PARASITOLOGICAL EXAMINATION OF SOME READY TO EAT FRUITS SOLD AT OYE EMENE ENUGU STATE NIGERIA

BY

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MB/2008/408

DEPARTMENT OF MICROBIOLOGY AND BIOTECHNOLOGY

FACULTY OF NATURAL SCIENCE

CARITAS UNIVERSITY

AMORJI-NIKE EMENE, ENUGU,

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A PROJECT RESEARCH WORK IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC).

SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY AND BIOTECHNOLOGY FACULTY OF NATURAL SCIENCES, CARITAS UNIVERSITY, AMORJI-NIKE EMENE, ENUGU, ENUGU STATE, NIGERIA.

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APPROVAL PAGE

This project work has been approved as meeting the requirement of the department of Microbiology and Biotechnology, Caritas University, Amorji-Nike Enugu, for the award of Bachelor of Science Degree (B.Sc.).

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Dr. M. U. Orji — Date
(Project supervisor)

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(External Supervisor) — Date

DEDICATION

This work is humbly dedicated to the Sacred Heart of Jesus and Immaculate Heart of Mary the two inseparable Hearts of love and to God almighty my refuge and stronghold for His divine love and mercies for me right from the date of my conception till date.

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ABSTRACT

Some locally available fruits (garden egg, orange, banana, guava, avocado, pawpaw and pineapple).sold in Oye Emene Enugu, Enugu State was examined for parasitological contamination. A total of 87 samples were examined using sedimentation and concentration methods. 11(12.6%) of the 87 fruits were positive for intestinal parasites microscopically. Among these fruits, guava had the highest number of intestinal parasites which was 6 (35.3% positive and the lowest was banana 2(8%). Parasite implicated were ova of *Ascaris lumbricoides* 8(9.2%), cysts of Amoeba 3(10%), yeast cells were seen on pineapple and water melon. There is no significant difference between the two methods used. Results of the current study shows a significant level of fruits contamination with pathogenic parasite from different places in Oye Emene in Enugu, Enugu State suggesting existence of a great risk of acquiring intestinal parasites by eating improperly washed fruits.

CHAPTER ONE

INTRODUCTION

Among horticultural crops, fruits are of great importance for an adequate and balance human diet. In certain part of the world, fruits are the major dietary staple. Apart from being a rich source of vitamins, and minerals, the production of fruits also contributes significantly to regional and national economics through national and international trade. The term fruit has many different meaning depending on the context.

A fruit is a ripened ovary together with seeds of a flowering plant. Fruits are the means through which flowering plants disseminate seeds (Lewis, 2002).

In cuisine when food items are called “fruits” the term is most often used for those plants fruits that are edible, sweet, and fresh examples are: apples, and oranges.

Fruits are cultivated in areas where the environmental factors are suitable for their growth. Emene in Enugu state is considered one of the areas that have good cultivating land for great yield of fruits and this is done usually during rainy season or use of irrigation during dry season. Irrigation water is achieved by different sources like lake, stream, river, ponds etc. which may be polluted with animal and human feaces. Due to high number of eggs, cyst and larvae of human intestinal parasites present in the waste

water, the use of excreta polluted water is a health risk to both the farmers and the consumers that eat the produce raw and fresh, like apples, guava, pear and mango (Scolf, 1992).

Pollination is a vital part of fruit culture, and in few species, they may develop in the absence of pollination/fertilization, a process known as "parthernocarpy" such fruits are seedless (Mauseth &James 2003). Many foods are botanically fruits, but are treated as vegetables in cooking and food preparations. Examples are: Tomatoes, eggplant, pumpkin and pears etc. (Mcgee and Harold 2004). Ethylene causes ripening in fruits.

There are three types of fruits

1. Simple fruit
2. Aggregate fruit and
3. Multiple fruit

Simple fruit can be either dry or fresh, Example carrot, wheat, tomato, avocado, banana etc.(Schelegel,2002).

