INCIDENCE OF PARASITES ON SOME RAW EATEN VEGETABLES SOLD IN MINNA MARKETS, NIGER STATE, NIGERIA

BY

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ABSTRACT

Vegetables are essential part of human healthy diet and are rich in vitamins. However, consumption of raw vegetables has been associated with transmission of parasites. This study investigated the incidence of parasites on five commonly consumed raw vegetables in Minna, Nigeria. A total of 2500 samples of the five different types of raw vegetables (lettuce, tomatoes, cucumber, carrots and cabbage) were purchased from five markets in Minna (Kure market, Bosso market, Maikunkele market, Maitunbi market and Gwari market) and analyzed for the presence of helminthes and protozoan parasites using concentration method. Out of the 2500 samples examined, 397 (15.88%) were found to be contaminated with various stages of parasites such as ova, cysts, eggs or a complete developed parasite. Highest rate of parasitic contamination was detected in carrots 116 (29.22%), followed by tomato 100 (25.19%), lettuce 79 (19.90%), cabbage 66 (16.63%) with cucumber 36 (9.07%) having the least contamination. Among the sampled markets Gwari market 88 (17.60%) recorded the highest prevalence while Bosso market 54 (10.80%) had the lowest. The seven parasitic stages detected were *Schistosoma masoni* 116 (29.22%) as the most prevalent followed by the eggs of *Teania* 80 (20.15%), cyst of *Schistosoma masoni* 82 (20.06%), cyst of *Schistosoma japonicum* 35 (8.82%), cyst of *Entamoeba histolytica* 32 (8.06%), Cyst of hookworm 26 (6.55%) and *Ascaris lumbricoides* 26 (6.55%) in that order Findings from this study show that a considerably number of commonly consumed raw vegetables in Minna are contaminated with a wide variety of parasites, indicating a major health risk to consumers. As such public enlightenment campaigns on the danger of consuming inadequately washed and raw vegetables should be carried out by local health authorities.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Vegetables are annual or perennial horticultural crops, with certain sections (roots, stalks, flowers, fruits or leaves.) that can be consumed wholly or partially, cooked or raw (Welbaum, 2015). They are essential parts of human healthy diet and are great sources of vitamins, fibre and minerals (Ulger *et al*., 2018). Their raw consumption has been associated with transmission of the parasites. It is also useful in reducing the risk of cardiovascular diseases, strokes or cancer. (Abougrain *et al*., 2010). Some vegetables that are eaten raw like *Dacus carota* (carrot), *Cucumis sativus* (cucumber), *Brassica oleracea* (cabbage), *Salanum lypoersicum* (tomatoes) and *Lactuca sativa* (lettuse) contain nutrients and non-nutritive phytochemicals (Ulger *et al.,* 2018). The raw consumption of these nutrient source (vegetables is encouraged to be eaten raw to avoid heat labile nutrients been destroyed by cooking resulting to increase transmission of the parasites. However, the consumption of uncooked vegetables is associated with infectious parasitic diseases (Adamu *et al*., 2012). There had been constantly reported cases of food borne diseases that were linked to eating of raw vegetables. Vegetables may be contaminated before harvesting in the field, Orchards, transportation, processing or distribution and marketing (Adanir and Tasci, 2013). The use of waste water to irrigate farm is the major driver of microbial contamination (Adanir and Tasci, 2013).

Human consumption patterns like eating raw vegetables from street vendors that have little or no regards or are entirely ignorant of food safety can increase the risk of infectious parasitic diseases and other ailments (Ajeagah, 2013).

In the past the risk of human parasitic diseases was limited to defined geographical locations because of selective definitive host or intermediate host and also particular environmental conditions. The conditions have been breached by travelers and international refrigerated foods which became available everywhere. The travelers will harbour the parasites or cysts or ova and travel back to their locations and spread it through feacal pollution through water or soil (Amahmid *et al*., 2002). Therefore, the presence of infectious parasite in vegetable is a global concern.

1.2 Statement of the Research Problem

Vegetables consumed raw are means of maintaining healthy life but often times also act as source and route for the spread of various parasitic infections. The transmission of parasitic infection may cause immune suppression as they lower the resistance of such individuals to other infections and render active immunization procedure less effective.

The incidence of parasitic infection remains a leading public health problem in the world and is neglected especially in tropical and sub-tropical regions because of their convalescence nature. This pose serious health threat and limitation to productivity of human resulting in economic losses.

1.3 Justification for the Study

Understanding the role played by the consumption of raw vegetables in the transmission chain of parasitic diseases as well as the possible sources of contamination is an important step in the effort toward the control of spread of the parasites. Hence this study would reveal the types of parasites and possible simple control measures that would reduce contamination.

1.4 Aim and Objectives of the Study

The aim of the study was to investigate incidence of parasites in some raw eaten vegetables sold in Minna, Niger State, Nigeria and the objectives were to:

i. detect the presence of some parasites in some raw eaten vegetables (carrots, tomatoes, lettuce, cabbage, cucumber) sold in Minna Markets, Niger State, north central zone, Nigeria.

ii. determine the prevalence of ova or cyst in some raw eaten vegetables sold in Minna Markets, Niger State, north central zone, Nigeria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description and Importance of Vegetables

Vegetables are commonly referred to as fresh edible portions of herbaceous plant roots, stems, leaves or fruits. In many countries, vegetables are eaten raw or slightly cooked to preserve nutrients and taste and this practice may favor the transmission of food-borne parasitic infections (Ozlem and Sener, 2005). Dias (2012) reported that Vegetables make up a major portion of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phytonutriceuticals: vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and phytochemicals. Some phytochemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic diseases by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumorigenesis (Wargovich, 2000; Dias, 2011).

Vegetables in the daily diet have been strongly associated with overall good health, improvement of gastrointestinal health and vision, reduced risk for some forms of cancer, heart disease, stroke, diabetes, anaemia, gastric ulcer, rheumatoid arthritis, and other chronic diseases (Prior and Cao, 2000).

Dias (2012) reported that the world vegetable survey showed that 402 vegetable crops are cultivated worldwide, representing 69 families and 230 genera. Leafy vegetables of which the leaves or young leafy shoots are consumed were the most often utilized (53 % of the total), followed by vegetable fruits (15 %), and vegetables with below ground edible organs

constituting 17 %. Many vegetable crops have more than one useful part. Most of the vegetables are marketed fresh with only a small proportion processed because most vegetables are perishable. Consumption shortly after harvest guarantees optimal vegetable quality (Dias, 2012).

