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Original Research

Zoonotic parasite contamination of some green, leafy and spicy vegetables sold in major markets of Makurdi metropolis, Benue state

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ABSTRACT

Consumption of contaminated vegetables can result in parasite ingestion with subsequent health problems. The spread of zoonotic parasites through contaminated vegetables is a complicated problem that requires attention to ensure food safety and protect public health. This study investigated the zoonotic parasitic contamination of some green, leafy, and spicy vegetables sold in Makurdi metropolis, Benue State. A cross-sectional study was designed in which four hundred green, leafy, and spicy vegetables consisting of five different vegetables, were collected from five markets in Makurdi town, Benue state. Sediments obtained by washing the vegetables with normal saline were centrifuged at 1500 rpm for five minutes before being subjected to flotation, sedimentation, and modified Ziehl-Neelsen techniques, and viewed under the microscope at various magnifications. The overall prevalence of zoonotic parasites was 59.75%. *Strongyloides* spp. larvae (40.5%), *Cryptosporidium* spp. Oocyst (0.5%), *Balantidium coli* cyst and trophozoite (7%), *Ascaris* spp (5.75%), *Taenia* spp. (9%), Strongyle ova (4.5%), and *Giardia lamblia* cyst (1.5%) were identified. Results showed a significant relationship between vegetable type and parasite contamination of vegetables ($p < 0.05$), but no significant relationship between market location and parasite contamination ($p > 0.05$). The high prevalence of zoonotic parasites observed in this study highlights the potential health risks associated with consuming contaminated vegetables. The study recommends public enlightenment of vegetable sellers and consumers in Makurdi on the importance of hand hygiene and safe handling of vegetables to prevent exposure to pathogenic microorganisms.

Keywords: Contamination, green leafy, Makurdi, prevalence, vegetables, zoonotic parasites

INTRODUCTION

Vegetables are important component of human and animal diet. Vegetables provide adequate amounts of many vitamins like beta-carotene, ascorbic acid, riboflavin, folic acid, and minerals such as calcium, iron, and phosphorus, carbohydrates, proteins, fibers, and antioxidant properties that support general health (Randhawa *et al*., 2015, Kumar *et al*., 2020). Vegetables are consumed either in their raw state or after various culinary preparations (Damen *et al*., 2007). Regular intake of vegetables is linked to a decreased risk of numerous non-communicable diseases, including cardiovascular diseases, stroke, and specific types of cancer (Dehghan *et al*., 2011). The World Health Organisation (WHO) recommends consuming at least 400 g of fruits and vegetables daily, particularly in less developed countries (WHO, 2011). However, these vegetables can serve as potential carriers of various pathogens due to their intricate surface characteristics and porosity which, unfortunately, makes them conducive for pathogen attachment and survival (Alegbeleye *et al*., 2018). Contamination of vegetables can occur at pre-harvest, harvest, and post-harvest. Expectedly, handling post-harvest contributes significantly to vegetable contamination. Several surveys in Nigeria (Damen *et al*., 2007; Adejumoke and Morenikeji, 2015; Lema, 2016; Ola-fadunsin *et al*., 2022; Eberemu *et al*., 2023) have reported fruits and vegetables as viable sources for transmission of different parasite developmental forms to humans.

Benue is well-known for its consistent supply of fruits and vegetables throughout the year, partly due to the presence of the Benue River and its tributaries, which facilitate