The examples of an aggregate fruits are pineapple, bread fruit, etc. some fruits have coat covered with Spikes or hooked burrs, either to prevent themselves from being eaten by animals, using them as dispersal agent. eg. unicorn plant (Heiser and Charles 2003). Many fruits are used to make beverages, such as fruit juice, (orange juice, apple juice, grape juice

etc).or alcoholic beverages, such as wine or brandy. Apples are often used to make vinegar. Mcgee (2004) fruits are contaminated with parasites and some parasite infections which have direct life cycle and do not need an intermediate host to infect a new host are via feacal-orally transmitted parasites. Infections acquired through direct ingestion of infective egg or cyst is intimately linked with level of personal hygiene and sanitation in the community. Factors like the lack of latrine and adequate sewage disposal facilities have been known to contribute to the spread of the infective states of the parasites thereby bringing about a wide spread contamination of foods. Infection can be acquired through contaminated unwashed fingers, insects, circulation of currency and by wind during dry season. Contamination of fruits with eggs and cyst especially those hawked by fruit vendors may also serve as a source of infection to consumers of such fruits items. These parasites includes: *entamoeba histolystica, Giardia duodenace, Trichuris trichura, Ascaris Lumbricoide*s and *Benterobius vermiculais*. (World Health Organization [WHO],2000).

Amoebiasis is known to cause about 450 million infections per annum in developing countries with an incidence of about 50 million and 100,000 deaths. Giardiais is more common in children and has a world wide prevalence of about 1-30% (Wov and Paterson, 1986). *Ascaris* is the commonest nematodes of man especially in tropical Africa with a prevalence of about 40% in Enugu State

(Reonthalaer, 1988). And may be as high as 96-100% in the rural community in Enugu State.The resistance capacity of the eggs and cyst of these parasites is a feature of profound influence on the epidemiology.

Eggs of *Ascaris* can remain viable for up to six years. (Njom,2002). Many people who eat fresh fruit as part of an overall health diet are likely to have reduced risk of some chronic diseases. Eating a diet rich in fruits and vegetables reduce risk for stroke, diabetes, certain cancers such as (mouth), heart, diseases, developing kidney stone, and decreases bone loss.

Fruit reduces the risk of neural tube defects, spina bifida and anecephaly during fetal development

(Ayer, 2001). Fruits are very important in human consumption especially health wise. Parasites that affect fruits need to be controlled in a proper process in order not to affect those that are of medially important. The major way of selecting the appropriate intervention stops to reduce population to pathogenic microorganism on fruits is to identify sources of contamination and ecology of the pathogens as it is affected by processing practices. Organism like; *Samonella, cryptosproridium, cyclospora, Giardia,* are among the disease causing organisms that have been transferred via fresh fruits (Sushow, 1997).The use of disinfectant like chlorine, in wash water can also help to prevent both host harvest disease and food borne illnesses.

The evaluation of these medically important parasites found in fruits will depend on the knowledge of the factors contributing to the spread of such parasites including the activities of fruit vendors at Orie emene market and then affect on the distribution of these parasites.

AIMS AND OBJECTIVES

1. To determine the parasite of medical importance in fruit sold at Orie Emene market in Enugu State.
2. To determine whether washing of fruits with untreated water eliminates the parasites of medical importance (pathogenic parasites) from them.
3. To determine whether fruits are safe to be eaten without washing them as some people do.

CHAPTER TWO

2.0 LITERATURE REVIEW

Parasites are organisms which have adapted themselves in or on another organism which is called a host, and lives at the expenses of the tissue and fluid of the host deriving their nutrient and protection from the host, thereby harming or being of no advantage to the host. (Crew, 1999).

They increase their fitness by exploiting host for food, habitat and dispersal. Parasites may be transmitted from animals to humans, from humans to humans, or from humans to animals. Several parasites have emerged as significant cause of food borne and water borne disease in the whole world.