The promotion of healthy vegetable products has coincided with a surging number of consumers interested in the healthy functionality of food. There is an increasing awareness among the general public of the advantages of diets rich in vegetables to ensure an adequate intake of most vitamins and micronutrients, dietary fibers, and phytochemicals that promote health. Consumers' interest in whole foods with enhanced nutritional qualities is at an all-time high, and more consumers are choosing foods on the basis of their health benefits (Dias, 2012).

There are general beliefs among nutritionists and health professionals that the health benefit of vegetables should not be linked to only one binocular or one type of vegetable, but rather a balanced diet that includes more than one type of vegetable and which is likely to provide better protection. All the vegetables may offer protection to humans against chronic diseases. With the exception of glucosinolates and thiosulfides, which are unique to the crucifers and alliums, the phytonutriceuticals content of a number of other vegetables consist primarily of vitamin C, fiber, selenium, folate and polyphenolics (carotenoids and flavonoids) (Dias, 2012). The main difference is that each vegetable group contains a unique combination and amount of these phytonutriceuticals, which distinguishes them from other groups and vegetables within their own group. For example the *Apiaceae* family (e.g. celery, parsley, carrot) is rich in flavonoids, carotenoids, vitamin C, and vitamin E. Celery and parsley for example are among the best vegetables sources for the flavonoid apigenin and

vitamin E (Nielson *et al.,* 1999), and carrots have an unique combination of three flavonoids: kaempferol, quercetin, and luteolin (Lila, 2004).

2.2 Cultivation of Vegetable and Sources of Contamination

The cultivation of vegetables in many parts of the word has been amplified with the application of fertilizer and or manure. In Africa, the transmission of intestinal parasitic infection has been considered to increase successfully due to the frequent use of untreated human or animal dung as manure in cultivation by the local farmers, which serves as a source of enhancement of zoonotic parasitic infection. (Luca *et al*., 2000).

Consumption of raw or unhygienically prepared vegetables such as cabbage *(Brassica oleracea*), lettuce (*Lactuca sativa*), okra (*Abelmoschus esculentus*), garden egg (*Solanium macrocarpon*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*), water leaf (*Talinum triangulare*), pumpkin (*Cucurbita pepo*), spinach (*Spinacia oleracea*), tomatoes (*Salanum lycoersicum*), etc., is considered to be a risk factor for human parasitic infections (Chessbrough, 1991).

The cultivation of vegetables for commercial and domestic purposes in Nigeria is mostly carried out by peasant farmers depend on irrigation or natural rainfall (Luca, *et al.,* 2000). These vegetables though seasonal, are cultivated in the same piece of land every year. As a result of this continuous land usage there is depletion of nutrient hence the need for fertilizer or manure. Most farmers use untreated animals and human faeces as manure, which are known to conntain various species of parasites that are of medical and veterinary importance Dias, 2012).

Indiscriminate faecal disposition in bushes, farm lands and even in present farms with a belief of enriching the lands is also a common practice by farmers and unlearned citizens.

Some of the water bodies used for irrigation are also polluted with parasites infeczted excreta, that could lead to recycling of infection (Ayres *et al*., 2015)

Altekruse (2014) reported that the potential risks factors for human intestinal parasitic infection, for example*; Ascaris lumbericoides, Trichuris trichuria, Ancylostoma duodenale, Necator americanus, Balantidium coli, Giardia intestinalis, Blastocystic hominis* involve unhygienic associations with unhygienic environment.

2.3 Empirical Evidences about Parasitic Contamination of Raw Vegetables

The consumption of and vegetables contaminated with the eggs of parasites may be the cause of parasitic infections in humans (Padmanandan *et al.*, 2016). A study was conducted in 2009 by Klapec and Borecka to evaluate the contamination of vegetables, fruits and soil with zoonotic parasitic eggs on organic and conventional farms in south eastern Poland. Selected fruits and vegetables were included in the study and the study finally revealed that highest number of contamination was found in samples from conventional farms in comparison to the organic farms. The eggs were of *toxocara, ascaris* and *trichuris*. The study also concluded that stricter sanitary standards on farms of all types may limit the incidence of parasitic zoonoses (Klapec and Borecka, 2012).

Soil-transmitted helminth infection is endemic in many parts of the world principally in developing countries where there is poor environmental sanitation and hygiene. A study was conducted by Uga *et al.* (2009) to find out the parasitic egg contamination of vegetables from a suburban markets in Vietnam. Ten (10) out of 317 vegetables, 82 samples were found to be contaminated and contamination was highest in leafy vegetables followed by root vegetables and fruit vegetables. Throughout the survey, eggs of *Ascaris, Trichuris, Toxocara, Taenia, Ascaridia galli* were found.

According to Padmanandan *et al.* (2016), the study interviewed 149 villagers out of which 121 stated that they not only use animal feces as fertilizer but also human feces as well. Contamination was highest during the dry season than rainy season. The study concluded that considering the eating habits of Vietnamese and 17 % embryonation rate of the parasites, vegetables play an important role in transmitting soil-related helminthic infection. The results suggested the necessity for nation-wide control measures against parasitic infection.

Raw vegetable consumption is a threat to public health by transmission of intestinal parasites to humans. A study was conducted by Said *e al*. (2012), during the year 2010-11 in Egypt to find out parasitic contamination in commonly consumed raw vegetables and it was found that intestinal parasites were detected in 31.7 % of the examined samples and cryptosporidium was found to be present in most of the samples. The findings of this study may have important implications for global food safety and emphasize the importance of raw vegetables in threatening public health by transmission of intestinal parasites to humans (Said, 2012).

In order to find out the prevalence of parasitic contamination of raw vegetables, Ezatpour *et al*. (2013) conducted a study in Iran to find out the prevalence of parasitic contamination in raw vegetables. This study showed a considerably high level of contamination in green vegetables (56.2 % in spring and 26 %in winter) (Ezatpour *et al.*, 2013).

Olyaei and Hajivandi (2013) reported that Vegetables are an important cause in transmission of parasites from study conducted in the year 2011-12 to detect the parasitological contamination of markets and farms in vegetables consumed in southern Iran. It was found that 38.5 % of market vegetables and 48.9 % of farm vegetables were contaminated. This

study also showed that prevalence of parasitic infection was higher in spring as compared to winter. This study also concluded that the importance of vegetables in the transmission of parasites is stressed and it is necessary to improve sanitary conditions. The inhabitants must be informed and educated with regards to food safety, good distribution practices and improving the safety concerns in farms and markets. People should also avoid using untreated water for irrigation of vegetables as this constitutes an important route of intestinal parasites transmission (Olyaei and Hajivandi, 2013)

From the above studies, it is seen that consumption of vegetables and fruits contaminated with the eggs of parasites are important cause of parasitosis in humans. The rate of parasitic contamination in vegetables is more during warm seasons than cold seasons. Soil transmitted helminth infection is endemic in many parts of the world principally in developing countries where there is poor environmental sanitation and hygiene.

