ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG, EASTERN REGION

PARASITIC CONTAMINATION OF COMMONLY CONSUMED SALAD VEGETABLES IN KOFORIDUA (NEW JUABEN MUNICIPAL)

BY

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DECLARATION

I hereby certify that except for reference to other people work, which I have duly cited, this project submitted to the Department of Community Health, Ensign College of Public Health, Kpong is the result of my own investigation, and has not been presented for any other degree elsewhere.

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DEDICATION

To God Almighty who has been faithful and sustained me from the beginning till the end of this work.

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ABBREVIATION

cm……………………………………………………………………………….centimetres

dl………………………………………………………………………………….decilitre

DALY………………………………………………………….Disability adjusted life years

g……………………………………………………………………………………….grams

GSS……………………………………………………………....Ghana Statistical Service

ml……………………………………………………………………………….….milliliter

mm……………………………………………………………………………….millimeter

$NaCl_2$……………………………………………………………………..Sodium Chloride

pH……………………………………………………………………potential of Hydrogen

STATA……………………………………………………Statistical software for data analysis

STH……………………………………………………………… Soil-transmitted Helminths

YLD……………………………………………………………Years lost due to disability

WASH…………………………………………………………Water, Sanitation and Hygiene

W.H.O……………………………………………………………....World Health Organization

/……………………………………………………………………………………………per

ABSTRACT

Fresh vegetables are good source of vital nutrients for healthy growth which we may not get when such vegetables are cooked. However, eating them raw may pose a great risk for transmissions of pathogens in humans because they may harbour a wide range of microbial contaminants.

Parasitic infections were reported to lead to about 300 million severely ill individuals with approximately 200,000 deaths occurring in poor-resourced nations. Prevalence of parasite among vegetables in Accra, Ghana was 36.0%.

A total of 360 vegetables consisting of cabbage, lettuce, spring onion, carrot and tomatoes were bought from two major markets in Koforidua. The samples were washed and the deposit examined microscopically for parasites.

The prevalence of parasitic contamination of commonly consumed salad vegetables was 57.5% of which spring onion was the most heavily contaminated (97.22%). *Strongyloides stercolaris* larvae was most common intestinal parasite (36.39%) infesting almost the vegetable. Use of different concentration of saline demonstrated that concentration of saline has no effect on parasitic recovering rate; however, serial washing of vegetables showed decreasing recovering rate of parasites in this particular study.

Vegetables in Koforidua markets are heavily contaminated with parasites and there is need for educating consumers, food venders and farmers on the dangers associated with consuming inadequately washed vegetable

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Chapter One

Introduction

1.1 Background Information

Vegetables are normally referred to as the fresh edible portion of herbaceous plant roots, stems, leaves or fruits. These plants can either be eaten raw or prepared in a number of ways (Damen *et al*., 2007b).

Fresh vegetables are important part of a healthy diet owing to their nutritional value. Raw vegetables are great sources of vitamins, dietary fibers and minerals; and their regular consumption is associated with a reduced risk of most non communicable diseases such as cardiovascular diseases, stroke and certain cancers. Some vegetables are eaten raw as salad to retain the natural taste and preserve heat labile nutrients (Said, 2012).

The consumption of raw vegetables without proper washing is an important route of transmission of parasitic diseases. These organisms find their ecological habitat living inside the host. Parasites that have been identified to be associated with vegetable –borne outbreaks are protozoan cyst and oocyst such as *Entamoeba*, *Giardia* and *Toxoplasma* and helminth eggs like that of *Ascaris*, *Fasciola*, *Hymenolopsis*, *Taenia*, *Toxocara*, *Trichostrongylus* and hookworm (Shahnazi and Jafari-Sabet, 2010).

Beuchat (2002) indicated that outbreaks of human infections associated with consumption of raw fruits and vegetables have occurred with increased frequency during the past decade. Factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immuno-compromised consumers. A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in part, to their inaccessibility to locations within structures and tissues that may harbour pathogens (Beuchat, 2002).

1.1.1 Common sources of parasitic contamination

It is a well-known fact that the use of excreta polluted irrigation water is a health risk to the farmer and consumers of crops so produced. Raw wastewater frequently contains high numbers of eggs of human intestinal nematodes (Ayres *et al*., 1992). The increasing use of wastewater for irrigation in the 1970s and early 1980s prompted a series of literature reviews and investigation into the global extent of waste water re-use and its associated human health risk. The infection was also reported to be due household affair where infected children or persons provide the chief source of soil contamination by their promiscuous defecation (Damen *et al*., 2007b).

Market vegetables were reported to be contaminated by eggs of human intestinal nematodes where night soil is extensively used as fertilizer or wastewater reuse is being practiced. The direct application of night soil, animal manure and wastewater as an agricultural fertilizer has been practiced for centuries in many parts of the world, particularly in the Asian sub-continent (Shuval, 1990). This practice is gaining prominence, in developing countries as a result of the growing cost of mineral fertilizers and because of the increasing demand for basic food supplies. Indirect reserve of river or flowing water which contains a substantial percentage of municipal sewage has also been reported to be going on in many developing countries and may have contributed greatly to the source of parasitic infestation (Damen *et al*., 2007b).

1.1.2 Burden of human parasitic infection

Since the dawn of man, intestinal parasitic infections continue to be the most common cause of chronic infections, in communities living in resource poor countries situated in the tropics and sub tropics. These diseases cause great sufferings in areas of high endemicity in deprived communities and have recently been categorized as one of the "Neglected Tropical Diseases" (NTDs) by World Health Organization. These disease conditions were classified by World Health Organization as NTDs because they are characterized by little or no attention from policymakers, lack of priority within health strategies, inadequate research, limited resource allocation and few interventions (W.H.O., 2010).

Parasitic infections has led to about 300 million severely ill individuals with approximately 200,000 deaths occurring in poor-resourced nations (Hotez *et al*., 2006). About one third of the world, more than two billion people, has beens infected with intestinal parasites (Brooker, 2010).

It has been estimated that *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura* infect 1,450 million, 1,300 million and 1,050 million people worldwide, respectively (W.H.O, 2002). Other estimates suggest that *Ascaris lumbricoides* can infect over a billion, *Trichuris trichiura* 795 million people and hookworms 740 million people (De Silva *et al.*, 2003).

Recent estimates indicate that there are more than 1.5 billion people or 24% of the world's population infected with at least one species of soil-transmitted helminths consisting of 807-1221 million cases of *Ascaris lumbricoides*, 604-795 million cases of *Trichuris trichiura* and 576-740 million cases of hookworm (Albonico *et al*., 2008, W.H.O., 2002).

1.1.3 Intestinal parasites common in Ghana

In Ghana, food and water have been reported as the major routes of diarrhea outbreaks with vegetables being major sources. Particular interest were intestinal protozoan infections like giardiasis, amoebiasis, cyclosporiasis and cryptosporidiosis which have caused high levels of morbidity and mortality (Donkor *et al*., 2010, Nkrumah and Nguah, 2011, Dawson, 2005, Amorós *et al*., 2010).

The prevalence of helminths infection among pregnant women in community based study in Ashanti region was estimated to be 17.6%: *Necator americanus* (13.9%), *Strongyloides stercolaris* (1.9%) , *Ascaris lumbricoidis* (0.9%) and *Trichiuris trichiura* (0.9%) (Baidoo *et al*., 2010). Ayeh- Kumi *et al* (2009) also reported prevalence of 21.6% among food vendors in Accra, Ghana. They recovered *Ascaris lumbricoides* (5.0%), *Strongyloides stercoralis* (4.4%), *Cryptosporidium parvum* (2.5%), *Enterobius vermicularis* (4.1%), *Giardia lamblia* (2.0%), *Ascaris duodonal* (2.0%), and *Entamoeba histolytica* (2.0%).

Work done on vegetables by Duedu *et al* (2014), has reported the prevalence of parasites such as *Strongyloides stercoralis* larvae (43%) and *Cryptosporidium parvum* oocyst (16%). Other parasites found includes Hookworm ova, *Entamoeba histolytica* cysts, *Giardia lamblia* cysts, *Cyclospora cayetanensis* oocysts, *Entamoeba coli* cysts, *Trichuris trichiuria* ova, *Enterobius vermicularis* ova, *Isospora belli* oocysts and *Fasciolopsis buski* ova (Duedu *et al*., 2014) .

1.1.4 Parasites common in vegetables

Intestinal parasites serve as one of the major public health problems especially in the tropical and sub-tropical countries in the world. Parasites that have been associated with vegetable-borned outbreaks are protozoa cysts and oocysts such as *Entamoeba*, *Giardia* and *Toxoplasma*; and helminth eggs such as *Ascaris*, *Fasciola*, *Hymenolepis*, *Taenia*, *Toxocara*, *Trichostrongylus*, and hookworms. *Cryptosporidium* and *Giardia* were also identified in salad vegetables in Spain by Amoros and the friends (Adamu *et al*., 2012, Al-Megrin, 2010, Amoah *et al*., 2006, Erdogˇrul and Şener, 2005, Uneke *et al*., 2007, Amorós *et al*., 2010).

1.1.5 Public health and clinical significance of intestinal parasites

The public health significance of intestinal parasites can actually be measured through quantitative methods such as years of potential life lost, number of healthy days lost, incidence rate of the disease, prevalence of the disease and fatality rate since most of the cases are asymptomatic.

The amount of harm caused by intestinal parasite to the health of the individual depends on the parasite species, the intensity, and nature of interactions between the parasite and concurrent infection, nutritional and immunological status, and other socio economic factors.

Although the prevalence of intestinal parasite is quite high, the mortality caused by the parasites is very few. Ascariasis often results in intestinal obstruction, hookworm in iron deficiency anemia and trichuiriasis is associated with chronic dysentery and rectal prolapse. Giardiasis has been reported to cause nausea, vomiting, malabsorption, diarrhea, and weight loss (Kucik *et al*., 2004).

Ascariasis was the cause of intestinal obstruction in 5-35% of the pediatric cases in a comparison studies conducted in the tropics, giardiasis was 9.9 % in children in Brazil and trichuiriasis causing about 4% rectal prolapse in children in West Indies (Norhayati *et al*., 2003).

1.2 Problem statement

Fresh vegetables are good source of vital nutrients for healthy growth which we may not get when such vegetables are usually cooked. However, eating them raw may pose a great risk for transmissions of pathogens in humans because they may harbour a wide range of microbial contaminants such as viruses, bacteria and parasites. Such risks may be influenced by the sources of the vegetables and washing techniques used. Consumption of raw vegetables has been associated with outbreaks of foodborne diseases in many countries (Daryani *et al*., 2008, Johannessen *et al*., 2002).

In Ghana, food and water have been reported as the major routes of diarrhea outbreaks with vegetables being major sources (Donkor *et al*., 2010, Nkrumah and Nguah, 2011).