This is achieved through consumption of contaminated food and water or by eating any raw fruits that has been contaminated with water or food. Parasites are of different types and ranges in size from tiny-single-celled, microscopic organisms (protozoa) to large multi-cellular worms, (helminthes) that may be seen with a microscope.

Some of the parasites are

Giardia lamblia (intestinalis) cryptosporidium parvum, Cyclospora-cayetanesis, Toxoplasma gonadii, and Trichnella Spiralis.

G. duodenalis formally called g.

Lamblia causes Giardiasis.

It is one celled microsiopic parasites that can live in the intestine of animals and people.

It is found in every region throughout the world and may cause chronic diarrhea, malabsorptoin, weight loss with symptoms for several months. (Hill, 1993). Giardiaisis is mainly acquired by transmission of cysts of *G. Intestiinalis* via soiled hands, contaminated with faeces (pentersen, 1988). Consumers get this disease by consuming food or water – contaminated with *G. duodenalis* cysts (infective stage of the organism) and by putting anything into the mouth that has touched the stool of a person or animal with giardiasis. This occur usually 1-2 weeks after ingestion of G. duodenalis cysts, which is the environmental survival form and infecting stages of the organism but may last for 4 to 6 weeks in healthy person.

There are cases of chronic illness lasting months or even years. People exposed to public places including those with HIV/AIDS infection are at risk for contracting giardiasis. (World Health Organization [WHO],2003).

Giardiasis can be prevented by washing hand with hot, soapy water before handling foods and eating, and after using the toilet diapering young children, and handling animals also by making sure infected individuals wash their hands frequently to reduce the spread of infection, and drinking water only from the treated municipal water supplies. When traveling to countries where the water supply may be unsafe to drink, either avoid drinking the water or boil to kill

parasites. Drinking bottled beverages or hot coffee and tea are safe alternative. Do not swallow water while swimming especially in community pools where there might be people's child or people suffring giardiasis. Drink only pasteurizes milk, juice or cider. Wash, peel, or cook raw fruits and vegetables before eating. Do not use untreated manure to fertilize fruits and vegetables.

Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection and is implicated in 25% of the cases of gastrointestinal diseases and may be present asymptomatically about 40% of those who are diagnosed with giardiasis demonstrate disaccharide intolerance during detectable infection and about six months after, the infection can no longer be detected. (John and Wily,1999).

Chronic cases of giardiasis in immunodeficient and normal individuals are frequently retractile to drug treatments (Nichole and Smith, 2002). Five outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated fruits and vegetables that are eaten raw cannot be excluded.

Cryptosporidiosis; it is a celled microscopic parasite, and a significant cause of water borne illness worldwide. It is found in the intestine of many herd animals people get cryptosporidiosis by

1. Consuming food or water contaminated with *C. parvum* oocysts, (infective stage).The oocysts is the environmental resistant stage of the organism and are shed in the faeces of a host into soil.
2. By putting anything into the mouth (faecal oral route) that have touched stool of infected person. The symptoms of this disease are watery diarrhea, stomach cramp, upset stomach and light fever. Some case may be without symptoms. Symptoms appear to ten days after ingestion of *C. parvum* oocysts. The illness usually goes away without medical intervention in three to four days. But in some outbreaks in day care centres diarrhea has lasted one to four weeks. In people with weakened immune system, cryptosporidiosis can be serious, long lasting and sometimes fatal. However, there is no known effective drug or medication whatsoever for the treatment of cryptosporidiosis (Millard, Gensheimer and Addis, 1994).

Cyclospora cayetanesis, causes of cyclosporidiasis is a one celled microscopic parasite. Currently little is known about this organism, although cases of cyclosporasis are reported from various countries with increasing frequency.