2.4 Zoonotic Parasites and Health Implication

Parasitic zoonoses are now well recognized as an important public health problem worldwide. In fact, one fourth of the known human infectious diseases, particularly that cause gastrointestinal problems in man are caused by the helminthic group (Cleaveland *et al.,* 2001).

Zoonotic infections, especially those related with helminth parasites are transmitted via faecal-oral route through contaminated water, soil and food and raw and minimally processed vegetables are known to serve as vehicles of human diseases (Idahosa, 2011). The direct application of animal dung as manure to soil and the use of untreated or waste water for irrigation of vegetable crop fields can pose a significant occupational and public health risk (De Sorvillo *et al.,* 2007). Vegetables may get exposed to parasitic contamination

during pre-harvest (cultivation, irrigation, livestock manure etc.), post-harvest handling, storage, transportation or while processing for consumption (Erkan and Vural, 2008).

Vegetables are reported to harbour intestinal parasites such as *Ascaris lumbricoides, Taenia* spp., *Fasciola hepatica, Hymenolepis nana, Echinococcus* spp., *Trichuris* spp., *Enterobius vermicularis, Trichostrongylus* spp., *Toxocara* spp. and *Strongyloides stercoralis* (Abougrain *et al.,* 2010).

An important characteristic of these organisms is the production of highly resistant cysts and ova that can survive for very long time in the wastewater. Though, mortality from these helminths is relatively low, but the indirect effects have a substantial impact on health and quality of life causing childhood diarrhoea and stunted growth (Slifko *et al.,* 2000).

The local health and environmental authorities should educate the people on the health hazards of eating fresh salad vegetables. The people must know the importance of washing and disinfecting them before consumption.

2.5 Knowledge, Attitude and Practices of Food Safety among Food Handlers

Food shops and food stalls need continuous monitoring and food handlers should receive suitable training in the basic principles of food safety as well as rules of personal hygiene and approved practices in food handling (Padmanandan *et al.,* 2016). A survey was carried out by Cuprasitrut *et al.* (2011) during the year 2010 to determine the food safety situation among food shops and food stalls which sell foods to be given to monks for the purpose of making merit and to assess the relationship between knowledge, attitude and practice of food safety in Bangkok. It was found that both microbiological and chemical contamination was present in 13 food shops and 55 food stalls examined. It also showed that only 13 % of

food handlers had good knowledge, 18.5 % had good attitude and 15.2 % had good practices (Cuprasitrut *et al.,* 2011).

There is a lack of food safety knowledge and practices among home food preparers. A cross-sectional study was carried out by Farahat *et al*. (2015) to find out the food safety knowledge and practices among 811 Saudi women. It was found that the women reported better food safety practices than food safety knowledge in all parameters except cooking where they recorded lowest mean practice score. Working women showed higher mean knowledge and practice scores when compared to non-working women. The study was done on the following parameters: (i) Food safety knowledge and practices concerning purchasing and storage, (ii) Food safety knowledge and practices concerning preparation, (iii) Food safety knowledge and practices concerning cooking, (iv), Food safety knowledge and practices concerning utensils and equipment and (v) Food safety knowledge and practices concerning cooking.

The present study revealed gaps between food safety knowledge and practices that were significant in most parameters among Saudi women with secondary and bachelor education, from East and Middle provinces, in the age groups 20-, 30- and 40-years as well as among both working and non-working women (Padmanandan *et al.,* 2016).

Better food safety practices indicated that some women used to do the right practices although their knowledge was deficient. Even the personal hygiene where the interviewed women reported better knowledge and practice, its score needs further improvements. This necessitates launching a food safety training program from the responsible authorities emphasizing all studied food safety parameters. Motivation for proper food handling

practices requires that the consumer view the mishandling of food as a direct threat to their health (Padmanandan *et al.*, 2016).

The limitations of the study were that Saudi women were easily accessible by students and hence results cannot be generalized to all women and also that food safety practices were assessed through self-reporting that may over-estimate the actual practices as reported by Padmanandan *et al. (*2016).

2.6 General Knowledge of Food-Borne Parasites

The World Health Organization (WHO) categories parasites among the six most harmful infective diseases of man and parasitic infections outrank cancer as the number one killer in the world. Parasites can be contracted by eating contaminated under-cooked beef, pork, fish or other flesh foods, eating unclean raw fruits and vegetables or drinking infected water as reported by Wafaa and Hussein, 2017).

Parasites may be present in food or in water and can be identified as causes of food-borne or waterborne illness. Numerous parasites can be transmitted by food including many protozoa and helminthes. There are about 107 known species of parasites that can be food-borne (Wafaa and Hussein, 2017). In the United States, the most common food-borne parasites are protozoa such as *Cryptosporidium* spp., *Giardia intestinalis*, *Cyclospora cayetanensis*, and *Toxoplasma gondii*; roundworms such as *Trichinella* spp. and *Anisakis* spp.; and tapeworms such as *Diphyllobothrium* spp. and *Taenia* spp. A wide variety of helminthic roundworms, tapeworms, and flukes are transmitted in foods such as (undercooked fish, crabs, and mollusks; undercooked meat; raw aquatic plants such as watercress and raw vegetables that have been contaminated by human or animal feces) (Wafaa and Hussein, 2017). Some foods are contaminated by food service workers who practice poor hygiene or who work in

unsanitary facilities. Symptoms of food-borne parasitic infections vary greatly depending on the type of parasite. Protozoa such as *Cryptosporidium* spp., *Giardia intestinalis*, and *Cyclospora cayetanensis* most commonly cause diarrhea and other gastrointestinal symptoms. Helminthic infections can cause abdominal pain, diarrhea, muscle pain, cough, skin lesions, malnutrition, weight loss, neurological and many other symptoms depending on the particular organism and burden of infection. Treatment is available for most of the food-borne parasitic organisms (CDC, 2015).