1.3 Rationale of the study

The usual practice at home is washing vegetables with either raw or pipe-borne water, and in some cases with saline water (salt solution).What is usually done is by fetching some quantity of salt either by hand or spoon, dissolving it in immeasurable amount of water for washing vegetables. Thus, the concentration, duration, and the number of washing to be done for effective removal of parasites are not known by the locals.

This study tried to explore the use of different saline concentration and normal water for washing of vegetables to assess its effectiveness in recovering of parasites from vegetables.

1.4 The Conceptual Framework

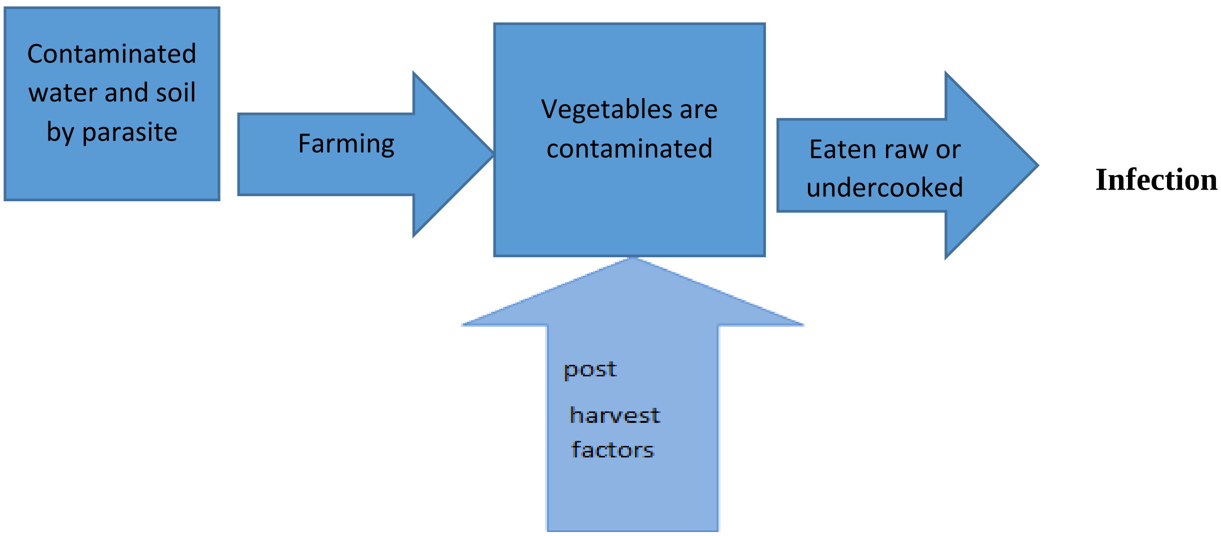

Figure 1.0: Author's own construct

A conceptual framework is used to outline possible sources of vegetable contamination. This conceptual framework shows how contaminated soil and water due to poor sanitation practices coupled with postharvest factors such as harvesting tools, single water use for washing of vegetables and transportation medium lead to contamination of vegetables on the farmlands. When these vegetables are eaten raw without proper washing with water or saline, there is the possibility of getting parasitic infection.

1.5 Research Questions

1. What is the prevalence of parasitic infection in vegetables?
2. What is the prominent parasitic infection among the vegetables types?
3. Is there any difference in using raw water and various saline concentrations in parasitic recovering rate?

4. What are some of the factors associated with parasitic contamination?

1.6 Main objective

To determine the parasitic prevalence rate in vegetables.

1.7 Secondary objectives

1. To identify the prominent parasitic infection of fresh vegetables.
2. To compare parasitic prevalence using different concentrations of saline water, and raw water for vegetable treatment.
3. To identify factors that are associated with vegetable contamination.

1.8 Profile of the study area

New Juaben is a Municipality in the Eastern Region of Ghana. It is the smallest of the 26 districts in the region and houses the Regional Capital. It's boundary to the North by East Akim District, South by Akwapim North District, and East by Yilo Krobo District and West by Suhum Kraboa Coaltar District. Estimated growth rate of 1.2% and the total population of 195547 (Municipal health profile report, 2016).

1.9 Scope of the Study

The study covers vegetable sellers that sell their produce in Koforidua, New Juaben Municipal for the purpose of estimating the prevalence of parasites in commonly consumed salad vegetables.

1.10 Organization of the Report

Chapter one comprised general information on vegetables, parasites that are common in Ghana, those that contaminate vegetables, public and clinical significance of parasitic infection and the objectives of this study. Chapter two entailed reviewed literature on parasites, emerging foodborne parasites, zoonotic parasites, vegetables and the importance in promoting health. Other things considered were sources of parasitic infection in vegetables, its prevention and control. Chapter three was about the type of research, sample size, sampling method, and the laboratory investigation methods for the detection of parasites in vegetables. Chapter four, five and six explained the outcome of the study, discussion in situation to other studies and inference from the study respectively.

Chapter Two

Literature Review

2.1 Parasites

Parasites are organisms that found their ecological niche living in or on organisms of distinct species, called host. Parasitic infections are mostly caused by helminths and protozoan parasites, and the infection caused by these parasites continue to be a global health problem, particularly affecting the wellbeing of children in poor communities in developing countries since their immune system is not fully developed (Gonçalves *et al*., 2003). The infection is characterized by malabsorption, diarrhea, blood loss, impaired work capacity and reduced growth rate in affected individuals. Epidemiological assessment of parasitic infections is focused on estimation of the prevalence. This is because clinical consequences of parasitic infections are very mild (Norhayati *et al*., 2003).

Helminths are among the most common and widespread causes of human infections. They are worms with many cells, metazoa. Nematodes (round worms), cestodes (tapeworms), and trematodes (flatworms) and soil transmitted helminthic parasites: *Ascaris lumbricoides* (roundworm), *Trichuris trichiuria* (whipworm), *Ancylostoma duodenale*, and *Necator americanicus* (hookworm) are among the most common helminths that inhabit human guts and rarely cause death but rather clinical disorders in humans (Bethony *et al*., 2006).

Intestinal helminths of importance to man are *Enterobius vermicularis* (pinworm), soil-transmitted helminths (STH) - *Ascaris lumbricoides* (round worm), *Trichuris trichiura* (whip worm), *Necator americanus* and *Ascaris duodenale* (hookworm) and *Strongyloides stercolaris* (threadworm). The other intestinal nematodes (*Anisakis sp*., *Capillaria philippinensis*), trematodes and cestodes are less widespread in man. Their distribution is limited to certain areas in the world and the infections are usually confined to certain communities (Norhayati *et al*., 2003).

Protozoan parasites have only one cell and can multiply inside the human body. The most common types are *Giardia instestinalis*, *Entamoeba histolytica and Cryptosporiduim sp.* which cause waterborne disease outbreak associated with diarrhea (Ananthakrishnan and Das, 2001). Intestinal protozoa of importance to man are *Entamoeba histolytica* and *Giardia duodenalis*. Currently, *Blastocystis hominis* and opportunistic protozoa such as *Cryptosporidium sp*. and *Isospora sp*. have been identified as the causes of diarrhea in children and immune-compromised patients. Other protozoal intestinal infections have restricted distribution (*Balantidium coli*) or are widely distributed but not pathogenic (*Entamoeba coli*, *Dientamoeba fragilis*, *Trichomonas hominis*). The common parasitic infections of vegetables are mostly soil transmitted helminthes and protozoan parasites (Norhayati *et al*., 2003).

2.2 Soil-transmitted Helminths (STH)

The three main soil-transmitted helminth infections, ascariasis, trichuriasis, and hookworm, are common clinical disorders in man. The gastrointestinal tract of a child living in poverty in a less developed country is likely to be parasitized with at least one, and in many cases all three soil-transmitted helminths, with resultant impairments in physical, intellectual, cognitive development and contributing to the perpetuation of the poverty-disease cycle (Bethony *et al*., 2006).

2.2.1 Ascariasis

Ascaris lumbricoides, causal agent of ascariasis is transmitted through the ingestion of infective eggs from contaminated food, hands or water (Crompton *et al*., 1985). An adult female worm living inside an infected person produces on average about 240 000 eggs per day for about a year, which are passed in the faeces. The eggs develop in the soil within 2 to 3 weeks, given optimal temperatures, presence of oxygen, and moisture. On being swallowed, each egg develops into a larval worm in the small intestine. The larvae migrate through the body via the hepatic portal system to the liver and lungs where they develop further for 1 to 2 weeks.

Then, they return to the small intestine and attain sexual maturity. The release of eggs by the female worms begins about 2 months after ingestion of infective eggs. Adult worms are large, with the male worms measuring up to 20 cm and females up to 45 cm in length.

Ascaris lumbricoides is highly specific for man and the infection does not produce a strong protective immunity. For its survival the parasite depends greatly on a high reservoir of infective eggs in an environment and thrives in areas where there is a lack of sanitation, particularly where people defecate indiscriminately around human settlements and where human excrement (night-soil) is used as a fertilizer in agriculture. The eggs are able to survive in adverse environmental conditions owing to their protective shell, and this further helps the parasite to persist.

Several types of complication are associated with ascariasis. Intestinal obstruction may be produced by a bolus of worms, or adult worms may migrate from the small intestine into the bile and pancreatic ducts, respiratory passages, and peritoneum. These conditions may cause medical or surgical emergencies. *Ascaris pneumonitis* due to larval migration is probably quite common although it is rarely detected clinically. *Ascaris lumbricoides* releases powerful allergens which may induce hypersensitivity. Faecal examination is necessary for diagnosis. Imaging with ultrasound or endoscopy can aid in diagnosis (Crompton *et al*., 1985, Committee, 1987).

2.2.2 Trichuriasis

The nematode *Trichuris trichiura* has a simple life-cycle, with eggs serving as the infective stage. The adult worms survive for as long as 5 years, firmly attached to the epithelial lining of the large intestine, with the caecum being the most commonly affected region. Each female worm has been estimated to produce from about 2000 to 14 000 eggs per day and these leave the host in the stools and contaminate the human environment, as do the eggs of *Ascaris lumbricoides* and the hookworms. Under suitable conditions, infective larvae develop inside the eggs in about 3 weeks and some may retain their viability for months. About 70-90 days after infective eggs are swallowed; the host begins to pass *Trichuris trichiura* eggs, indicating that adult worms are present in the large intestine.

The morbidity associated with trichuriasis is due to the worms' unique mode of attachment to the wall of the large intestine. Each worm is about 50 mm long and has a thin anterior part with which it burrows into the intestinal wall where it feeds on the intestinal tissues. The degree of morbidity is related to the intensity of the infection. Chronic impairment of the host's nutritional status (malabsorption and malnutrition) should be suspected when diarrhoea, hypoalbuminaemia, and iron-deficiency anemia are observed in association with the presence of the parasite. Diagnosis is mostly by faecal investigation for the presence of eggs (Committee, 1987).