Consumption of contaminated fruits and food in general with *C. cayetanesis* causes this disease. Symptoms includes; watery-diarrhea sometimes explosive, Stomach cramps, nausa, vomiting, muscle ache, low grade fever and fatigue. Some cases are more severe in persons with weakened immune system and appear about

one week after the ingestion of *C. caetanensis* oocysts. Persons of all ages are at risk for infection. Untreated manure should not be used to fertilize fruits. The parasite that are mainly found in fruits more than vegetables are *cryptosporidium*, *cyclospora*, and *Giardia* (Nichols and Smith, 2002). Outbreaks linked to these protozoa and multi-cellular worms – has been associate with fruits. Fruits and vegetables normally carry a non-pathogenic epiphytic micro-flora. Hence, there are certain factors which contribute the microbiological contamination of these products with pathogens. Consequences of treating soil with organic fertilizers such as manure, and savage sludge from irrigation water harvesting, cutting, slicing, etc the inner tissues of fruits are usually regarded as sterile (Lund, 1992).

Bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures or contaminated with human pathogens.

Fruits and vegetables can become contaminated while growing in fields or during harvesting, handling processing, distribution and use. (Beuchat, 1995).

At the point when an intact part of a plant is marketed e.g. carrot, lettuce etc. any microbial contamination present is likely to reflect the environment through which the product has passed, information compiled by Beuchat (1998) provides an overview of food borne pathogens. In different fruits products, a conclusion of the report is that the presence of pathogenic – microorganisms on raw fruits varies

considerably. Surveys of the presence of parasite are fewer because of the lack of adequate detection methods that can be applied to fruits. Fresh fruits continue to respire consuming oxygen and producing carbon dioxide and water vapour.

The increasing use of waste water for irrigation in the 1970s and early 1980s prompted a series of literature reviews and investigations into the global extent of waste water re-use and its association with human health risk.

The infection can also be a house hold affair where infected children or person provide the chief source of soil contamination by their promiscuous defecation in the soils.

The climate, vegetation and topography of Plateau state are suitable for the cultivation of fruits, using rain during wet season and irrigation during which water is derived from two sources, rivers and ponds during dry season.

It is a known fact that the use of execrate polluted irrigation water is a health risk to farmers and consumers of crops so produced.

Raw waste water frequently contains high number of eggs of human intestinal nematodes where night soil is extensively used as fertilizers or waste water re-use is practiced.

This work is designed to detect the human intestinal parasites that improperly washed fruits contain and ways in which they could be controlled.

More and more people are concerned these days about the presence of parasites of medical importance in fresh fruits. According to the numerous studies nearly 85% Americans have parasites in their body. (World Health Organization [WHO],1990). Intestinal parasitic helminthes and protozoa infections are among the most common infections world-wide. In spite of the fact that the mortality rate among these infections is rather low in the case of *Ascaris lumbricoides* two per 1,000,000 people due to the high prevalence, these infections are regarded as a serious public health problem.

There are several types of intestinal worms, the most commonly noticed parasites are roundworms, hookworms, Threadworms, and tape worms.

Roundworms are giant intestinal parasites measuring 15 to 25 cm in length. They are of white in colour and are most frequently infect Chidren, Eggs produced by them are passed in stools. Roundworms eggs may remain alive in the soil for many months. They may give rise to inflammation of the intestine and lungs prepared food such as salad, canned Salmon, ice etc have also been implicated in food borne outbreak (Nichols, 2002).

G.lamblia-cyst have been detected in flies that may serve as a vector for contamination of foods (Graczyk, Grimes and Veal, 2003).

Of 39 outbreaks of the disease associated with drinking water reported to the CDC in 1999 to 2000, 6 outbreaks affecting 52people, were traced to the presence of G.

intestinalis in inadequately treated well or driver water or in water contaminated by cross connections with pipes containing sewage (Campbell and Wallis, 2003). *Ascaris lumbricoide* is a common intestinal roundworm parasite infecting an estimated one quarter of the world's population (Han, 2003).

Humans are the only known host for the roundworms.