2.7 Scientific Facts of Food-borne Parasites

Food-borne illnesses caused by parasites are prevalent in all parts of the world. They usually causes nausea and vomiting, diarrhea, abdominal cramps and fever may lead to more serious complications or even death. Over recent decades, parasitic protozoa have been recognized as having great potential to cause water-borne and food-borne disease. The organisms of greatest concern in food production worldwide are *Cryptosporidium*, *Cyclospora*, *Giardia*, and *Toxoplasma*. Although other parasitic protozoa can be spread by food or water, current epidemiological evidence suggests that these four parasites present the largest risks (Wafaa and Hussein, 2017). The major modes of transmission of protozoa include consumption of water, exposure to contaminated water, animal-to-person contact and person-to-person contact (Speer, 1997). However, the epidemiology of protozoa most commonly associated with human infections, namely *Giardia*, *Entamoeba*, *Toxoplasma*, *Sarcocystis*, *Isopora*, *Cryptosporidium*, *Eimeria* and *Cyclospora*, is not fully understood (Goodgame, 1996). While the life cycles of each of these parasites differ, however all require passage through an animal or human host. Shedding of cysts or spores into feces which may then, directly or indirectly (e.g. via sewage or irrigation water), contaminate raw fruits and vegetables occurs

on a global scale as reported by risks Wafaa and Hussein (2017). Outbreaks of protozoan infections in humans have been linked to raw fruits and vegetables. Epidemiological evidence has implicated an asymptomatic food handler as the probable source of *Giardia lamblia* and raw sliced vegetables as the vehicle of transmission in an outbreak of *Giardiasis* (Mintz *et al*., 1993).

A survey of vegetables has revealed the presence of *Cryptosporidium* oocysts on cilantro, lettuce, radish, tomato, cucumber and carrot (Monge and Chinchilla, 1996). The presence of these protozoa on raw fruits and vegetables is likely to be due to contact with animal or human feces, sewage, water containing untreated sewage and sludge from primary or secondary municipal water treatment facilities. While *Cryptosporidium*, *Giardia* and other parasites in water are quite resistant to chlorine and other disinfectants, little is known about the efficacy of these disinfectants in killing or removing parasites from the surface or tissues of fruits and vegetables. Surveys have shown that there is a high incidence of the parasitic roundworm, *Ascraris*, in the sewage sludge of many cities (Jackson *et al*., 1997).

Intestinal parasitic infections are widely distributed throughout the world causing substantial intimidation to the public health, economy, and physical and cognitive development particularly among children in developing countries (Wafaa and Hussein, 2017).

2.8 Diagnosis of Parasitic Infectious Diseases

Currently, the detection and diagnosis of parasite infections rely on several laboratory methods in addition to clinical symptoms, clinical history, travel history, and geographic location of patient. The primary tests currently used to diagnose many parasitic diseases have changed little since the development of the microscope in the 15th century by Antonie van Leeuwenhoek. Furthermore, most of the current tests cannot distinguish between past,

latent, acute, and reactivated infections and are not useful for following response to therapy or for prognosis (Ndao, 2009).

According to the report of Ndao (2009), there were basically three methods of detecting parasites.

2.8.1 Microscopy

For many years, microscopy has been the only tool available for the detection of parasites through inspection of blood smears (Duffy and Fried, 2005), tissue specimens (Cobo *et al*., 2007), feces, lymph node aspirates (Chappuis *et al*., 2006), bone marrow (Cruz *et al*., 2006), and even cerebrospinal fluid (Croft *et al*., 2006). However, sample preparation for direct observation is time-consuming, labour intensive, and proper diagnosis depends on qualified laboratory technicians. In the case of slide reading, a second independent reading is preferable, but not always required for accurate diagnosis. If need be, divided readings are resolved by a third reader. In endemic regions, where resources are limited, this proves to be difficult and misdiagnosis can significantly impact patient care. In reality, all major intestinal helminth infections are still solely dependent on microscopy or diagnosis. As for other parasite infections, many are confirmed by the use of microscopy in conjunction to other methods of diagnosis including serology-based assays and more recently molecular-based assays as reported by Ndao (2009).

2.8.2 Serology-based assays

In situations where biologic samples or tissue specimens are unavailable, serology alone is the gold standard for diagnosis. Serology-based diagnosis tools can be divided into two categories: antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and

all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Other assays include the hemagglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA) tests, complement fixation (CF) test, and immunoblotting and rapid diagnostic tests (RDTs) as reported by Ndao (2009).

2.8.3 Molecular-based approaches

The many limitations of microscopy and serology-based assays have influenced parasitologists towards the use of gene amplification methods made possible with the advent of the polymerase chain reaction (PCR). Besides the traditional PCR, including nested and multiplexed PCR, we have seen the implementation of the real-time PCR (RT-PCR) for the detection of several parasitic infections (Ndao, 2009). Newer technologies such as loop-mediated isothermal amplification and Luminex-based assays have also emerged as possible new approaches for the diagnosis of parasitic diseases. Molecular-based approaches based on nucleic acids offer greater sensitivity and specificity over the existing diagnostic tests. They permit the detection of infections from very low parasitized samples including those from asymptomatic patient's samples (Mens *et al*., 2007). Moreover, multiplexed PCR allows for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections simultaneously (Zarlenga and Higgins, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Minna, which lies between the Latitude of 9.5836^{O}N and Longitude of 6.5463^{O}E (Figure 3.1), and is the capital City of Niger state, North central zone of Nigeria. A Cross-sectional study was conducted to determine the level of parasitic contamination of vegetables in selected local markets in Minna including Kure, Bosso, Maikunkele, Gwari and Maitumbi markets (Figure 3.2)

Figure 3.1: Map of Nigeria showing Niger state (Source: Mustafa *et al*., 2020)