2.2.3 Hookworm

The hookworm life-cycle is direct and begins with the eggs being released by the female worms into the lumen of the small intestine and being passed in the faeces. The embryos within the eggs develop rapidly, given moisture, warmth, and oxygen, and skin-penetrating, third-stage infective larvae are formed within 5-10 days after the deposition of the eggs. Infection occurs when the larvae enter the body through the skin, most commonly through the feet. Larvae of *Ascairis duodenale* are also infective by mouth.

In an endemic area, contaminated soil may continually or seasonally bear large numbers of infective larvae, which are found at the surface of the ground when the soil is damp. Lack of sanitation, indiscriminate defecation, and high egg production ensure constant exposure to infection, as do the practices of using the same places for defecation and going barefoot. The skin-penetrating larvae probably do not survive for more than a month under tropical conditions, but adult *Ascaris duodenale* and *Necator americanus* are believed to be capable of surviving for on average about 1 and 4 years, respectively.

Hookworm infection causes chronic blood loss and depletion of the body's iron stores, leading to iron-deficiency anaemia. It can also cause hypoproteinamia and anasarca. Presence of larvae or egg in stool is significant for diagnosis (Committee, 1987).

2.2.4 Nematodes

Strongyloides stercoralis is a nematode with a unique, complex lifecycle that causes an autoinfection in the host. The infection can persist for decades, even after departure from endemic areas, and under certain conditions, *Strongyloides stercolaris* can trigger a life-threatening form of strongyloidiasis, (the so-called hyper infection syndrome) and disseminated strongyloidiasis. Although usually associated with immunosuppression or debilitating diseases, risk factors for the severe forms of the disease have not been thoroughly evaluated. Symptoms include abdominal pain, diarrhea, weight loss and other non-specific complaints (Al-Bahrani *et al*., 1995). Definitive diagnosis requires direct visualization of the parasite, but because no test is 100% sensitive there is no absolute reference standard. Treatment is also difficult. Only total eradication eliminates the threat of potentially serious disease, and relapses are frequent with standard drugs such as thiabendazole and ivermectin (Sánchez *et al*., 2001).

2.3 Protozoan food contamination

The Giardia species infecting humans (and other mammals) and causing giardiasis is *Giardia duodenalis*, sometimes referred to as *Giardia lamblia* or *Giardia intestinalis* (regarded by some as a trace T of *G. duodenalis*). Other species for example, *Giardia muris* only infect other animals (rodents, birds and reptiles). In developed countries, giardiasisis particularly associated with foreign travel, and a common route of infection is through faecal or oral transmission. Giardia has a two-stage life cycle: a reproductive trophozoite and an environmentally resistant cyst stage. An ingested cyst passes into the duodenum, where excystation occurs, and releasing two trophozoites. These then multiply rapidly via asexual reproduction and colonise the small intestine. It is during the trophozoite stage that clinical symptoms occur, as a result of damage to the mucous membrane (Smith, 1993).

The infective dose for Giardia is between 10 and100 cysts. The incubation period in humans for Giardia is typically 1 to 2 weeks. The (oo) cysts are very resistant to environmental and water treatment stresses, assisting in their dissemination, and their potential to be transmitted from non-human to human hosts and vice versa. Cool and moist conditions favor their survival (Smith *et* *al*., 2007). Symptoms typically include watery diarrhea, bloating and flatulence. Stools are often fatty, malabsorption and weight loss can be significant. When the disease is untreated, it lasts for at least 5 days and potentially much longer. It can also reoccur, and can assume asymptomatic condition. Giardiasis can be diagnosed by stool examination for trophozoites and stool antigen immunoassays.

2.4 Emerging food borne parasites

Waterborne parasites can be transmitted by contaminated food. Water is a major vehicle for many environmental stages of parasites. These stages can contaminate foodstuffs such as fruits and vegetables as well as shellfish. Among emerging waterborne parasitic infections that may be acquired by food are *Cyclospora cayetanensis*, *Giardia sp.*, and *Cryptosporidium sp*.

2.4.1 *Cyclospora cayetanensis*

Outbreaks of *Cyclospora cayetanensis*, a coccidian parasite, which infects the small intestine and can cause watery diarrhoea, nausea and vomiting in humans, have been increasingly observed since the 1990s, especially in North America and Asia. Other symptoms include loss of appetite, weight loss, bloating, increased flatus, stomach cramps and muscle aches. However, some infected individuals may be asymptomatic. Almost 1500 cases occurred in the United States (US) and Canada in 1996 associated with eating raspberries from Guatemala (Herwaldt and Ackers, 1997). A year later, over 100 laboratory confirmed cases occurred in 19 clusters throughout the United State and Canada and on a cruise ship. Again, raspberries from Guatemala were implicated as the probable vehicle of infection for these clusters although preventive measures had been implemented. Other outbreaks were thought to have been spread through lettuce (CDC, 1997).

2.4.2 Cryptosporidium and Giardia

Cryptosporidium and Giardia were major cause of diarrhoeal disease in humans especially in immune compromised individuals worldwide and were major cause of protozoan waterborne diseases (Smith *et al*., 2007). *Cryptosporidium sp*. mostly affects cows, goats, and wild animals. Sixteen 'valid' Cryptosporidium species and a further 33 genotypes have been described, but only few have been reported to be zoonotic of which *Cryptosporidium parvum* is the major zoonotic Cryptosporidium species. With regard to Giardia, only two Assemblages (A and B) seem to be zoonotic, although recent findings suggested that zoonotic transmission occurs more rarely than previously believed (Monis *et al*., 2009).

Life cycles of Cryptosporidium and Giardia are completed within a single host, with transmission by the faecal–oral route. The transmissive stages, *Cryptosporidium parvum oocysts* or Giardia cysts, are produced in large numbers and are infectious when excreted. These parasites have low infective doses, which make them particularly adapted for environmental infection. In addition, the (oo) cysts are very resistant to environmental and water treatment stresses, which assists their spreading, and have the potential to be transmitted from non-human to human hosts and vice versa, enhancing the reservoir of (oo) cysts markedly (Smith *et al*., 2007). Waterborne outbreaks as well as infections through handling animals, contact with children and through recreational watershave been reported. (Oo) cysts of these parasites may also contaminate soft fruits and salad vegetables and cause infections in humans (Anh *et al*., 2007).

2.5 Zoonotic Parasites

2.5.1 *Fasciola sp.*

Fascioliasis is an emerging or re-emerging parasitic disease in many countries as a consequence of many phenomena related to environmental changes as well as man-made modifications (Mas-Coma *et al*., 2005). Fascioliasis, caused by Fasciola species, is a disease of herbivorous animals. It is a trematode which has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. Fascioliasis may occasionally affect man. Human infection causes serious hepatic pathological sequences. Two clinical stages have been recognized in human: fascioliasis which is an acute stage which coincides with the larval migration and worm maturation in the hepatic tissue; and a chronic stage which coincides with the persistence of Fasciola worms in the bile ducts. The parasitological diagnosis is based on identification of eggs in stool, duodenal contents or bile, also by the recovery of adult worm during surgical exploration, after treatment or at autopsy. However, the eggs may be present in very small number at irregular intervals, hence difficult to be detected. Besides, the eggs may be transiently present in stool after ingestion of raw or undercooked liver from infected animals. The symptoms may be present for several weeks before eggs are recovered in stool. Thus, the serologic tests are the alternative method of confirming early and extra biliary human fascioliasis. However, cross-reactions with other helminthic antigen may confuse the interpretation of the results (Haseeb *et al*., 2002).

2.5.2 *Balantidium Coli*

Balantidium coli is ciliate known to infect humans and cause dysentery. Balantidium species are found in variety of animals. *Balantidium coli* can be found in a trophozoite and cyst stage. The parasite is transmitted by oral-fecal route and cyst in infective stage. Multiplication is usually by binary fission and elongation of organism. In chronic disease patients, they experience frequent bowel movements alternating with periods of constipation; asymptomatic carriers do harbour parasites and continue to pass cysts in feces (Zaman and Cox, 1998).

Pigs are its reservoir hosts, and humans become infected through direct or indirect contact with pigs. In rural areas and in some developing countries where pig and human fecal matter contaminates the water supply, there is a greater likelihood that balantidiosis may develop in humans. The infection may be subclinical in humans, as it mostly is in pigs, or may develop as a fulminant infection with bloody and mucus-containing diarrhea; this can lead to perforation of the colon. The disease responds to treatment with tetracycline or metronidazole. Balantidiosis is a disease that need never exist given access to clean water and a public health infrastructure that monitors the water supply and tracks infections (Schuster and Ramirez-Avila, 2008).

2.6 Global Burden of Parasites

Parasitic infections lead to about 300 million severely ill individuals with approximately 200,000 deaths occurring in poor-resourced nations (Hotez *et al*., 2006). About one third of the world, more than two billion people, are infected with intestinal parasites (Brooker, 2010). It has been estimated that *Ascaris lumbricoides*, *hookworm*, and *Trichuris trichiura* infect 1,450 million, 1,300 million and 1,050 million people worldwide, respectively (Committee, 2002). Other estimates suggest that *Ascaris lumbricoides* can infect over a billion, *Trichuris trichiura* 795 million people and hookworms 740 million people (De Silva *et al*., 2003). Later in 2008, estimates indicate that there are more than 1.5 billion people or 24% of the world’s population infected with at least one species of soil-transmitted helminths consisting of 807-1221 million cases of *Ascaris lumbricoides*, 604-795 million cases of *Trichuris trichiura* and 576-740 million cases of hookworm (Albonico *et al*., 2008, W.H.O, 2002).

Recent estimate through review of 118 countries revealed globally, an estimated 438.9 million people (95% CI, 406.3 - 480.2 million) were infected with hookworm in 2010, 819.0 million (95% CI, 771.7 – 891.6 million) with *Ascaris lumbricoides* and 464.6 million (95% CI, 429.6 – 508.0 million) with *Trichuris trichiura*. Of the 4.98 million YLDs attributable to STH globally in 2010, 65% are attributable to hookworm, 22% to *Ascaris lumbricoides* and the remaining 13% to *Trichuris trichiura*. There were an estimated 2,824 deaths attributable to *Ascaris lumbricoides* in 2010, with most occurring in populations from Asia and south Asia, increasing the global DALYs attributable to *Ascaris lumbricoides* to 1.31 million (0.71 – 2.35 million). For hookworm and *Trichuris trichiura* –for which no deaths are attributed – the YLDs represent the total DALYs contributed by these infections. This brings the DALYs contributed by STH to 5.18 million in 2010, with 3.23 caused by hookworm, 1.31 by *Ascaris lumbricoides* and 0.64 by *Trichuris trichiura*. In sub-Sahara Africa, the total prevalence for hookworm infection is 14.1% (YLDs is 456 823), *A.lumbricoides* is 15.2% (YLDs is168, 652) and *Trichuris trichiura* is 21.0% (YLDs is 134,055) (Pullan *et al*., 2014).