Techniques for the detection and enumeration of *Ascaris* egg on fruits have been developed and may be useful for screening fresh fruits for contamination (Hebbari,Tifrouli and Mandel 2000). The real cause of intestinal worms however is the use of contaminated food or water. The eggs of these worms can breed in the intestinal only if they find suitable medium for propagation. This medium is an intestinal tract clogged with morbid matter and systemic refuse to winging feeding habitat Robertson et al.

Epidemiological studies have demonstrated that drinking water is a important vehicle for transmission in underdeveloped countries when there was a breakdown in a water purification or cross contamination between sewage and drinking water pipes (Barwick et al, 2002).

Food borne transmission is often due to handler and also occurs hen produce is freshened or crops are irrigated with contaminated water.

2.1 INCIDENCE OF WATER BORNE DISEASE WITH SPECIAL EMPHASIS ON RE-USE OF WASTE WATER IN AGRICULTURE

The use of contaminated water has caused some incidence of water borne diseases in Nigeria. Okoronkwo (2000) in Jos, Plateau State, Nigeria worked on 120 samples each ofvarious vegetables crops as follows: tomatoes, lettuce, carrot, sprinarch, and cabbage.

The result showed that varying amounts of viable eggs of *Ascaris, Trichuris,* and *Ancyclostoma – dudodenale* per 100 gram of the various vegetables were recovered, hence, the need to enforce the use of treatment technology which can produce high level of parasite, bacterial and viral removal from waste water used to irrigate vegetables farmlands.

In Africa, the growing demand for fresh and perishable agricultural produce in the major cities is deriving the development of pre-urban agriculture.

This demand is not seasonal, necessitating years-round production, heavily dependent on irrigation.

Recycling solid waste and waste water into Petri-urban horticultural production contribute to cleaning the environment.

However, this is associated with potential health risk which calls for careful agronomic practices including water quality and waste water management (Hebbari et al, 1999). Investigated the cases of helminthic infections associated

with the use of raw waste water for agricultural purposes in Beni Mella, Morocco. Children attending primary schools were used because schools are very easily accessible for most suitable study and disease recurrence is usually high. Seven hindered and forty were savaged from five schools where waste water re-use for agriculture is practiced. World Health Organization (2001) made it clear that for the two parasites (*Ascaris lumbricoides and T. trichura*.).

Infection can be classified as light intensity infections (<5000 egg and <1000 egg per gram, respectively). While mixed infections by two or three parasites was observed among 2.9% of the waste water exposed children, none occurred among the children living in the control. Among the children infected by two or three parasite, 40.9 and were infected by a. *lumbricoides, E. Vermiculairs, H.nana* and 4.5% by *tribhura and E. Vermicularis* in the area exposed to waste water irrigation *A. lumbricoides, T. trichiura and T. saginata* affected 21.10%. 0.4%, 6.0% 8.5% and 2.5% of boys and girls respectively. No statistical significant sex difference was found except for *Taenia baginata*.

2.2 INDICATOR ORGANISMS

Faecal coliform and faecal streptococcus are the conventional indication of faecal pollution of water is used (Colwell et al, 1978). Some more reliable indicator organisms of faecal pollution are *coliphagea, clisotridium perfringes* and

bitido bacteria (Abnelli, 1989). They should be present in feacal in greater numbers than any pathogen, yet be unable to proliferate in water to any extent. More over, they should be more resistant than other pathogen to the stress of the aquatic environment and disinfection process

(Mackie and Mac, 1999).As a result of this difficulty, for direct search for pathogens in water, microbiologist have evolved simple and rapid test for the detection of normal intestinal organism known as faecal indicator organisms, which account for the highest source of pollution especially in developing countries like Nigeria. The organisms used as indicators are *E.coli, S. faecalis, Salmonella SPP*, *Bitidobacteria Vibrio Cholera*. The most widely used are coliforms bacteria. The total coliforms that got narrowed down to the faecal Streptococcus coliforms, and the faecal streptococcus faecal numbers that usually disappear at he same rate as *E. coli* but quick than coliforms.