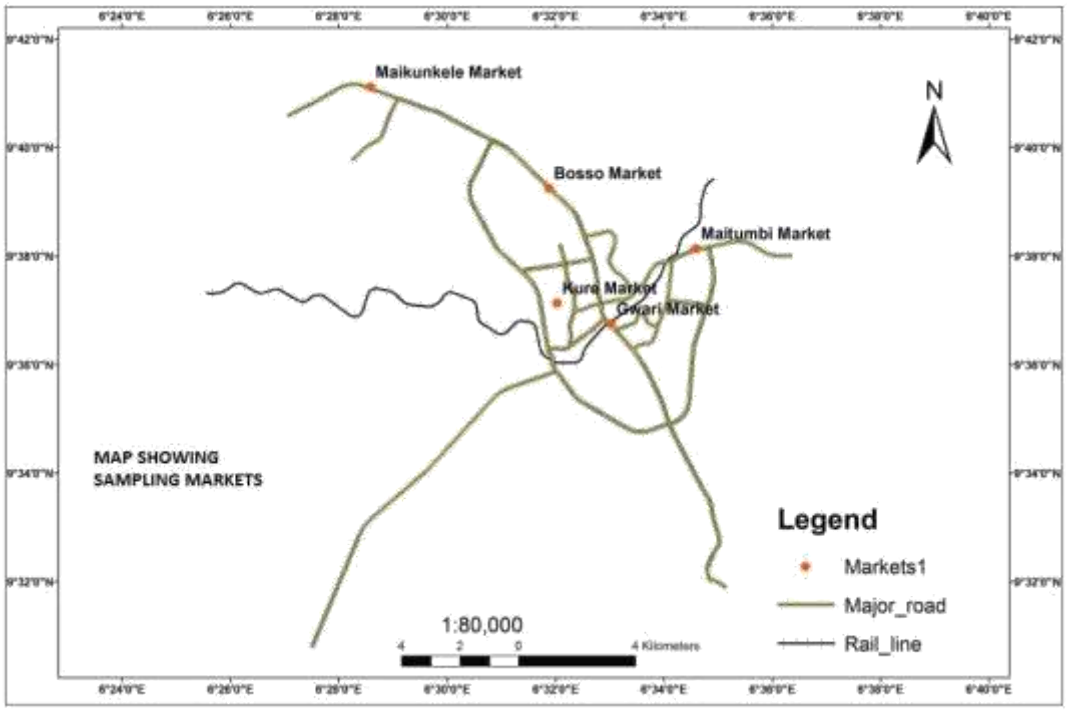

Figure 3.2: Map of Minna showing 5 different Markets where samples were purchased.

(Source: Generated using ArcGIS 10.1 software)

3.2 Sample Size Determination

The method of sample size determination for the detection of intestinal parasites was done according to Isah (2016). Using the formula as follows:

Where; N is the sample size

Z is the standard normal deviation setting at 1.96 corresponding to 95% confident interval

P is the estimated proportion of the attribute under study

Q is derived from 1-P and Family E is the precession

level (0.05)

3.3 Sample Collection

Five hundred (500) samples of each Vegetable (carrot, cucumber, tomatoes, lettuce and cabbage) were analyzed. The vegetables were bought from the Markets and transported inside polyethylene bags, the polythene was prevented by contact with all possible material to prevent external contamination and placed inside a cold chamber to the Centre for Genetic Engineering and Bioexploration (CGEB) laboratory Federal University of Technology Minna for microbiological analysis.

Plate I: Vegetable sample, (a) Cabbage (b) Carrots (c) Cucumber (d) Lettuce and (e) Tomatoes

3.4 Methods of Detection Of Parasites

3.4.1 Detection of intestinal parasites using formol ether sedimentation method

In this method, 1.0g of finely chopped vegetable thoroughly sample was mixed in 4.0 mL of 10 % formalin solution and allowed to stand for five (5) minutes before filtration. The filtrate was added to 4.0 mL of diethyl ether and mixed together for sixty (60) seconds. The mixture was centrifuged at 2000 rpm for five (5) minutes. The two layers that is ether and formal water layers were discarded, while the sediment was mixed with 1 % iodine solution on glass slide and covered with cover slip. The prepared slide was viewed with X10 and X40 objective lenses of microscope (Model; SDB2-180M, Proway Electronic Co., ltd) and the result was recorded (Ariyibi, 2001).

3.4.2 Detection of intestinal parasites using saturated glucose solution method

One hundred (100) grams of glucose was dissolved in one hundred (100) mL of distilled water. Twenty (20) mL of the solution was homogenized with three of sliced vegetable for two minutes and then sieved. Ten (10) mL of the sieved liquid mixture was centrifuged for five (5) minutes at 2000 rpm. After centrifuging, the supernatant was discarded; sediment was placed on slide and examined at X10 and X40 objective lenses using binocular microscope. The results obtained were recorded (Amahmid *et al.,* 2002).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Parasites isolated from Gwari Market

The samples analyzed for parasitic contamination of Gwari market revealed that carrots had the highest parasitic contaminants with a total of 37 different cyst and eggs of different parasites (Table 4.1). It is relatively followed by tomatoes with 28 different parasites and the least in cucumber with only 6 different parasites.

Table 4.1: Parasitic contamination of vegetables in Gwari market

PARASITE	CR	CU	CB	TM	LT	TOTAL	% FR
Teania egg	6	2	4	8	1	17	19.32
Cyst of *Entomoeaba hystolytica*	1	2	5	0	0	8	9.10
Eggs of hook worm	0	0	0	1	0	1	1.14
Ascaris lumbricoides	0	0	0	0	0	0	0
Eggs Schistosoma masoni	13	1	5	12	3	29	32.96
Schistosoma japanicum	3	0	3	3	1	10	11.36
Cyst of *Schistosoma masoni*	14	1	2	4	2	23	26.14
TOTAL	**37**	**6**	**19**	**28**	**7**	**88**	100

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; % FR: Percentage frequency

4.1.2 Parasites isolated from Kure Market

The highest contamination of cyst and egg of different parasites with a total of 25, while cucumber had the least contaminations with a total of 6 different cyst and eggs of different parasites.

Table 4.2: Parasitic contamination of vegetables in Kure market

PARASITE	CR	CU	CB	TM	LT	TOTAL	%FR
Teania egg	4	0	6	5	2	17	19.70
Cyst of *Entamoeaba histolytica*	0	1	6	1	3	11	12.79
Eggs of hookworm	1	1	0	0	5	7	8.14
Ascaris lumbricoides	1	3	0	0	5	9	10.47
Cyst Schistosoma masoni	9	1	11	1	0	22	25.58
Schistoma japanicum	1	0	1	4	5	11	12.79
Cyst of *Schistosoma masoni*	2	0	1	2	4	9	10.47
TOTAL	**18**	**6**	**25**	**13**	**24**	**86**	**100**

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; %FR: Percentage frequency

4.1.3 Parasites isolated from Maikunkele Market

The cyst of *Schistoma masoni* (25.76%) had the highest frequency of occurrence, with the least been the cyst of *Schistoma histolytica* and *Schistoma japanicum* both with a frequency of 9.10%.

Table 4.3: Parasitic contamination of vegetables in Maikunkele Market

PARASITE	CR	CU	CB	TM	LT	TOTAL	% FR
Teania egg	3	1	3	2	0	9	13.64
Cyst of *Entomoeaba histolytica*	1	1	0	3	1	6	9.10
Cyst of hook worm	0	0	0	1	2	3	4.55
Ascaris lumbricoides	0	6	2	1	3	12	18.18
Schistosoma mosoni	6	2	3	5	5	13	19.70
Schistosoma japanicum	0	0	1	2	3	6	9.10
Cyst of *Schistosoma masoni*	8	2	0	3	6	17	25.76
TOTAL	**18**	**12**	**9**	**15**	**20**	**88**	100

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; % FR: Percentage frequency

4.1.4 Parasites isolated from Maitunbi Market

Cyst of *Schistoma manoni* (26.25%) had the highest contamination rate followed by *Schistoma masoni* (23.75%) and the least rate was recorded with *Ascaris dumbricoides* (32.75%).