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productive activities such as irrigation farming. Hence, indigenous people consume vegetables highly due to their abundance, nutritional value, and affordability. Some surveys demonstrated the occurrence of parasites in some fruits and vegetables sold in Benue state (Anyebe *et al*., 2014; Amuta *et al*., 2017; Yandev *et al*., 2019). However, there is a dearth of information about the occurrence of zoonotic parasites on spicy and aromatic vegetables sold in Benue state, used in the preparation of special native soups. Therefore, the current study aimed to determine zoonotic parasite contamination of some green, leafy, and spicy vegetables sold in Makurdi metropolis, Benue state.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Makurdi (Figure I), the capital of Benue State, Nigeria, located in the Guinea-savannah region of North Central Nigeria. The metropolis is bordered by the Benue River and its tributaries, covering 26 km. Currently, Makurdi has a projected population of 472,000, (Macrotrends, 2024). The annual temperature ranges from 22.5°C to 40°C (Temi and Tor, 2006). The rainy season starts in April and ends in October, while the dry season spans from November to March. Benue is renowned for its consistent supply of fruits and vegetables throughout the year, partly due to the presence of the Benue River and its tributaries, which facilitate productive activities like irrigation farming. Residents primarily engage in agriculture, specializing in cultivating cash and subsistence crops, and fishing.

STUDY DESIGN

The project employed a cross-sectional study design to determine the contamination of green, leafy, and spicy vegetables with zoonotic parasites, sold in major markets within the Makurdi metropolis, Benue state, Nigeria.

SAMPLE POPULATION AND SAMPLING

Samples were collected from five major markets in Makurdi, namely: Modern market, Wurukum market, High-level market, Railway market, and North-bank market. These are well-established markets located in strategic areas of Makurdi metropolis. They cater to the needs of both residents and visitors, offering a wide variety of agricultural products, including fresh green vegetables. Vegetables sampled included *Piper guineense* (Uziza), *Gongronema latifolium* (Utazi), *Gnetum africanum* (Okazi), *Pterocarpus mildbraedii* (Oha), and *Corchorus olitorius* (Jute). Using convenience sampling method (based on the availability of the vegetables), samples were collected for each vegetable type from each of the five major markets by visiting different vendors and selecting representative samples of each vegetable type. A total of 400 samples (80 samples per market, 80 samples per vegetable type) were collected. Samples were placed into clean and sterile zip lock bags to avoid contamination, labeled appropriately and transported to the Parasitology laboratory of the Veterinary Teaching Hospital, and Parasitology laboratory of the College of Veterinary Medicine, Joseph Sarwuan Tarka University Makurdi (JOSTUM) for processing. Sampling was done from August 2023 to December 2023.

SAMPLE PROCESSING

Before subjecting the samples to further processing, the vegetables were visually inspected to remove non-edible parts following usual household practices (Kudah et al., 2018). About 100 grams of each vegetable was weighed and washed with clean water to remove surface dirt and debris. After washing, the samples were cut into smaller pieces to facilitate the extraction of parasites and promote homogeneity. Each sample was washed vigorously with normal saline (containing 0.85% sodium chloride) in a 250 ml round bottom clean plastic bowl. The suspension was strained to remove debris and allowed to stand on the bench a few hours to allow for proper sedimentation. The supernatant was discarded, leaving about 15 ml at the bottom. The mixture (in two parts) was transferred into a centrifuge tube and spun at 1500 rpm for five minutes. The supernatant was decanted, leaving the sediment at the bottom of the tube. Subsequently, the sediment was mixed and divided into three portions for examination using floatation, sedimentation, and modified Ziehl-Neelson techniques (Cheesbrough, 2005).

FLOTATION TECHNIQUE

A saturated salt solution with a specific gravity (SG) of 1.2 was prepared. Sediment was mixed with the flotation solution in a tube and left to stand for 15 minutes. A coverslip was placed on top of the test tube and transferred onto a microscope slide for further examination at x4, x10, and x40 objectives.

SEDIMENTATION METHOD

Drops of the sediment were placed in duplicates on a clean grease-free glass slide. A drop of Lugol’s iodine solution was added to the sediments and was gently covered with a clean coverslip, avoiding air bubbles and overflooding. The smear was examined under a light microscope at x4, x10, and x40 magnifications.