2.7 Sources of Pathogens and Factors that Influences their Survival

The survival of pathogen is greatly influenced by the organism, produce item and the environmental conditions in the field and thereafter, including storage conditions. These factors are grouped into pre-harvest and postharvest factors (Harris *et al*., 2003). The environmental route of transmission is important for many protozoan and helminth parasites, with water, food and soil being particularly significant. The potential for producing large numbers of transmissive stages and their environmental robustness and the parasites being able to survive in moist microclimates for prolonged periods of time, pose a persistent threat to public health. The increased demands on natural resources increase the likelihood of encountering environments and produce contaminated with parasites.

For waterborne diseases, the protozoa, Cryptosporidium, Giardia and Toxoplasma, are the most significant causes, yet, with the exception of Toxoplasma, the contribution of zoonotic transmission remains unclear due to the absence of ‘standardized’ methods. The microsporidia have been documented in one waterborne outbreak, but the role of animals as the cause of contamination was not elucidated.

In food, surface contamination is associated with the faecal–oral pathogens, and some data are available to indicate that animal wastes remain an important source of contamination (e.g. cattle faeces and apple cider outbreaks). Review of some literature indicated that that typhoid fever, infectious hepatitis, fascioliasis, and cholera are the diseases that have been most frequently transmitted by foods contaminated by sewage or irrigation water in agricultural or aquacultural practices. Vegetables contaminated by night soil or raw or partially treated sewage were reported as vehicles in some outbreaks. There is an increasing recognition of the burden of human fascioliasis and it is now recognized as an emerging zoonosis by the WHO (Harris *et al*., 2003).

2.7.1 Pre-harvest Factors of Vegetable Contamination

Soil, irrigation water, inadequate composted manure, air or dust, wild or domestic animals, human handling and water used for irrigation were considered as possible sources of parasitic contamination of fresh vegetables. In evaluations of many risk factors for preharvest-level produce contamination, the quality assessment of the reviewed studies confirmed the existence of solid evidence for only some of them, including growing produce on clay-type soil, the application of contaminated or non–pH-stabilized manure, and the use of spray irrigation with contaminated water, with a particular risk of contamination on the lower leaf surface (Park *et al*., 2012).

2.7.2 Postharvest Factors of Vegetable Contamination

Post harvesting processes, ranging from storage and rinsing to cutting, are also possible sources of contamination. Contamination of produce in the harvesting process can result from transfer of pathogens from farm workers or from unsanitary harvest containers. Series of washes that do reuse wash water, the harvesting bin and containers or tool that comes in contact with produce not kept clean and not sanitized prior to use. Poor sanitation of the packing area, domestic and wild animals (especially rodents) being permitted in the wash area, improper sanitized vehicles that are used for the transport of fresh produce to the market improper packaging, Cross contamination of vegetables and improper handling after wholesale purchase have been identified as sources of parasitic contamination of fresh produce (Berger *et al*., 2010, Buck *et al*., 2003).

2.8 Importance of Raw Vegetables

Vegetables in its broadest sense, refers to any kind of plant life or plant products. It is also commonly referred to as the fresh edible portion of herbaceous plants roots, stems, leaves or fruits. Fresh vegetables are an important part of a healthy diet owing to their nutritional value. Raw vegetables are great source of vitamins, dietary fiber and minerals; and their regular consumption is associated with a reduced risk of cardiovascular diseases, stroke and certain cancers. Freshly eaten vegetables are a major source of nutrients like vitamins (vitamin B-complex, Vitamin-C, vitamin A, and vitamin K) and minerals (calcium, magnesium, potassium, iron, beta-carotene) as well as dietary fiber.

They are known to reduce the risk of several diseases like bacterial, fungal, viral and cancer and serve as phytochemicals which function as antioxidants and anti-inflammatory agents reducing the risk of cardiovascular diseases, stroke and certain cancers (Slavin and Lloyd, 2012, Poiroux-Gonord *et al*., 2010). The anti-oxidant in vegetables helps protect the human body from oxidant stress which causes intravascular haemolysis.

The fibers also absorb water in the colon, retain good amount of moisture in the faecal matter and help in smooth passage out of the body, thereby offering protection from conditions like constipation, hemorrhoids, and rectal fissures among others. The folate and iron helps in formation of red blood cell and boost the haemoglobin level thus preventing anaemia in adults and reduces the risk of neural tube defect, spin bifida and anencephaly during fetal development in pregnant women. The potassium actually helps in maintaining healthy blood pressure(Alade *et al*., 2013).

2.8.1 Carrot

It is parsley family and originates from middle Asia around Afghanistan and slowly spreads to Mediterranean area. Carrots as a vegetable can grow in any environment with nutrient dense soil, sunlight and water (Wood-Meon, 2011). Orange root of carrot containing pigments carotene were found by a research to contain calcium, potassium, vitamin A, B and C with their medical properties. Carrot is rich in alkaline elements which purify and revitalize the blood. Contents of carrots are vitamin A, B1, B6 and K, manganese and phosphate. Carrot juice has found to be a cure for early cancer formation(Fabiyi *et al.*, 2015). Vitamin B6 helps in production of an important brain chemicals and hormones like serotonin and melatonin needed for good sleep and mood stability. It also aids in metabolic processes and function of the immune system. Vitamin A is good for good vision (Wood-Meon, 2011). Eating carrot is also good for allergies, anemia, rheumatism, tonic for nervous system and stimulates milk during lactation (WCM, 2008).

2.8.2 Tomatoes

The evidence for a benefit was strongest for cancers of the prostate, lung, and stomach. Data were also suggestive of a benefit for cancers of the pancreas, colon and rectum, esophagus, oral cavity, breast, and cervix. A cause-effect relationship, however, could not be established because data were from observational studies. However, the consistency of the results across numerous studies in diverse populations, for case-control and prospective studies, and for dietary-based and blood-based investigations argues against bias or confounding as the explanation for these findings. Lycopene may account for or contribute to these benefits, but this possibility is not yet proven and requires further study. Numerous other potentially beneficial compounds are present in tomatoes, and, conceivably, complex interactions among multiple components may contribute to the anticancer properties of tomatoes (Giovannucci, 1999).

2.8.3 Green Leafy Vegetables

All green vegetables contribute to diet in terms of terms of potassium, calcium and magnesium (Kawashima and Soares, 2003). They have been known to be indispensable ingredients in traditional sauces that accompany carbohydrate stables as well. Leafy green vegetables such as lettuce and cabbage contain water, protein, fat, sugar, dietary fibers, energy and micro nutrients such as vitamin C, folate, carotenoids, calcium and iron. The iron actually helps in production of red blood cells and therefore boosting the haemoglobin level. Dietary fiber is linked to less cardio vascular diseases and probably has a role in obesity prevention (Slavin and Lloyd, 2012).The antioxidant compounds, polyphenols and vitamin C, have been determined in five varieties of lettuce (iceberg, romaine, continental, red oak leaf, lollorosso) (Llorach *et al*., 2008).

Fibre cleanses the digestive tract, by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the food and prevents the intake of excess starchy food and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus (Mensah *et al*., 2008).

2.9 Factors that are Associated with Vegetable Contamination

Food borne diseases according to Blackburn and McClure (2002) to continue to be a common and serious threat to public health all over the world and are a major cause of morbidity. They further stated that, outbreaks of human infections due to the consumption of raw fruits and vegetables have occurred with increased frequency during the past decade. To buttress the above, Al-Megrm (2010) posits that, the consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic foodborne diseases. This in the view of Wakid (2009) results in an increase in intestinal parasites in humans and is among the main public health problems around the world especially in tropical and subtropical countries (Al-Megrm, 2010, Blackburn and McClure, 2002, Wakid, 2009).

Studies have also shown that *Ascaris lumbricoides*, *Cryptosporidium sp.*, *Entamoeba histolytica*, *Enterobius vermicularis, Fasciola sp., Giardia intestinalis*, hookworm, *Hymenolepis sp., Taenia* *sp.*, *Trichuris trichiura*, and *Toxocara sp*., can infect humans who consume contaminated, uncooked, or improperly washed vegetables and fruits (Kozan *et al*., 2005).

In recent years, due to the increase in the number of reported cases of foodborne illness linked to consumption of fresh vegetables, some studies have been carried out to identify some of the factors that are associated with vegetable contamination. Some of the factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immune-compromised consumers. Also, a general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in part, to their inaccessibility to locations within structures and tissues that may harbour pathogens (Beuchat, 2002).

The extent of contamination has also been identified to be dependent on several factors that include among others, using contaminated water for irrigation, applying untreated or improperly composed manure as fertilizer, fecal contamination from domestic animals and human beings, post-harvest handling and hygienic conditions of preparation in food service or home settings (Damen *et al*., 2007b, Gharavi *et al*., 2002). Other researchers such as Opara and Udoidung (2002); Dalomo (2003); and Ekwunife & Akolisa (2009) also avers that, environmental sanitation and personal hygiene of the people are major contributory factors to the spread of food-borne illnesses, which is a major and serious problem in most developing countries. Most parasites according to them rely on being swallowed in order to infect a human host.

The broad range in prevalence could be further be attributed to other factors such as geographical location, type and number of vegetable samples examined, methods used for detection of the intestinal parasites, type of water used for irrigation, and post-harvest handling methods of such vegetables which are different from one country to another. Other factors that can affect parasitic transmission may also include population related hygienic habits, sanitary facilitations, climatic conditions, and range of foodborne parasites endemicity in certain countries

Epidemiological studies have also shown that, in parts of the world where parasitic diseases are prevalent in the population and where wastewater is used to irrigate vegetables which are eaten raw, the intake of wastewater irrigated vegetables without proper washing may lead to parasitic infection (Damen *et al*., 2007b). These parasites may be acquired through the consumption of these vegetables, especially when not hygienically grown and adequately prepared before consumption.

Therefore, to reduce the incidence of the consumption of parasite infected vegetables, residents must be taught how to properly disinfect vegetables before consuming them raw. Rapt attention must also be paid to effective and comprehensive prevention and treatment measures such as thoroughly washing vegetables before eating to ensure food safety. Sanitation conditions of farms and also sales points must also be considered to help reduce infestation.

2.9 Control and Prevention of Parasitic Infection

The most effective control program of intestinal parasitic infections is an integrated approach with community participation. The long-term objectives to reduce the prevalence, intensity of infection and severity of intestinal parasitic infections to levels at which they cease to be of public health significance. The infections can be controlled and prevented by improvement in environmental sanitation such as safe methods of faeces and waste disposal and provision of safe water supplies and health education on health promotion of personal and food hygiene.