2.3 SANITATION OF FRUITS

The use of a disinfectant in wash water can help to prevent both post harvest disease and food borne illnesses.

Chlorine in the form of hypochloride solution or as dry powered calcium hypochlorite can be used in hydro-cooling or wash water as a disinfectant.

Chlorine is routinely used as a sanitizer in wash, spray, and flums waste used in the fresh and vegetable industry.

The efficacy of chlorine in killing pathogenic microorganisms has been extensively studied. As noted by (Lund, 2002).

The inaccessibility of chlorine to microbial cells in crevices, ceases, Pockets and natural openings in the skin also undoubtedly contributes to the overall lack of effectiveness of chlorine in killing pathogens. The hydrophobic nature of the waxy cuticle on tissue surface cuticle on tissue surface protects surface contaminants from exposure to chlorine and other produce sanitizers that do not penetrate or dissolve these waxes. Surface active agents lessen the hydrophobic of fruit skin as well as the surface of edible leaves, stems and flowers, but they may also cause deterioration of sensory qualities.

Sanitizer that contain a solvent that would remove the waxy cuticle layer, adversely affecting sensory characteristics would hold greater potential than chlorinated water in reducing microbial populations on whole raw produce, such sanitizers may be limited to use on produce that will be further processed into juice or cut products, or on whole fruits vegetables, or plants or on whole fruits, vegetables, or plant parts. Destined for immediate consumption, since their application adversely affect visual appearances.

Currently, chlorine at concentration permitted for use by the industry to wash fresh fruits and vegetables cannot be relied upon to eliminate pathogens. Ozonizatoin is another technology that can be sued to sanitize produce. A naturally occurring molecule, ozone is a powerful disinfectant and has long been used to sanitize drinking water. Fruits and vegetable growers also have begun using it in dump tanks. Ozone not only kills whatever food borne pathogen might be present, it also destroys microbes responsible for spoilage.

The use of mesh produce bag in an old washing machine set to spine cycle is recommended (Newen house, 1998).

2.4 IMPORTANCE OF CLEANING AND SANITATION OF FRUITS

Clean, well designed and maintained equipment is less likely to cause damage to fresh produce and to introduce spillage and pathogenic microorganism (Brackett, 1992). And keeping the harvested product under controlled environmental conditions will help retard growth of post harvest spoilage (Brackett, 1992, and pathogenic microorganisms. Adequate post harvest treatment of fruits including handling, storage, Transportation and cleaning; helps to reduce cross contamination of the produce from other agricultural materials or from the workers.

Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption.

The presence of cut or damaged surface provides an opportunity for contamination and growth of microorganism and ingress into the plant tissue (Francis et al, 1999). Washing of fruits remove most of the adhering soil and dirt, hence, it should be recognized that first washing may also be a source of microbial contamination so should be done properly. An examples is the contamination of mangoes exported to the United States from Braciwerer found to be infecting consumers with salmonella (sivapalasingam and Barwich, 2000) cleaning and sanitizing food surfaces and equipment extend shelf life and increase protection against financial loss. The method of infestation applied in determining contamination in fruit is carried out in the laboratory following the procedures and or steps explained below:

CHAPTER THREE

MATERIALS AND METHOD

The reagent used in this work includes zine sulphate solution, iodine solution, eosine solution, normal saline and deionized water.

3.0 SAMPLE COLLECTION

Samples of banana, garden eggs, guava etc were purchased from their dealers at Oye Emene, Enugu. The samples of each were purchased from different sellers at different locations in the market.

Plastic buckets with cover sterilized by dusting with cotton wool dipped in 75% ethanol were used to receive the samples from the sellers and taken to the laboratory for analysis.

3.1 PARASITOLOGICAL EXAMINATION OF THE FRUITS

Each of the fruit samples (1g) was put into a small plastic bucket containing 50ml of normal saline (0.85g sodium chloride salt in 100ml of water).