MODIFIED ZIEHL-NEELSON:

For each sample, a thick smear of the sediment was prepared, allowed to air dry, and subsequently heat fixed for a few seconds. The slide was stained with 3% cold carbol fuchsin for 15 minutes. After staining, the slides were dipped in a 1% hydrochloric acid-ethanol solution for 15 seconds and washed with distilled water. Subsequently, the slides

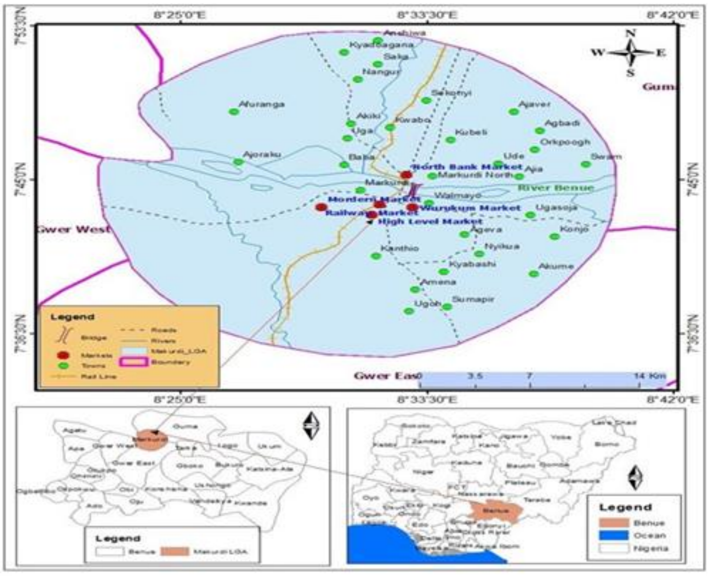

Figure I. Map of study area (Sourcee: GIS Lab Department of Geography and Environmental management ABU Zaria)

were counterstained with 0.3% methylene blue for 30 seconds. The slides were rinsed with water, allowed to air dry, and examined microscopically using a 100X objective with oil immersion.

IDENTIFICATION OF PARASITES

Parasite eggs, oocysts, larvae, and segments were identified based on their morphological characteristics. The size, shape, color, and other distinctive features of the parasite were observed and compared with reference images and descriptions of known parasites (Cheesbrough, 2005). Identification of the parasites was done by a parasitologist.

DATA ANALYSIS

The data obtained from the study were entered into Google Sheets. Descriptive statistics such as frequency and percentages were calculated using Google Sheets, while inferential statistics was done using IBM SPSS® Statistics version 25.0 (New York, USA). The prevalence of parasites in the leafy vegetable samples was calculated by dividing the number of positive samples (containing parasites) by the total number of samples examined. Specific prevalence/contamination for vegetable type and market location was also calculated. Chi-Square test of significance was used to test for significance of association between specific variables (vegetable type and market location) and contamination status of the vegetables. Statistical significance at a probability of 5 % ($p < 0.05$) with a confidence interval of 95 % was adopted.

RESULTS

Out of 400 vegetable samples, 274 (68.5%) samples were contaminated with at least one type of parasite. Parasites observed included *Strongyloides* spp larvae, oocyst of *Cryptosporidium* and *Eimeria* spp., *Balantidium coli* cyst and trophozoite, *Giardia lamblia* cyst, ova of *Ascaris* spp, *Taenia* spp, *Toxascaris* spp., *Enterobius* spp, and Strongyle-type ova, mites and unidentified ova. Results revealed that out of 400 samples, 239 (59.75%) samples were contaminated with at least one type of zoonotic parasite. The zoonotic parasites observed included *Strongyloides* spp larvae, *Cryptosporidium* spp., *Balantidium coli*, *Giardia lamblia* cyst *and* ova of *Ascaris* spp., *Taenia* spp, and Strongyle ova. The most common zoonotic parasite was *Strongyloides* spp larvae, and the least occurring was *Cryptosporidium* spp. (Table I).

Results showed a significant ($p = 0.003$ association between vegetable type and contamination status of the vegetables (Table II). *Corchorus olitorius* (Jute) had the highest contamination (85%), while *Piper guineense* (uziza leaf) was the least contaminated. Although no significant ($p = 0.099$) association was observed between market location and contamination status of the vegetables (Table III), Railway market had the highest contamination (76.25%), while Northbank market was the least contaminated.