In recent years the availability and treatment with of single-dose broad-spectrum antihelminthics has helped in reducing the worm burden in endemic communities. Studies have shown that periodic chemotherapy strategy has successfully lowered the intensity of Ascaris and hookworm infections. Water, Sanitation and Hygiene (WASH) access and practices were reported to be generally associated with reduced odds of STH infection. Pooled estimates from all meta-analyses, except for two, indicated at least a 33% reduction in odds of infection associated with individual WASH practices or access (Strunz *et al*., 2014).

Access to improved sanitation, hygiene in the home should be prioritized alongside preventive chemotherapy and health education to achieve a durable reduction of the burden of helminthiases. Availability and use of sanitation facilities and water treatment is associated with lower odds of intestinal protozoa infections as well (Ziegelbauer *et al*., 2012, Speich *et al*., 2016).

Findings of some research studies also outline other control and preventive measures of parasitic infection. For example Wegayehu *et al.* (2013) conducted a study to assess the magnitude and pattern of intestinal parasitism in highland and lowland dwellers in Gamo area, South Ethiopia. Community-based cross-sectional study was conducted between September 2010 and July 2011 at Lante, Kolla Shelle, Dorze and Geressie kebeles of Gamo Gofa Zone, South Ethiopia. The study sites and study participants were selected using multistage sampling method. Data was gathered through house-to-house survey.

A total of 858 stool specimens were collected and processed using direct wet mount and formol-ether concentration techniques for the presence of parasite. Out of the total examined subjects, 342(39.9%) were found positive for at least one intestinal parasite. The prevalence of *Entamoeba histolytica/dispar* was the highest 98(11.4%), followed by *Giardia lamblia* 91(10.6%), *Ascaris* *lumbricoides* 67(7.8%), *Strongyloides stercoralis* 51(5.9%), hookworm 42(4.9%), *Trichuris trichiura* 24(2.8%), *Taenia sp*. 18(2.1%), *Hymenolepis nana* 7(0.6%) and *Schistosoma mansoni* 1(0.12%). No statistically significant difference was observed in the prevalence of intestinal parasitic infections among lowland (37.9%) and highland dwellers (42.3%) (P = 0.185). Also, no statistically significant difference of infection was observed among the age groups (P = 0.228) but it was higher in reproductive age group. At the end of the study, to control and prevent parasitic infections, it was recommended that, enhancing socioeconomic status, improving sanitation facilities, instilling health education and promoting ways of keeping personal hygiene can be good strategies to control these infections in the area (Wegayehu *et al*., 2013).

Also, Tabatabaei *et al* (2013) evaluated the parasitic pollution of raw vegetables in Amol city of Iran. The study was descriptive in nature compromised of 200 samples from 10 different vegetables including spinach, garden cress, parsley, lettuce, peppermint, radish, green onion, basil, coriander and scallion. The samples were evaluated using timely-sediment method and then centrifuging concentrating. The obtained results showed that 93(46.5%) of all vegetables (collected from 20 regions) were contaminated with pathologic and non-pathologic parasites. Among these, spinach and coriander were the highest (17.2%) and the lowest (3.2%) contaminated samples, respectively. The isolated parasites were nematode larva 25.8%, Giardia cyst 22.5%, *Entamoeba coli* cyst 19.3%. However, the obtained results from 100 restaurants and kebab samples did not show any parasitic contamination. Based on the results, it was concluded that, using organized and new methods for irrigating agricultural fields, preventing animals to enter the vegetable's farmlands and also improving people's knowledge on proper washing of vegetables can be useful in decreasing parasites prevalence (Siyadatpanah *et al*., 2013).

Tafera *et al*. (2014) in their study aimed at determining the prevalence and predictors of parasitic contamination of fruits and vegetables collected from local markets in Jimma Town, Ethiopia between April and May 2013. A total of 360 samples of fruits and vegetables were examined by sedimentation concentration after washing using normal saline. The overall prevalence of parasitic contamination was 57.8%. Strongyloides like parasite (21.9%) was the most frequent parasitic contaminant followed by *Toxocara Sp.* (14.7%), *Cryptosporidium Sp.* (12.8%), *Hymenolepis nana* (8.3%), *Giardiadia lamblia* (7.5%), *Ascaris lumbricoides* (6.7%), *Entamoeba histolytica/dispar* (5.3%), *Cyclospora sp.* (5.0%), and *H. diminuta* (1.4%). Washing of the fruits and vegetables before display for selling was significantly associated with decreased parasitic contamination. Since fruits and vegetables are potential sources of transmission for intestinal parasites, consumers were advised to avoid acquiring parasitic infection from contaminated fruits and vegetables through proper cleaning and cooking.

Further, using a total of 112 samples made up of cabbage (17), mint (11), coriander leaves (11), spinach (15), onion (10), carrot (10), potato (10), ginger (15), beet root (7) and tomato (6) which were collected from a retail market at Mannuthy, Kerala, Sunil *et al*. (2014) assessed the parasitic contamination of raw vegetables retailed at Mannuthy in Thrissur district of Kerala state, India. The samples collected were washed with physiological saline solution. The washings were collected and examined under light microscopy. The results indicated that, Helminthic eggs were detected in three (2.7%) of 112 samples. Two samples of cabbage (1.8%) and one sample of onion (0.9%) were positive for ova of *Ascaris sp*. The study was concluded on the bases that, since vegetables can act as potential sources of gastrointestinal parasitic infections, there is the need to proper washing of vegetables before they are consumed or cooked(Sunil *et al*., 2014).

In this study Amoah *et al*. (2016) broadened the concept of infection risks in the exposure assessments by measurements of the concentration of STHs both in wastewater used for irrigation and the soil, as well as the actual load of STHs ova in the stool of farmers and their family members (165 and 127 in the wet and dry seasons respectively) and a control group of non-farmers (100 and 52 in the wet and dry seasons, respectively). Odds ratios were calculated for exposure and non-exposure to wastewater irrigation. The results obtained indicate positive correlation between STH concentrations in irrigation water/soil and STHs ova as measured in the stool of the exposed farmer population. The correlations are based on reinfection during a 3 months period after prior confirmed deworming. Farmers and family members exposed to irrigation water were three times more likely as compared to the control group of non-farmers to be infected with *Ascaris sp.* (OR = 3.9, 95% CI, 1.15–13.86) and hookworm (OR = 3.07, 95% CI, 0.87–10.82). This study therefore contributed to the evidence-based conclusion that wastewater irrigation contributes to a higher incidence of STHs infection for farmers exposed annually, with higher odds of infection in the wet season. Thus, the results obtained show an epidemiological link between wastewater irrigation and helminth infection in Ghana. It was therefore recommended that, more emphasis were needed for regulations and interventions aimed at making the practice safer for the farmers which in turn would contribute significantly in breaking the cycle of infection (Amoah *et al*., 2016).

Finally, in finding ways to reduce vegetable contaminations, Amoah *et al*. (2007) tried to analyse and improve the effectiveness of common indigenous washing methods for the reduction of faecal coliform populations on the surface of wastewater-irrigated vegetables and to determine simple factors affecting their efficacy. Questionnaire interviews were used to gather information on common methods used for washing vegetables in seven West African countries.

The efficacy of the most common decontamination methods was measured in terms of log reductions in FC populations on homogenised contaminated lettuce, cabbage and spring onion samples. Findings revealed that, the large majority of urban households and restaurants in the sub-region are aware of vegetable-related health risks and wash vegetables before consumption. Methods used varied widely within and between Ghana and its neighboring francophone West African countries (Amoah *et al*., 2007).

However, several of the most common methods did not reduce the contamination to any desirable level. Significantly, different log reductions were achieved depending on the washing method, contact time and water temperature. Tests to improve the apparent ineffective methods were especially promising in view of the relatively expensive vinegar. However, up to 3 log units reduction was also possible at a much lower price with 'Eau de Javel' (household bleach), which is commonly used in francophone West Africa. Per the findings, the authors recommended that, washing vegetables before consumption was an important component of a multiple barrier approach for health risk reduction. The high risk perception among consumers necessitated the need to make more information available on the appropriate use of these washing methods. This is because any washing method would need complementary efforts to reduce contamination before the vegetables enter the kitchen, such as safer irrigation practices (Amoah *et al*., 2007).

Chapter Three

Methodology

3.1 Study design and sample size

Quantitative, cross-sectional and experimental study was conducted with a sample size of 360 based on a 36% prevalence obtained from a similar study conducted by Duedu *et al.* in Accra, Ghana with a margin error of 5% (Duedu *et al*., 2014). The actual calculated sample size was 354 but equal number (72 samples) of each vegetable type was used for analysis from the two market sites.

$$n = \frac{Z^2(pq)}{e^2}$$

$$\mathrm{n} = \frac{1.96^2\,(0.36 \times 0.64)}{0.05^2} = 354$$

Where,

p = prevalence;

q =1-p;

e = error margin at 95% confidence interval;

z = critical value and

n = sample size

3.2 Data collection techniques

3.2.1 Collection of samples for the study

Quantities of each vegetable (tomatoes, cabbage, lettuce, spring onion and carrot were picked conveniently from two retail market sites in Koforidua (Agathar market and the main market) by traditional buying method.

3.2.2 Sample preparation and examination

The vegetables were transported to the laboratory in plastic bags. First, the edible part was separated according to household practice. About 200g (big vegetable) of each vegetable was weighed, chopped and washed with raw water and different concentrations of physiological saline (0.45%, 0.9%, 1.5%NaCl). The washing water was left for 12 hours for it to sediment after using gauze to remove visible particles, the top water was discarded and the remaining water was centrifuged at 1500 rpm for 10 minutes. The supernatant was removed and the residue carefully collected in the test tube by tapping the bottom of the tube and transferring unto a labeled clean slides for examination purposes (Cheesbrough, 2006). Each vegetable type was washed twice at each level of saline concentration and the saline used for the preparations were autoclaved at 121^0c for 20 minutes.

3.2.3 Macroscopic and microscopic examination

Each of the vegetable was examined carefully for the presence of segment of cestodes and adult nematodes.

The sediment was mixed and examined as follow:

(1) Simple smear: a drop of the sediment was applied on the center of a clean grease-free slide. A clean cover slip was placed gently to avoid air bubbles and over flooding. The preparation was examined under a light microscope using ×10 and ×40 objectives.

(2) Iodine smear: the drop of the sediment was mixed with a drop of Lugol's Iodine solution and examined as in simple smear for the purpose of parasite confirmation.

Simple and iodine smears were used for detection of parasitic eggs, cysts and larva. Two (2) slides were prepared for each sediment. Eggs, cysts and oocysts of parasites found under the light microscope were identified.