The fruits were shacked well to ensure that as much material in the surface of the fruits as possible were discharge into the normal saline.

Examination for protozoa parasites, the sedimentation and concentration method was used with the aid of a centrifuge (World Health Organization [WHO], 1991, Ramink, 2006).

The normal saline wash of each of the fruits samples obtained by washing the fruits in normal saline was sieved into test tubes using a fine mesh (mosquito net). The sieved contents were centrifuged and the supernatant fluid poured off. The deposit was re-suspended in more saline and centrifuged again. This was repeated until the supernatant fluid was clear.

The deposit was than examined directly on a slide by covering a drop of the deposit with cover slip and examined under X10 and X40 objectives. To facilitate better visualization iodine (Dobell's iodine) preparation was done by adding a drop of Dobell's iodine in the saline preparation before examination under the microscope.

3.2 TABLE ONE;

S/N	Sample	Total no of Samples Collected from Orie Emene Market	No of Samples Used for the Test	Weight of the test Samples
1	Bananas	15	15	100gm
2	Pineapple	4 portion	4 portion	100gm
3	Water melon	2	A portion	100gm
4	apple	1	1	100gm
5	Garden egg	30	30	100gm
6	Papaw	1	A portion	100gm
7	Orange	4	4	100gm
8	pear	7	7	100gm
9	Guava	23	23	100gm

3.2.1 CONCENTRATION OF EGGS OR LARVA OF CYSTS

This will be done using zinc sulphate flotation method (cheesibrough, 2005]. The techniques are recommended for concentrating the eggs of parasite.

1ml from the 45 ml of the washed fruit sample from step one above is added to the one quarter full zinc.

Sulphate solution in a test tube and emulsify

The tube was then filled with the zinc sulphate solution and mixed.

The suspension was then stained to remove large particles

Using a pasture pipette, further solution of zinc sulphate was added ensuring that the tube was filled to the brim.

A clean grease free cover slip was then carefully placed on top of the tube avoiding air bubbles and then left undisturbed for 30 minutes upwards and then placed face down on a clean grease free microscope slide.

After 30 minutes the cover slip was then carefully lifted upwards and than placed free down on a clean grease free microscopic slide.

The preparation was then examined under a low power [x10] objective for ova, cysts, or larva of parasites. This was then confirmed by using [x40] objectives. One drop of iodine solution or eosine was also included in the duplication preparations for the identification of cysts.

Concentration is needed to quantify cysts of intestinal parasites.

CHAPTER FOUR

4.0 RESULTS

Pathogenic parasites were isolated in some of the fruit samples examined. The parasitic protozoa isolated are shown in table two. Ascaris egg was isolated from some of the fruits types examined while cyst of Amoeba was isolated only in garden egg.

Three (3) cysts of Amoeba were seen in garden egg.

Six (6) eggs of worms were seen in guava.

Four (4) eggs of worms were seen in banana.

No egg, cysts or ova/larva of worms was seen in apple, carrot, pineapple, pawpaw and pears.

4.1 CALCULATIONS USING STOLL'S TECHNIQUE

To calculate the number of eggs/cysts or larva per 100gm of raw fruits = multiplication of number of parasites in 100gm of fruits

Garden egg = 3 cysts of Amoeba was seen

= 3x5 = 15 cyst of Amoeba per 100gm of fruits.

Guava egg = 6 eggs of worms was seen

= 6x5 = 30 eggs of worms per 100gm of guava fruit

Banana = 4 eggs of worms was seen

= 4x5 = 20 eggs of worms per 100gm of banana fruits.

QUANTIFICATION (CALCULATION OF PARASITE OVA/EGG/CYST)

This is done by using the standard modified stool's techniques.