Table 4.4: Parasitic contamination of vegetables in Maitumbi Market

PARASITE	CR	CU	CB	TM	LT	TOTAL	% FR
Teania egg	7	1	3	4	1	16	20.00
Cyst of *Entomoeaba histolytica*	1	1	2	3	1	8	10.00
Cyst of hookworm	1	0	1	2	2	6	7.50
Ascaris lumbricoides	0	0	1	2	0	3	3.75
Schistosoma masoni	11	1	3	2	2	18	23.75
Schistosoma japanicum	2	0	1	2	2	7	8.75
Cyst of *Schistosoma masoni*	12	1	2	3	3	21	26.25
TOTAL	**34**	**4**	**13**	**18**	**11**	**80**	100

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; % FR: Percentage frequency

4.1.5 Parasites isolated from Bosso Market

Teania eggs (31.48%) had the highest frequency of occurrence followed by cyst of hook worm (18.52%), *Schistoma mansoni* (18.52%) and the lowest frequency of occurrence was observed in cyst of *Schistoma japanicum* (1.85%).

Table 4.5: Parasitic contamination of vegetables in Bosso Market

PARASITE	CR	CU	CB	TM	LT	TOTAL	% FR
Teania egg	5	0	2	7	3	17	31.48
Cyst of *Entamoeaba hystolytica*	0	0	1	3	1	5	9.26
Cyst of hookworm	0	1	3	4	2	10	18.52
Ascaris lumbricoides	0	0	0	0	1	1	1.85
Schistosoma masoni	1	2	0	4	3	10	18.52
Cyst of *schistosoma japanicum*	0	0	1	0	0	1	1.85
Cyst of *schistosoma masoni*	3	1	2	1	3	10	18.52
Total	**9**	**4**	**9**	**19**	**13**	**54**	**100**

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; % FR: Percentage frequency

4.1.6: Distribution of Parasites in all the markets

Carrots has the highest parasitic contamination rate with a total number of 116 out of 500 samples analyzed, followed by tomatoes with total parasitic contamination of 100. The least of all is cucumber with a total of contamination rate of 36 samples out of 500 samples analyzed.

Table 4.6: Distribution of parasites found in vegetables in all the markets

PARASITE	CR	CU	CB	TM	LT	TOTAL	% FR
Teania egg	25	4	18	26	7	80	20.15
Cyst of *Entomoeba hystolytica*	3	5	9	10	5	32	8.06
Cyst of hookworm	2	2	4	7	11	26	6.55
Ascaris lumbricoides	1	9	3	3	10	26	6.55
Schistosoma mansoni	40	11	18	30	17	116	29.22
Cyst of *Schistosoma japanicum*	6	0	7	11	11	35	8.82
Cyst of *Schistosoma mansoni*	39	5	7	13	18	82	20.06
TOTAL	**116**	**36**	**66**	**100**	**79**	**397**	100

Key: CR: Carrot; CU: Cucumber; CB: Cabbage; TM: Tomato; LT: Lettuce; % FR: Percentage frequency

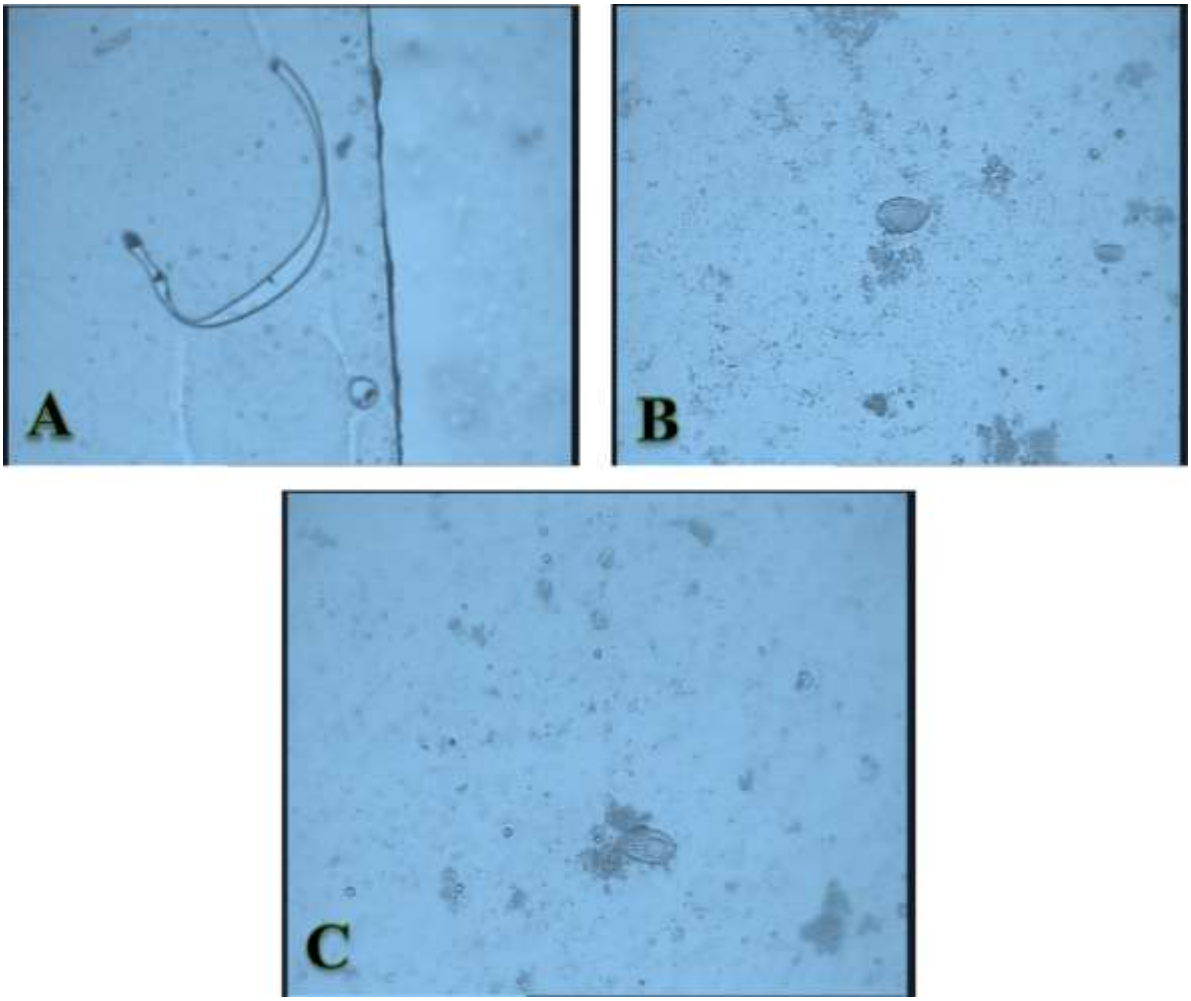

Plate II: Microscopic view of some parasitic features from samples analysed

(A) *Ascaris lumbricoides* (B) Cyst of *Schistosoma mansoni* and (C) Cyst of *Schistosoma haematobium*