DISCUSSION

The current study reported an overall contamination of green, leafy and spicy vegetables to be 68.5%, while contamination with zoonotic parasites was 59.75%. Comparing the findings of this study to previous studies in the same region (Amuta *et al*., 2017; Apuu *et al.*, 2022), and beyond Benue state (Damen *et al*., 2007; Lema, 2016; Obebe *et al*., 2020; Okwuonu *et al*., 2022), higher contamination was observed. Similarly, the current study reported a higher overall prevalence when compared with some studies in different countries, 29% in Iran (Daryani *et al*., 2008) and 57.7% in Ghana (Kudah *et al.*, 2018). Factors that may have contributed to the differences in contamination rates among the various studies include differences in agricultural practices, environmental contamination, hygienic practices, cross-contamination during transportation, handling of vegetables in the market, sample size, sampling methods and laboratory methods used in analysing the samples. The current study findings suggest that there is a need for thorough washing of vegetables (in water either with salt or vinegar) before consumption due to the possibility of zoonotic parasites being present, as parasites were found even after normal household practice of washing vegetables before use.

It is important to note that the vegetables analyzed in the current study differ from previous studies in Makurdi. Green, leafy and spicy-aromatic vegetables were studied. These

Table I: Frequency Distribution of Parasites Observed in Vegetables Sampled From Five Major Markets of Makurdi, Nigeria, 2023 (n = 400)

Parasite	Vegetable type *Corchorus olitorius* (ewedu)	*Piper guineense* (uziza)	*Pterocarpus mildbraedii* (oha)	*Gnetum africanum* (okazi)	*Gongronema latifolium* (utazi)	Total (%)
Strongyloides larva	38	32	43	32	17	162 (40.5)
Taenia spp	8	7	0	1	20	36 (9.0)
Balantidium coli	14	2	3	2	7	28 (7.0)
Ascaris spp ova	5	5	4	5	4	23 (5.75)
Strongyle ova	10	0	0	3	5	18 (4.5)
Toxoascaris spp	3	1	0	1	1	6 (1.5)
Mite	1	0	0	1	0	2 (0.5)
Enterobius spp	0	2	0	0	3	5 (1.25)
Gardia lamblia cyst	2	1	1	2	0	6 (1.5)
Eimeria oocyst	3	2	2	0	0	7 (1.75)
Cryptosporidium oocyst	0	0	0	2	0	2 (0.5)
Unidentified ova	19	2	5	1	1	28 (7.0)

Table II: Association Between Vegetable Type and Contamination of Sampled Vegetables, Makurdi, 2023

Vegetable Type	Total	Positive	% Positive	P value
C. olitorius (ewedu)	80	68	85.0	0.003
P. guineense (uziza)	80	48	60.0	
P. mildbraedii (oha)	80	58	72.5	
G. africanum (okazi)	80	50	62.5	
G. latifolium (utazi)	80	50	62.5	
Total	400	274	68.5	

χ^2 = 16.035, df = 4, α = 0.05

Table III: Association Between Market Location and Contamination of Sampled Vegetables, Makurdi, 2023

Market Location	Total	Positive	% Positive	P value
High Level	80	55	68.8	0.099
Railway	80	60	75.0	
Wurukum	80	58	72.5	
Modern market	80	56	70.0	
North bank	80	45	56.3	
Total	400	274	68.5	

χ2 = 7.809, df = 4, α = 0.05

vegetables are used in preparing special native meals in different parts of Nigeria. Three out of the vegetables (common names: uziza, okazi, and utazi) can be purchased in their dried form in the markets. The method of drying, environmental contamination, handling, and hand hygiene practices play important factors in the level of contamination of these vegetables before they get to the final consumer and into the food of its consumers. On the other hand, several studies reported similar higher prevalence of parasites on various vegetables; 68%, 79% and 65.5% in Libya, Iran and Kenya, respectively (Abougrain *et al*., 2010; Ezatpour *et al*., 2013; Nyarango *et al.*, 2008). These international comparisons underscore the global issue of parasitic contamination in vegetables and emphasize the importance of stringent food safety measures.

