carrot	3	01	3.3		**0.04**
	Pear	3	01	3.3		**0.04**
	Garden egg	30	—	—		—
***Schistosoma* sp.**	Cucumber	30	—	—	**4.5**	—
	Carrot	30	02	6.7		**0.07**

	Pear	30	03	10		**0.12**
	Garden egg	30	—	—		—
***Fasciola* sp.**	Cucumber	30	—	—	**5.5**	—
	Carrot	30	03	3		**0.11**
	Pear	30	03	3		**0.12**
	Garden egg	30	—	—		—
Heterophyes heterophyes	Cucumber	30	—	—	**0.9**	—
	Carrot	30	01	3.3		**0.04**
	Pear	30	—	—		—
	Garden egg	30	—	—		—
Nematode						
Ascaris lumbricoides	Cucumber	30	24	80	**60**	**0.83**
	Carrot	30	14	46.7		**0.52**
	Pear	30	13	43.3		**0.50**
	Garden egg	30	15	50		**0.54**
Ancylostoma duodenale	Cucumber	30	13	43.3	**31.8**	**0.45**
	Carrot	30	05	16.7		**0.19**
	Pear	30	07	23.3		**0.27**
	Garden egg	30	10	33.3		**0.36**
Trichuris trichiura	Cucumber	30	—	—	**4.5**	—
	Carrot	30	02	6.7		**0.07**
	Pear	30	02	6.7		**0.08**
	Garden egg	30	01	3.3		**0.04**
Enterobius vermicularis	Cucumber	30	09	30	**13.6**	**0.31**
	Carrot	30	03	10		**0.11**
	Pear	30	02	6		**0.08**
	Garden egg	30	01	3.3		**0.04**
Strongyloides stercoralis	Cucumber	30	05	16.7	**18.2**	**0.17**
	Carrot	30	06	20		**0.22**
	Pear	30	05	16.7		**0.19**
	Garden	30	04	13.3		**0.14**

	egg					
***Trichostrongylus* sp..**	*Cucumber*	30	—	—	**0.9**	—
	Carrot	30	01	3.3		**0.04**
	Pear	30	—	—		—
	Garden egg	30	—	—		—
Unidentified nematode eggs	Cucumber	30	—	—	**3.6**	—
	Carrot	30	—	—		—
	Pear	30	02	6.7		**0.08**
	Garden egg	30	02	6.7		**0.07**
Unidentified nematode larvae	Cucumber	30	04	13.3	**19.1**	**0.14**
	Carrot	30	04	13.3		**0.15**
	Pear	30	10	33.3		**0.38**
	Garden egg	30	03	10		**0.11**

CHAPTER FIVE

5.0 DISCUSSION

The fruit samples examined in this study were collected fresh in the early hours of the day**.**

A high (91.67%) overall prevalence of parasite contamination was recorded in the fruits examined in this study. The four types of fruits investigated were infected with at least one parasite. Cucumber (*Cucumis sativus)* had the highest prevalence (96.7%) of parasite contamination while African pear (*Dacryodes edulis*) had the least prevalence (93.3%) of parasite contamination in this study.

The high prevalence of parasite contamination observed could be due to certain factors which include the poor hygiene in the market where these fruit samples were purchased, the environment which was noticed at Efehi street was very poor in hygiene, most of the fruits were displayed on obviously dirty floors at the market. Another factor which can be considered to be highly pivotal in the transmission of parasite juvenile stages (eggs, larvae and cysts) to these fruits is the process of transportation. Based on the information acquired from the sellers, most of these fruits were transported to Benin from the North. It was also observed that there was no proper and adequate measures for refuse disposal, the refuse were dumped close to where the fruits were displayed for sales, this probably led to the high rate of contamination, a lot of flies and other carriers of parasites which could be associated with refuse dumped close to the market were also and this could possibly be one of the factors which led to the high prevalence of parasites in the fruit samples.

Cucumber (*Cucumis sativus*) which had the highest prevalence of parasite contamination in this study is a fruit crop of the family *Cucurbitacae* (Akoani *et al.,* 2023). It is cultivated in nearly all countries of the world (Tatolioglu, 1993). It is considered an annual crop which originated in

Asia but now grows on many continents, it is very common in Nigeria where its fruits are eaten raw or added to dishes.

Carrot (*Daucus carota*) is a biennial plant belonging to the Apiaceae family native to Europe and Southwestern Asia but also grown in some parts of Nigeria. The characteristic orange colour is as a result of beta-carotene which makes carrots a rich source of Vitamin A. As a common source of beta-carotene in diets, carrots are a provitamin A source (Tamg 2012). Carrots can be eaten in many ways thus the high consumption, the high consumption of Carrots without proper washing which is very common aids in the transmission of parasites. As opposed to the results from the study on parasitic contamination of fruits and vegetables collected from four local markets of Jimma town in Ethiopia conducted by Tefera *et al* in 2014 where carrots used in the study were recorded to have 13.5% prevalence of intestinal parasites , the carrots in this study had a higher prevalence of parasitic contamination which could be due to the difference in locations.

Dacryodes edulis, also known as African pear is a member of the family *Burseracaea* native to Africa (Bascom *et al.,* 1951). It is predominantly known In Edo State, Southern Nigeria where it is highly consumed as 'Ube'. It is majorly eaten either raw, cooked in salt water or roasted, the texture of the cooked flesh of the fruit is similar to butter and is often eaten on bread (Omogbai *et al .,* 2010).

Solanum aethiopicum , commonly known as Garden egg in Nigeria is a highly variable fruit which is eaten both raw and cooked. The fruits are usually harvested while still green, before the skin becomes thick. In Nigeria, Garden egg is also used by Igbos as a substitute for Kola nut, this is mostly done without proper washing and passed from hands to hands which aids in the spread of parasites.

In this study, ova of *Ascaris lumbricoides* was the most predominant parasite with prevalence of 59.1. The high prevalence of *Ascaris lumbricoides* could be due to poor sanitary conditions, probably perpetuated by contamination of soil by human fence or use of untreated feces as fertilizer (Bokhari, 2021). The juvenile stage of *Ascaris* has a resistant shell and this probably enhances its survival. Proper washing of fruits with saline (a mixture of salt and water) can decrease the rate of parasite contamination

CONCLUSION

The fruits investigated in this study were contaminated with helminthes and protozoan parasites which could infect consumers especially when eaten without proper washing or cooking. Fruit vendors, farmers and end-users (consumers) can aid in decreasing contamination by practicing proper hygiene, neat storage of fruits, improvement of sanitary environments in and around the markets where fruits are sold and proper washing of fruits with saline solution before ingestion by consumers. Adequate education of farmers, vendors and consumers on the dangers of improperly handling fruits meant for consumption is also of utmost importance.

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