4.1.7: Distribution of Parasites in all the markets

The distribution of parasites in all the markets is shown in Table 4.7. The total samples analyzed and the number of samples contaminated and the total percentage prevalence for each sample.

Table 4.7: Parasitic contamination of vegetables from the Markets

Raw vegetable	No analysed	No contaminated	% Prevalence of parasite
Carrots	500	116	29.22
Cucumber	500	36	7.20
Cabbage	500	66	13.20
Tomato	500	100	20.00
Lettuce	500	79	15.80
Total	**2500**	**397**	**15.88**

4.2 Discussion

Consumption of raw vegetables without proper handling and processing can lead to infection with various parasites, which can be the cause of a wide clinical spectrum ranging from symptomless infections to life-threatening conditions, particularly in developing countries (Escobedo *et al*., 2008). This study assessed the contamination and prevalence of

parasites/parasitic stages on raw commonly consumed vegetables purchased from five markets in Minna (Maikunkele market, Kure market, Gwari market, Maitunbi market and Bosso market). Finding from this study, it showed that a considerably number of commonly consumed raw vegetables in Minna are contaminated with a wide variety of parasites, which may represent a risk to the health of consumers of raw vegetables from the city. Among the two thousand five hundred (2500) samples of raw consumable vegetables analysed, 397 (15.88 %) was found to be contaminated with different stages of parasites either their ova, cyst, egg or a complete developed parasite. The result is similar to those of other studies carried out by Adejanya and Morenikeji (2015) in Ibadan where 11.6 % parasitic contamination of raw vegetables was recorded. Al-Megrin (2010) and Mohamed *et al* (2016) also reported similar prevalence of 16.2 % parasite contaminating raw eaten vegetables in Saudi Arabia, 13.5 % in Khartoum and Sudan respectively. Higher prevalence of 31.70 % of *Cyclospora* spp. oocyst, 39.1 % of *Microsporidia* spp. spores, 57.5 % of *Cryptosporidia* spp. oocysts, 57.8 % of *Ascaria* eggs and 79 % of *Toxocara* eggs respectively was recorded in Alexandria, Egypt (Said, 2012), Ethiopia (Alemu *et al*., 2020), Dutsen-Ma town Katsina State (Auta *et al*., 2017), Ilorin (Fumilayo *et al*., 2020), Ghana (Kudah *et al*., 2018), Ethiopia (Tefera *et al*., 2014) and Iran (Ezatpour *et al*. 2013) respectively. The difference in the prevalence of the parasite recorded in this study and those of previous studies done in other location could be attributed to variations in geographical locations, climatic and environmental conditions, differences in the 500 samples examined, sampling techniques employed, sanitary status of community, methods used for detection of parasites, and socioeconomic status.

In the present study five types of commonly consumed raw vegetable in Minna ware examined; carrots, cabbage, cucumber, lettuce and tomatoes. Of all these vegetables, carrots had the highest parasitic contamination with a prevalence of 29.22 % due its rough surface body, followed by Tomato (25.19 %) this may be as a result constantly moist easily perishable condition, lettuce (19.90 %), cabbage (16.63 %) with cucumber (9.07 %) having the least amount of contamination. Parasitic contamination of these vegetables may occur in a variety of ways such as contact with the soil, use of contaminated water for irrigation, exposure to non-hygienic conditions in markets and from contaminated hands of vendor and retailers. The rate of contamination seems to vary with the nature of the vegetable surface depending on whether it is smooth (tomatoes, cucumber), uneven (lettuce, cabbage) or root crops (carrots). Factors such as improper handling, washing with contaminated water or other unhygienic practices might have also been responsible for the high level of parasitic contamination in carrots and tomatoes. In addition crops growing close to the ground in general, are more susceptible to contamination by water, soil or animals. From this study the prevalence of parasites in relation to vegetables revealed that carrots had the highest prevalence of 29.22 % which is close to the prevalence of 33.33 % reported by Dawet *et al* (2019) from carrots samples in Jos and 29.00 % reported by Alemu *et al* (2019) in Ethiopia. Lower prevalence of parasitic contamination of carrots was reported by Ishaku *et al* (2012) who recorded a prevalence of 15 % from carrots samples obtained from Alhamis market in lafia metropolis, also Dawet *et al* (2019) recorded a prevalence of just 2.40 % from carrots sold in Jos, Plateau state. Higher parasitic prevalence of 62.2 % was reported by Tefera *et al* (2014) in carrots obtained from some markets in Ethiopia. Contamination could be attributed to Post-harvest faecal contamination which may occur during handling and transport,

usually due splashing the vegetables with contaminated water from dirty containers or unhygienic handling in order to keep them fresh.

Prevalence of parasitic contamination in cabbage (16.63 %) and lettuce (19.90 %) was relatively high in this study. This prevalence however, is significantly lower compared to those from other studies; prevalence of 21.67 %, 66.67 %, 50.00 % and 43.1 % was reported in cabbage by Ishaku *et al* (2012), Dawet *et al* (2019), Auta *et al* (2017) and Kudah *et al* (2018) respectively, while the prevalence in lettuce recorded by Dawet *et al* (2019), Ishaku *et al* (2012), Said (2012), Mohamed *et al* (2016) and Kudah *et al* (2018) was 33.33 %, 31.67 %, 45 %, 36.40 % and 70.80 % respectively. Higher prevalence of parasitic contamination in cabbage and lettuce is usually reported due to direct contact of these vegetables with contaminated soil and water since they are found nearer to the soil surface. It has also been reported that the large and uneven surfaces of cabbage and lettuce aids the parasites in easily attaching to their surfaces (Rawaa *et al*., 2011; Luz *et al*., 2017; Dawet *et al*., 2019). Idrissa *et al*. (2010) reported that vegetables with dense foliage are usually more contaminated than those growing on the surface as the dense foliage would protect the helminth eggs against unfavourable conditions such as sunlight, desiccation, and wind ensuring their persistence and survival.