(3) Staining of sediment smear by Modified Ziehl–Neelsen to detect protozoal parasitic (oo) cysts and spores of *microsporidium sp*.: A thick smear of the sediment was done, air dried and fixed with methanol for 3 minutes. The slides were stained with cold carbol fuschin for 15 minutes and then differentiated in 1% hydrochloric acid-ethanol solution for 15 seconds after washing off excess carbol fuschin with distilled water. The slides were rinsed with distilled water and counter stained with 0.3% methylene blue for 30 seconds. The slides were rinsed with water; air dried, and examined microscopically using 100X objective oil immersion lens.

(4) Staining of sediment smear by Florescence staining to detect (oo) cysts and spores of *microsporidium sp*.: A thin smear of the sediment was done, air dried and fixed with methanol for 3 minutes. The slides were stained with Auramine O-phenol for 20 minutes, after which the slides were washed with distilled water, and then differentiated in 0.5 % hydrochloric acid-ethanol solution for 3 minutes. The slides were rinsed in distilled water and counter stained with 0.5% potassium per manganate for 30 seconds. The slides were rinsed with water; air dried, and examined microscopically using 40x objective lens of florescence microscope.

3.2.4 Questionnaires

Questionnaires were administered to the vegetable sellers to assess the source of produce and other possible sources of parasitic contamination. This was adapted and modified from the work done by Antwi and others (Antwi-Agyei *et al*., 2015). Observations was also made of mode of display of vegetables for sale.

3.3 Study site

In Ghana, the large open-aired markets are the most patronized and serve as a supply point for most shops, stalls and household consumables. Samples were taken from Koforidua main market and Agartha market. These markets were considered because majority of the farmers from different villages in New Juaben convey their farm produce, which include carrot, tomatoes, lettuce, cabbage, green leaves, green pepper, and other vegetables for sale on market days which are Mondays and Thursdays.

Map 1: Map of New Juaben Municipal showing various districts (extract from GSS 2010 report)

3.4 Study variables

Vegetables that were used in this study were tomatoes (*Lycopersicon Esculentum*), lettuce (*Lactuca Sativa*), carrots (*Daucus Carota*), cabbage (*Brassica Oleracea*), and spring onion (*Allium Fistulosum*). The dependent variable was presence of parasites and independent variables were saline concentration, market site, vegetable type, performance of hand hygiene, and mode of display of vegetable.

3.5 Sampling

Quantities (36) of each vegetable were picked conveniently from two retail market sites in Koforidua by means of normal buying process in the market from January to March, 2017.

3.6 Pretesting

Pretesting of the questionnaire was done in Nkruakan market with 3 sellers where 10 vegetables were bought. Pre-testing revealed the weakness of the data collection tools. Corrections were made to address the weaknesses observed.

3.7 Data Handling

Data collected was entered and cleaned in Excel 2016

3.8 Data Analysis

Data was imported into, analyzed using STATA version 14.0 and R version 3.3.1

Prevalence calculation, Chi square test and logistic regression (outcome variable was presence of parasite and the explanatory variables were vegetable types, market type, demographic characteristics of vendors, mode of display, hand hygiene performance) were done using STATA.

Because the number of parasites recorded in the study represented count data, Poisson regression was also done. And to account for over dispersion in the variance, quasi-likelihood (Quasi-Poisson) regression was used to fit the data using Generalised Linear Model (GLM) in R statistical programming environment.

3.9 Ethical consideration

Ethical approval was obtained from the Ethics Review Committee of Ensign College of Public Health.

Approval was sought from the municipal director of health and the head of Regional Hospital, Laboratory.

Written consent was sought from each seller before administration of questionnaires.

Each category of vegetable was put into polythene bag and labeled with unique identification number as well as the questionnaires.

3.10 Limitation

Cluster effects of vegetables, therefore vegetables were bought from vast sellers to reduce this effect on the study.

Identification of *cryptosporidium sp.* was difficult with the saline solution and Modified Ziehl–Neelsen staining. We relied solely on the florescence staining and the experience of the microscopist.

3.11 Assumption

Each vegetable was identified with a unique seller. Each vegetable was treated as potential vegetable with parasites.

Chapter Four

Result

A total of 360 samples of salad vegetables which included tomatoes, carrot, lettuce, cabbage, spring onion were bought from two major market sites in Koforidua and examined for parasitological contamination at the Regional Hospital Laboratory, Koforidua. The result of the study showed that 207 samples were identified to be contaminated with at least one type of parasite which gave rise to overall prevalence of 57.50 %.These include 97.2% of spring onion, 70.8% of lettuce, 50.0% of tomatoes, 43.1% of cabbage and 26.4% of carrot. The detail is as shown in Table 4.1. The most contaminated vegetable was spring onion and the least contaminated salad vegetable was carrot.

Table 4.1: Prevalence of parasite among vegetable types (January 2nd to March 7th, 2017)

Vegetable Type	Number Examined	Number Positive (%)
Cabbage	72	31 (43.1)
Lettuce	72	51 (70.8)
Carrot	72	19 (26.4)
spring onion	72	70 (97.2)
Tomatoes	72	36 (50.0)
Total	**360**	**207 (57.5)**

Table 4.2: Frequency distribution of parasites among vegetables in two major markets in Koforidua from Jan 2nd to March 7th 2017

Vegetable Type	Number Examined	*Strongyloides stercolaris* n(%)	*Balantidium coli* n (%)	*Fasciola sp.* n (%)	*Flagellate* n (%)	*Cryptosporiduim sp.* n (%)
Cabbage	72	8 (11.11)	21 (29.17)	0 (0)	10 (13.89)	12 (16.67)
Lettuce	72	39 (54.17)	3 (4.17)	26 (36.11)	0 (0)	15 (20.83)
Carrot	72	14 (19.44)	2 (2.78)	0 (0)	3 (4.17)	4 (5.56)
Spring onion	72	70 (97.22)	1 (1.39)	0 (0)	0 (0)	8 (11.11)
Tomatoes	72	0 (0)	22 (30.56)	0 (0)	15 (20.83)	1 (1.39)
Total	**360**	**131(36.39)**	**49 (13.61)**	**26 (7.22)**	**28 (7.78)**	**40 (11.11)**

n = Total number of vegetable type contaminated

The different types of parasites identified were *Strongyloides stercolaris* larvae, *Fasciola sp*., *Cryptosporiduim sp*. oocyst, *Balantiduim coli*, and flagellates. The most frequent occurring parasite was *Strongyloides stercolaris* larvae (36.39%) and the least occurring was *Fasciola sp.* (7.22%). *Strongyloides stercolaris* larvae were detected in all the vegetable types except tomatoes. *Fasciola sp.* was a common parasite of lettuce. *Cryptosporidium sp*. and *Balantiduim* *coli* were common in among all the vegetable types considered. Flagellates were recovered from cabbage, carrot, tomatoes and not in lettuce and spring onion (Table 4.2).

Table 4.3: Chi-square test of factors associated with parasitic contamination of salad vegetables in two major markets in Koforidua from Jan 2nd to March 7th 2017.

Variables (n= 360)	Positive (%)	Total	chi-square	p-value
MARKET			15.5612	< 0.001
Agartha	122 (67.78)	180		
Main Market	85 (47.22)	180		
VEGETABLE TYPE			88.0477	< 0.001
Cabbage	31 (43.06)	72		
Lettuce	51 (70.83)	72		
Carrot	19 (26.39)	72		
Spring Onion	70 (97.22)	72		
Tomatoes	36 (50.00)	72		
HAND HYGIENE AT MARKET			0.6874	0.407
Soap And Water	139 (56.05)	248		
Water Only	68 (60.71)	112		
SOURCE OF PRODUCE			15.9724	< 0.001
Farm Gate	35 (39.77)	88		
Wholesale	87 (60.42)	144		
Retailers	85 (66.41)	128		
SALINE CONCENTRATION(g/dl)			1.7619	0.623
0	50 (55.56)	90		
0.45	57 (63.33)	90		
0.9	49 (54.44)	90		
1.5	49 (54.44)	90		

The parasitic contamination rate among the different vegetable types; tomatoes, cabbage, lettuce, spring onion and carrot were reasonably high. The test of association between the vegetable types and parasitic contamination was very significant different (p value < 0.001).

Different saline concentrations (0.0, 0.45, 0.9, 1.5 g/dl) were used to wash the vegetables and deposits were used for parasite detection. Testing association between different saline concentration that were used for the washing of vegetables and parasitic contamination has no significant difference (p value =0.623).

In addition to parasitological investigation, factors that associated with contamination of vegetables were assessed. These factors were assessed by interviewing 13 vegetable sellers, where each vegetable result was linked with a particular seller and used for the analysis, and by observation in the two market places in Koforidua. The mean age was 40 (±) 7.The educational statuses of the sellers were also established. Majority (69.35%) of vegetables from those who had secondary or A-level form of education had their vegetable contaminated by at least one parasite.

Vegetables were collected from two market sites, namely Agartha and main market. The study showed that parasitic contamination was higher for vegetables from Agartha market (67.78%) than that from the main market (47.22%). There was however, a significant difference (p<0.001) for test of association between parasite detected and market type (Table 4.3).

Another factor considered was hand hygiene performance by vendors when in the market. Hand hygiene was normally done by majority of the total vendors interviewed. Hand hygiene is performed with soap and water to wash the hands or use of sanitizer. Parasites were identified in 56.05% of vegetables of the vendors who performed hand hygiene, the test of association was not found significant (p= 0.407) (Table 4.3).

The sources of vegetables considered were farm gate, retailers and wholesale. Retailers had their produce from whole sellers and whole sale dealer had their produce from distant town like Kumasi and therefore pass through different handlers and vehicles before getting to the consumers.

The least contaminated source was from the farm gate (39.77%).The source of vegetable was found to be associated with parasitic contamination of salad vegetables (p value < 0.001) (Table 4.3).

Table 4.4: Parasitic recovering rate after first and second wash of vegetables with different saline concentration

Saline Concentration(g/dl)	First Wash (%)	Second Wash (%)	Total
0	50 (55.56)	40 (44.44)	90
0.45	57 (63.33)	43 (47.78)	90
0.9	51 (56.67)	37 (41.11)	90
1.5	49 (54.44)	32 (35.56)	90
Total	**207 (57.50)**	**152 (42.22)**	**360**

Each particular vegetable in a given level of concentration was washed twice. The total recovering rate was however lower in the second wash than the first washing (Table 4.4). This indicates that serial washing of vegetables may reduce the parasitic load in the vegetables.