3mls of washed samples was added into 45mls of normal saline and shaked for good mixing. Using a pasture pipette, 0.15ml of the suspension was transferred to the slide and was examined under the microscope, the eggs or larvae were seen and was calculated, using stools method for the calculation of parasite in fluid specimen as reported in by (cheesbrough, 2005) which is = multiplication of number of parasite seen by 5 to get the number of parasite in 1000gm of fruits.

4.2 PERCENTAGE

To get the percentage

$$\frac{\text{The total no of parasite found in fruits}}{\text{The number of fruits samples used}} \times 100$$

Garden egg = $\frac{4}{30} \times \frac{100}{1} = 13.33\%$

Guava = $\frac{6}{23} \times \frac{100}{1} = 26.1\%$

Banana = $\frac{3}{15} \times \frac{100}{1} = 20\%$

4.2.1 TABLE 2; RESULTS OF FRUITS EXAMINED

No	Fruits samples	I drop of londine	2 drops of lodined
		No ova	No ova
1	Orange (citrus sinensis)	Of parasite seen	Of parasite seen.
2	Avocado (perseamericana)	No ova of parasite seen	On ova of parasite seen.
3.	Guava (pisdium guajava)	Six (6) eggs of worms was seen	Six (6) eggs of worms was seen
4	Pawpaw (arica papaya)	Yeast cells seen	Yeast cells seen
5.	Garden egg solanum melongena)	four (4) cysts of Amoeba was seen	Four (4) cysts of Amoeba seen.
6	Pineapple (ananas cosmosus)	Yeast cells seen	Yeast cells seen
7	Water melon (ctrullus lanatus)	Yeast cells seen	Yeast cells seen
8	Apple (Aberia cattra)	No ova of parasite seen	No ova of parasite seen
9	Banana (dusa accuminata)	three (3) eggs of worms was seen	three(3) eggs of worms seen

4.3 FINDINGS:

A total of nine (9) different types of fruits (over all number of 87 fruits) were examined for parasites contamination Banana has the highest contamination rate of 26.7%.

Guava has contamination rate of 26% and Garden egg has contamination rate of 10%.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

Microorganisms were isolated from some of the fruit samples examined. The isolation of intestinal parasitic protozoa from some of the fruits tends to suggest that fruits are possible sources of transmission of food borne diseases in man; their presence in those fruits may be associated with the water used in washing the fruits by their sellers. The presence of these parasites in the fruits may not be surprising when the water the sellers use in washing and sprinkled on the fruits are considered.

Guava and garden eggs tend to show the presence of isolates more than banana. This may be because the bananas as the other fruits were not sprinkled with contaminated water. It is known that faecal matters of animals and human begins harbour some of the protozoa/parasites isolated and the faecal matters are often used as manure for the cultivation of fruits.

It is therefore possible that some of protozoa, parasite isolates especially those on he garden eggs are contaminants from the field.

5.2 CONCLUSION

In conclusion, irrigation farming and use of night soil for agricultural purposes in some parts of Enugu State may lead to parasitic contamination of fruits more especially with protozoan.

The control measures includes treatment of irrigation waters before using for agricultural purposes, provision of goods sanitary system in both the urban and rural areas to prevent contamination of soil and water with parasite from poor deposition of feaces, covering of foods and water to prevent contamination from flies which act as cysts carriers, use of appropriate disinfectant to wash fruits before eating and mass education of people on dangers involved in eating unwashed and contaminated fruits.

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APPENDIX
APPARATUS AND EQUIPMENTS

Microscopes

Measuring cylinder

Microscopic slides

Cover slip

Sterile plastic bucket

Weighing balance

Syringes

REAGENTS

Dobell’s iodine 1g

Normal saline

Zinc sulphate 33%

Deionized water

Distilled water 50ml

PREPARATION OF NORMAL SALINE

Weigh 0.85g of powdered sodium

Chloride and add it into a 100ml of distilled water and then allow to dissolve

PREPARATION OF 33% ZINC SULPHATE

Using a weighing balance note the weight of soil and than weigh 33g of the powdered zinc sulp