Cucumber with a prevalence of 9.07 % had the least contamination of all vegetables examined, this is similar to the results obtained from other studies which reported low prevalence of parasitic contamination in cucumber presumably because of its smooth surface which reduces the rate of parasitic attachment. Prevalence of 3.39 %, 11.11 %, 0.0 %, 0.0 % and 0.00 % was recorded in cucumber by Ishaku *et al* (2012), Adejanya and Morenikeji (2015), Mohamed *et al* (2016), Auta *et al* (2017) and Dawet *et al* (2019) respectively.

Gwari market recorded a higher parasitic prevalence of (17.6 %) of all the market studied followed by Kure market (17.2 %), Maitunbi market (16 %), Maikunkele market (13.3 %) and Bosso market (10.8 %). The high prevalence of contamination with parasites found in the vegetables sold in the Gwari market could be attributed to the poor sanitary conditions, lack of water supplies and a frequent presence of piles of garbage that provide a fertile environmental for transmission of parasites (Nyarango *et al*., 2008). Vegetables may also become contaminated at the point of sale in markets, from contaminated hands or contaminated water used for washing these vegetables, contaminated containers and from insect that land on both food and garbage (Rawaa *et al*., 2011). Bosso market recorded the least parasitic contamination. This might be due to the fact that the water used in washing the vegetables was good portable water from boreholes and pipe bone water which has minimal parasitic contamination.

Different stages of parasites were observed during the course of this study, S*chistosoma masoni* (29.22 %), had the highest frequency of occurrence followed by the eggs of *Teania* (20.15 %), cyst of *Schistosoma masoni* (20.06 %), cyst of *Schistosoma japonicum* (8.82 %), Cyst of *Entamoeba histolytica* (8.06 %), Cyst of hookworm (6.55 %) and *Ascaris lumbricoides* (6.55 %). The presence of these parasitic species in raw commonly consumed vegetables demonstrates that the population of Minna is supposedly exposed to several parasitic diseases, such as hookworm infections, ascariasis, schistosomiasis and amoebiasis as most of these vegetables are processed and eaten uncooked. These parasites are indicator of poor socio-economic condition as wel as poor environmental and sanitation practices (Shural *et al*., 1986). Lack of modern toilet facilities, inadequate public health enlightenment and illiteracy makes people defecate indiscriminately, thereby resulting in pollution of water

and farmland, which inadvertently cause contamination of vegetables in the farmland (Damen *et al*., 2007) In addition, the practice of using human excreta as fertilizer, as well as the use of untreated sewage or irrigation water containing pathogens, are also responsible for contamination of vegetables.

However, parasites observed from this study differ from those of other studies carried out in other parts of Nigeria and other countries. This variation could be due to differences in geographical location of the study, climate conditions, type of soil, type and number of samples examined, methods used for detection and the handling methods of such vegetables. A study carried out in Akure by Fumilayo *et al*. (2017) reported ova of *Ascaris lumbricoides* (51.9 %) as the most frequent parasite followed by the ova of Hookworm (26 %), Cyst of *Entamoeba histolytica* (18.5 %) and eggs of *Enterobius vermicularis* (3.7 %). Adejanya and Morenikeji (2015) reported eggs of *Ascaris lumbricoides* (51.7 %), larvae of Hookworm (27.6 %) and larvae of *Strongyloides stercoralis* (20.7 %) as the commonly observed parasites in vegetables sold in major markets in in Ibadan. Amaechi *et al* (2016) also reported Eggs of *Ascaris lumbricoides* (28.0 %), *Entamoeba histolytica* (23.3 %), *Enterobius vermicularis* (11.7 %), cysts of *Giardia lambia* (5.0 %), *Fasciola hepatica* (6.7 %), *Trichuris trichiura* (3.3 %), *Strongyloides stercoralis* (10.0 %), *Balantidium coli* (3.3 %) and *Necator americanus* (8.3 %) in vegetables from Ilorin. In Ghana Kudah *et al* (2018) encountered *Strongyloides stercoralis* (36.4 %), *Balantidium coli* (13.6 %), *Fasciola eggs* (7.2 %), *Flagellates* (7.8 %) and *Cryptosporidium oocyst* (11.1 %). Mohammed *et al* (2016) identified *Entamoeba histolytica* (42.9 %), *Entamoeba coli* (14.3 %), *Giardia lamblia* (22.9 %), *Ascaris lumbricoides* (2.9 %), *Strongyloides stercoralis* (8.6 %), *Trichuris trichiura* (2.9

%) and hookworms (5.7 %) as parasitic contaminants of fresh vegetable in Khartoum, Sudan.

The consumption of raw vegetable plays an important role in the transmission of parasitic infections. The frequency of contamination of commonly consumed raw vegetables with different stages of parasites from this study is significant; this puts the public at a high risk of infection with various parasites. So, adopting control measures that cover guidelines of irrigation water quality, strategies to reduce the risk of disease transmission by food-borne parasites and avoiding to the use of untreated human feces as fertilizer is highly recommended. Effective and comprehensive prevention and treatment measures should be taken to ensure food safety. Washing procedures before eating raw vegetables regardless of the provider's sanitation should be performed to avoid transmission of parasites.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The total prevalence of parasite on vegetables sold in some Markets in Minna was 15.88%. Some of the vegetables analysed in the present study were moderately contaminated with intestinal parasites such as S*chistosoma masoni* (29.22%), Eggs of *Teania* (20.15%), cyst of *Schistosoma masoni* (20.06%), cyst of *Schistosoma japonicum* (8.82%), Cyst of *Entamoeba histolytica* (8.06%), cyst of hookworm (6.55%) and *Ascaris lumbricoides* (6.55%).

5.2 Recommendations

It is recommended that:

1. farmers should be enlightened on packaging and transportation to the market, because it one of the major source of contamination.
2. sellers and hawkers should wash the vegetables with good portable water and package it properly and also not stay in unhygienic areas to sell.
3. consumers should look of the point of sales and the seller are in hygienic condition before they buy the vegetables.
4. consumers should wash it properly, if possible with salty water so as to eliminate the cyst, ova eggs or even fully developed parasites before consumption of raw vegetables.
5. consumers should seek medical attention immediately when any of the symptoms start emerging after consuming the vegetables and drawing attention to include laboratory diagnosis especially for stool samples.

6. vegetables should be washed properly with vinegar. This will also eliminate the over or cyst present.

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