Table 4.5: Logistic regression of some factors associated with parasitic contamination (Jan 2nd to March 7th, 2017)

Variables (n=360)	Positive (%)	Total	COR	(95% CI)	AOR	(95%CI)
MARKET						
*Agartha	122(67.78)	180				
Main market	85(47.22)	180	-3.91	(-1.28;-.43)	-3.44	(-1.80;-0.49)
VEGETABLE TYPE						
*Cabbage	31(43.06)	72				
Lettuce	51(70.83)	72	3.32	(0.48;1.86)	**3.54**	(0.78;2.72)
Carrot	19(26.39)	72	-2.08	(-1.45;-.04)	-2.35	(-2.46;-0.22)
Spring onion	70(97.22)	72	5.08	(2.35;5.32)	**4.08**	(1.87; 5.32)
Tomatoes	36(50.00)	72	0.4	(-0.38;0.94)	-2.2	(-2.50;-0.14)
SOURCE OF PRODUCE						
*Farm gate	35(39.77)	88				
Wholesale	87(60.42)	144	3.03	(0.30;1.38)	0.48	(-1.04;1.71)
Retailers	85(66.41)	128	3.82	(0.53;1.66)	0.21	(-1.21;1.50)
SALINE CONCENTRATION(g/dl)						
*0	50(55.56)	90				
0.45	57(63.33)	90	1.06	(-0.27;0.92)	1.3	(-0.25;1.22)
0.9	49(54.44)	90	0.15	(-0.54;0.63)	0.19	(-0.66;0.80)
1.5	49(54.44)	90	-0.15	(-0.63;0.54)	-0.19	(-0.80;0.66)

**reference variable C I-confidence interval COR- crude odds ratio AOR- adjusted odds ratio*

Logistic analysis confirms that with reference to cabbage, spring onion is 4 times (AOR=4.08, 95 CI (1.87; 5.32) and lettuce is 3.5 times (AOR=3.54, 95 CI (0.78; 2.72)) associated with parasitic contamination (Table 4.5).

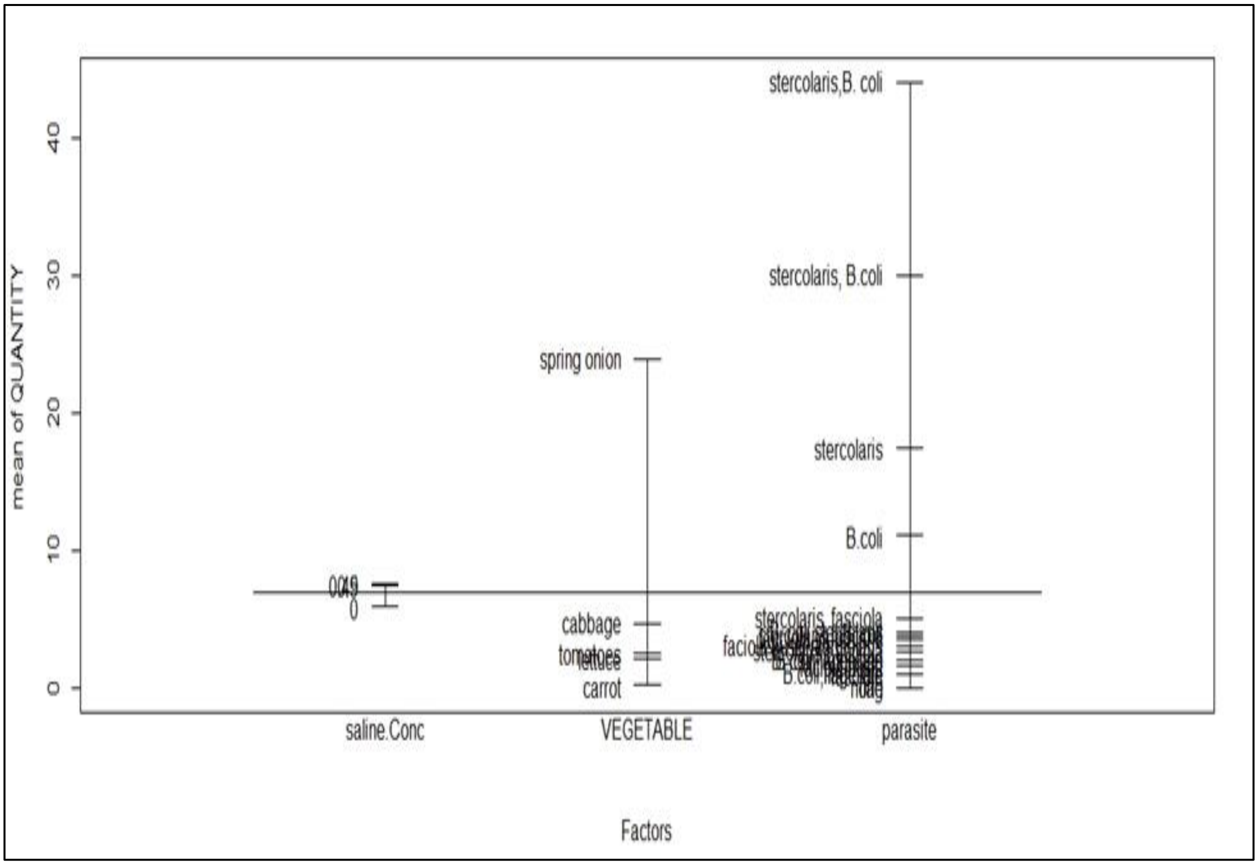

Figure 4.0: Mean number of parasites recovered plotted against the factor levels (Vegetable type and saline concentration)

From figure 4.0, it confirms saline concentration seems not to have significant impact on the number of parasites removed from the vegetables. The number of parasites removed rather appeared to depend on the parasite type and vegetable type which in this study was spring onion.

Chapter Five

Discussion

The study aimed to estimate parasitic contamination of commonly consumed salad vegetables sold in two major market sites in Koforidua, New Juaben Municipal. The overall prevalence was 57.50%.The highest contaminated vegetable being 97.22% of spring onion, followed by 70.83% of lettuce, 50.0% of tomatoes, and 43.06% of cabbage and the least being 26.39% of carrot.

This finding was consistent with previous report from Jimma Town, Southwest Ethiopia (57.8%) (Tefera *et al*., 2014). In similar studies done in Ghana, Accra, contamination rates was 36% which is relatively lower (Duedu *et al*., 2014). Other lower prevalence rates were detected in Alexandria, Egypt (Said, 2012) , in Ardabil, Iran (Daryani *et al*., 2008), and Nigeria (Damen *et al*., 2007b) where the contamination rates were (31.7%) , (29%), and (36%) respectively. Much lower rates of contamination were detected in Riyadh, Saudi Arabia (16.2%) (Al-Megrm, 2010), and Turkey (6.3%) (Adanir and Tasci, 2013). Higher prevalence rate were detected in Kenya (65.5%) (Nyarango *et al*., 2008), Tripoli, Libya (68%) (Abougrain *et al*., 2010) and in Khorramabad, Iran (79%) (Ezatpour *et al*., 2013) .This variation in contamination rates of the vegetables may, in part, be due to the differences in shape and surface of vegetables. Green leafy vegetables such as lettuce has uneven surfaces that probably facilitate sticking of parasitic eggs, cysts, and oocysts more readily, either at the farm or when washed with contaminated water. However, vegetables with smooth surface such as carrot had the lowest prevalence rates. The differences in the prevalence rate may also have been due to different sample size, sampling method, laboratory investigation methods used and cross contamination during transportation, or handling of vegetables in the market.

The most contaminated vegetable type identified was spring onion and the least being carrot. However, in similar study conducted by Duedu in Accra, he found lettuce to the most contaminated (61%) and tomatoes to be the least contaminated -18% (Duedu *et al*., 2014). Other works done in Benha, Egypt, and Saudi Arabia, identified lettuce as the most contaminated vegetable where prevalence were 45.5% and 27.8% respectively. This difference may be due to pre harvest factors such as contaminated water mostly used for irrigation and soil contaminate with human feces (Eraky *et al*., 2014, Al-Megrin, 2010).

The most frequent occurring parasite was *Strongyloides stercolaris* larvae (36.39%) and the least occurring was *Fasciola sp.* (7.22%), flagellate was (7.78%) , *Balantiduim coli* was 13.61%, and *cryptosporiduim sp* was 11.11%.

Strongyloides stercolaris was also detected as the most occurring parasite (43%) in Duedu's work (Duedu *et al*., 2014) and 45.8% was detected in south western Nigeria while as a much lower prevalence rate of 1.1% was detected by Aziz and others and 16.5% in Jos, Nigeria (Ogbolu *et al*., 2009, Damen *et al*., 2007a). *Strongyloides stercoralis* has been noted to be widely distributed in areas of poor sanitation. Infection may have occurred due to soil and water being contaminated by human defecation. The differences in the rates may have been due to different geographical locations as well.

Balantiduim coli was the second highest parasite detected in this study. A much lower prevalence of 8.19% and 0.8% were however detected in Ilorin, Nigeria and south western Nigeria respectively (Alade *et al*., 2013, Ogbolu *et al*., 2009). High contamination is possibly due to pig and human fecal matter contaminating the water supply used for irrigation.

Cryptosporidium sp. (11.11%) compared to similar study in Alexandria (29.3%), was lower (Said, 2012). But much lower rates were detected in south Ethiopia and Norway with the prevalence of 4.72% and 6 % respectively (Bekele *et al*., 2017, Robertson and Gjerde, 2001). Cryptosporidiasis is most likely due to contaminated water.

Fasciola sp. was identified as 7.2% in this study. Lower recovery rates of 5% was detected in similar studies in Iran (Daryani *et al*., 2008) and higher rate of 14.5% in Riyadh (Al-Megrm, 2010). *Fasciolas sp.* is a known zoonotic parasite. Other parasites like flagellates which were fast moving were recovered from almost all the vegetable types in this study.

Washing vegetables the second time recovered 42.22% parasites. This may be an indication that two wash alone is not enough to remove all the parasites from the vegetables. Duedu *et al.* (2014) has shown that, washing vegetables with just water was not enough to remove any contaminating parasites. He, however, recorded that using saline, phosphate buffered saline and tap water recovered 52%, 34% and 14% parasites respectively and use of saline was more effective in recovering of parasites.

Parasitic contamination was higher in Agartha market (67.78%) than the main market (47.22%). This might have been associated with mode of display of vegetable, exposure to dust and flies. Flies have been identified as a vector for transmission of *Cryptosporiduim sp.*(Meerburg *et al*., 2007).

Several factors may have contributed to such differences the parasitic prevalence such as geographical area, category of samples examined, methods used for detection of the parasites, type of water used for irrigation, and processing of such vegetables which are different from country to another.

Chapter Six

Conclusion and Recommendation

6.1 Conclusion

The result of this study indicated high parasitic contamination of commonly consumed salad vegetables compared to similar study done in Accra of which spring onion was the most heavily contaminated. *Strongyloides stercolaris* larvae was identified as the most common intestinal parasite infesting almost all the vegetable types considered in this study except for tomatoes. This may pose health risk leading to diarrheal diseases and abdominal pain among consumers. The findings also emphasized the public health implication of consumers of these vegetables being at high risk of strongyloidiasis, balantidiosis and Cryptosporidiasis.

To add to that , the use of different concentration of saline demonstrated that concentration of saline has no effect on removal of parasites however, serial washing of vegetables showed decreasing parasitic recovering rate from the selected vegetables in this particular study. This may serve as an educational tool for educating consumers on serial washing of raw vegetables before consumption.

Source of produce was also found to be associated with parasitic contamination and some of the parasites detected were zoonotic per this study. So therefore, protection of vegetables from the source of contamination is of great importance.

6.2 Recommendation

Vegetables in Koforidua markets are heavily contaminated with parasites and there is need for public enlightenment campaign on the danger of consuming inadequately washed vegetables. These include the consumers and food venders should be educated on serial washing of vegetables to reduce parasitic load in the vegetables.

Farmers should be educated use of properly treated manure, use of safe water for irrigation purposes and fencing of farmland to prevent passageway of animals by the environmental officers.

Further research should be done to identify the main source of contamination from the farm lands.

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APPENDIX A: RESEARCH TEAM MEMBERS

RESEARCH TEAM

Dr. SOVOE SIMEON	SUPERVISOR
CATHERINE KUDAH	PRINCIPAL INVESTIGATOR
ROGER LARYEA	LABORATORY TECHNICIAN
AUGUSTINE KYERE	LABORATORY TECHNICIAN

APPENDIX B: QUESTIONNAIRE

Raw produce (market) sample collection form

OBSERVATION

Date; Identification code:

Personal Information

Sex

Male ( ) Female ( )

Age (yrs.) ... ……..

Religion

Christian ( ) Muslim ( ) Traditional worshipper ( ) Other ( )

Educational background

No formal education ( ) Primary education ( ) JHS/MS ( ) SHS/A-level ( )

Polytechnic/University ( )

1. Type of market vendor

a) At main market (under shed) b) Open/street market (outside main market)

2. Mark if the sample was taken

Within 3m of faeces

Within 30m of latrine

With flies on food

Within 3m of sewage outfall

Or open drain

3. Produced exposed to Sunlight

Yes ( ) No ( )

4. How produce is displayed:

a) On the ground b) >1m above ground c) <1m above ground d) Other

QUESTIONNAIRE FOR MARKET VENDORS

A. Produce Hygiene conditions

1. Where do you normally store your produce before selling them?

At the market ( ) At home ( ) other ( ) specify

2. How do you normally store the following vegetables before selling them? (Indicate in the table below):

Vegetables	How storage is done
a) Lettuce	
b) Carrots	
c) Tomatoes	
d) Cabbage	
e) Green pepper	

Table top ( ) Basket/bowl ( ) material on ground ( ) bare ground ( )

4. Do you wash the vegetables before sales?

Yes ( ) No ( )

5. If yes, what is the source of water for washing the vegetables?

Piped water ( ) well water ( ) Tanker services ( ) Other (specify)...................

6. How much time do you spend on the following activities daily?

Activity	Time Spent
a) Washing of lettuce	
b) Washing of carrot	
c) Cutting or removal of waste parts of cabbages	
d) Removal of waste parts of spring onions	

7. Are customers normally happy with the quality of vegetables sold at the market?

Yes ( ) No ( )

8. What do customers normally complain of when at the market?

Write complaints...

....

B. Environmental Hygiene conditions

9. Where do you normally defaecate when you are at the market?

Public toilet ( ) Market toilet ( ) In a polythene bag ( ) Open Defecation ( )

10. What is your source of drinking water when working at the market?

Sachet water ( ) Bottled mineral water ( ) Piped water ( ) Water from home ()

11. Are you normally satisfied with refuse collection and management at the market?

Yes ( ) No ( )

12. If No, what are you not satisfied with?

Write down reason (s)..

13. Are you generally satisfied with drainage management at the market?

Yes ( ) No ( )

14. If No, what are you not satisfied with?

Write down reason (s)..

C. Hand washing and food Hygiene practices

15. How many times do you eat when at the market?

Once ( ) 2 times ( ) 3 times ( ) more than 3 times ( )

16. What is the source of the food you normally eat when at the Market?

Food from home ( ) street food ( ) local restaurant food ( ) Other ( ) specify..

17. Do you normally wash your hands when eating at the market?

Yes ( ) No ( )

18. If Yes, what do you wash your hands with?

Only water ( ) Soap and water ( )

D. Health Risk Awareness and Perceptions

19. Where do you buy the vegetables you sell from?

Farm gate ( ) Wholesale market ( ) Retail Market ( ) Other ( )

20. If farm gate, do you have any reason (s) why you buy from these farm gates?

Yes ( ) No ( )

21. If yes, what are your reasons?

List reasons...

22. Do you know of the source of water farmers use to irrigate their crops?

Yes ( )   No ( )

23. If you know the source is drain water, would you still buy the vegetables?

Yes ( ) No ( )

List reasons for Yes or No answer..

24. Are you aware of any health risks associated with the consumption of vegetables that are irrigated with drain water? Yes ( ) No ( )

25. If Yes, what health risks do you know of?

List health risks...

APPENDIX C: SALINE PREPARATION

0.45g/dl saline

Weigh 4.5 grams of sodium chloride and pour into 5 litre container.

Measure 1000 mls of distilled water with measuring cylinder

Pour one third into the container with the salt and mix well to dissolve

Add the rest of the distilled water

Label the container with concentration name and date of preparation.

0.9g/dl saline

Weigh 9.0 grams of sodium chloride and pour into 5 litre container.

Measure 1000 mls of distilled water with measuring cylinder

Pour one third into the container with the salt and mix well to dissolve

Add the rest of the distilled water

Label the container with concentration name and date of preparation.

1.5 g/dl saline

Weigh 15 grams of sodium chloride and pour into 5 litre container.

Measure 1000 mls of distilled water with measuring cylinder

Pour one third into the container with the salt and mix well to dissolve

Add the rest of the distilled water

Label the container with concentration name and date of preparation.

APPENDIX D: AUTOCLAVING OF SALINE

Saline was poured into autoclave bottles and labeled with the name of the saline concentrations.

The bottles were corked and put into the autoclave.

The wing nut were locked and the switched on.

The autoclaving was done at 121 ^{o}c , for 20 minutes.

Sufficient time was allowed to cool to at least 80 ^{o} c before opening the autoclave.

APPENDIX E: REAGENT PREPARATION

MODIFIED ZIEHL-NEELSEN STAIN

CARBOL FUHSIN STAIN

Basic fuchsin………………………..10g

Methanol……………………………..100ml

Phenol…………………………………50g

Distilled water…………………………1 litre

Weigh the basic fuchsin on a piece of clean paper and transfer the powder to a bottle of at least 2 litres.

Measure the methanol and add to the bottle. Mix at intervals until the basic fuchsin is fully dissolved.

With great care, weigh the phenol in a beaker. Measure the water, and add some of it to the beaker to dissolve the phenol. Transfer to the bottle of stain and mix well.

Add the remainder of the water and mix well.

Label and store at room temperature.

ACID ALCOHOL (Stock -3%)

Methanol…………………………..680 mls

Distilled water……………………….290 mls

Concentrated Hydrochloric acid……30mls

Measure the methanol and transfer to 1 litre leak proof bottle.

Measure the water; add to the alcohol and mix

Measure 30 mls of the concentrated hydrochloric acid, add to the solution and mix well.

Label the container with name and date and mark flammable.

WORKING SOLUTION (1 % Acid alcohol)

Add 30 mls of the 3 % acid alcohol to 60 mls of distilled water.

METHYLENE BLUE

Distilled water……………………………………100mls

Methylene blue……………………………………..1.0 g

Weigh methylene blue and transfer into a leak proof container

Measure water and add to the dye. Mix well till it dissolves

Label with name and date of preparation.

FLORESCENCE STAINS

0.1% AURAMINE SOLUTION

Solution A

Heat 30 g of phenol at 45^{o} c until crystals are completely molten

Add the molten phenol to 870 mls distilled water and mix.

Solution B

Dissolve 1g Auramine O in 100ml of ethanol. Mix thoroughly.

Add solution B to solution A

Mix gently and filter into brown place.

0.5% ACID ALCOHOL DECOLOURIZING SOLUTION

Ethanol………………………………….700ml

Distilled water…………………………..300ml

Concentrated hydrochloric acid…………5ml

Add 5ml concentrated hydrochloric acid into 1000ml of 70 % alcohol (700ml ethanol and 300ml distilled water)

Label with date and name.

0.5%POTASSUIM PERMANGANATE SOLUTION

Distilled water…………………………….1000ml

Potassium permanganate……………………5g

Dissolve 5g of Potassium permanganate in 1000 ml distilled water and put in a dark bottle.

Label with name and date.

APPENDIX F: INFORMED CONSENT

Project Title: PARASITIC CONTAMINATION OF COMMONLY CONSUMED SALAD VEGETABLES IN KOFORIDUA, NEW JUABEN MINICIPALITY.

Principal investigator: KUDAH CATHERINE Contact Of Principal Investigator: 0246838887

Email;essikudah@gamil.com. Name of Institution; Ensign College Of Public Health

General Information about the Research.

This research is being conducted in the two major markets within the municipality. The purpose of the study is to determine the parasitic contamination of tomatoes, lettuce, cabbage, spring onion and carrot. I am inviting you to participate in the study to determine parasitic contamination of the vegetables mentioned above. You were conveniently selected to participate in the study.

The inconvenience that the interview will cause you, the time you will send answering the question and some of the question may seem personal and sensitive, the information will be accessible only to me and would not be shared with anyone; it will be used only for the purpose of this study, your name will not be mentioned in the study of which nobody will be able to tract you answers back to you.

Even though there is no direct benefit, the information that will be obtained from this study will help in addressing issues relating to proper handling of fresh vegetables before consumption. Your participation in the study is entirely voluntary. If you agree to participate in the study, it may take 10-15 minutes to complete this questionnaire .You can also withdraw your consent in the study .If you decide not to participate in the study, there will be no penalty, loss of benefits or negative consequence whatsoever.

Anonymity and confidentiality

Any and all information you share in completing this questionnaire will be treated confidentially and no personal identifying information concerning you will be presented. Result of the study will be made available to the general public through publication, and general awareness program in collaboration with the municipal assembly. If you would like to find out more about the study or have any questions, please contact the principal investigator.

Participant agreement

The above document describing the benefits, risks and procedures for the research titled "PARASITIC CONTAMINATION OF COMMONLY CONSUMED SALAD VEGETABLES IN KOFORIDUA, NEW JUABEN MUNICIPAL "has been read and explained to me. I have been given an opportunity to have any question about the research answered to my satisfaction. I voluntarily agree to participate in the study.

………………………………………… ……………………………………

Date of consent signature of participant