Amongst the zoonotic parasites reported, *Strongyloides* spp larvae had the highest prevalence (40.5%). This result agrees with Ogbolu *et al*. (2009) and Kudah *et al*. (2018) who reported the prevalence of 45.8% and 36.4% respectively. However, Alade *et al*. (2013), Amuta *et al*. (2017), Apuu *et al*. (2022), Okwuonu *et al*. (2022) and Yahaya *et al*. (2023) reported the contrary, as *Ascaris* spp had the highest prevalence of 47.54%, 41.1%, 37.60%, 18.3%, and 67.6%, respectively. *Strongyloides* stercoralis is known to have a broad distribution, particularly prevalent in regions with inadequate sanitation conditions. Infections are often attributed to the contamination of soil and water sources by infected human or animals feacal matter. Based on the life of the parasite, *Strongyloides stercoralis* has a free-living state in the soil (Nutman, 2017). Therefore, in areas where sanitation is poor, and human and animal faeces are used as fertilizer, or open defeacation is practiced, there will be a higher risk of soil contamination. As a result, vegetables planted within the area will be contaminated.

Taenia spp. was the second-highest zoonotic parasite detected in this study. Vegetable contamination with *Taenia* spp may be attributed to the likelihood of faecal contamination of water used for irrigation, particularly faecal material of either pigs, cattle, or humans. This contamination could be because of inadequate sanitation, poor environmental and personal hygiene practices. The prevalence of *Taenia* eggs (9%) in this study was higher when compared to reports by Ogbolu *et al.* (2009) (4.2%). *Balantidium coli* is the third most prevalent zoonotic parasite found in this study (7%). When compared to studies conducted in southwestern Nigeria, the current study reported a higher prevalence of *B. coli* when compared to 0.8% and 2.3% reported by Ogbolu *et al*. (2009) and Ola-fadunsin *et al*. (2022) respectively. Alade *et al*. (2013) reported a higher prevalence (8.19%) of *B. coli* than the current study.

The contamination observed may be attributed to the likelihood of water used for irrigation being contaminated with faecal matter from both pigs and humans. *Cryptosporidium spp and Gardia lamblia* are protozoan parasites that cause diarrheal diseases in humans. Waterborne pathogens such as *Cryptosporidium* and *Gardia* are included in the WHO's "Neglected Disease Initiative" (Savioli *et al*., 2006). *Cryptosporidium* prevalence of 0.5% suggests a relatively low contamination rate. However, even low levels of *Cryptosporidium* can pose health risks, especially to individuals with weakened immune systems (Korpe *et al*., 2018). *Giardia lamblia* prevalence of 4.5% is relatively high and suggests a notable level of contamination. The presence of Strongyle (hookworm) ova in these vegetables indicates the possibility of vegetable sellers developing cutaneous larva migrans caused by zoonotic hookworms (Nezami *et al*., 2023).

The level of parasitic contamination was significantly different in the sampled vegetables, with *Corchorus olitorius* (ewedu) having the highest contamination of 85%. This means that contamination of the vegetables can be dependent on the type of vegetable. Factors that encourage this association may include pre-harvest and post-harvest handling of the vegetables and the characteristics of the leaf of the vegetable such as surface area and porosity. More so, amongst the vegetables sampled, *Corchorus olitorius* (ewedu) was the only vegetable that periodically came in contact with water which the vegetable vendors use to maintain its quality. It is possible that the water used for wetting the vegetables was contaminated, reflecting an increased contamination compared to the other vegetables.

The *Pterocarpus mildbraedii* (oha), although a leaf thought to be very clean due to its root being far from the soil, had high contamination. Contamination of this vegetable is more likely to occur during handling at post-harvest period, including long-distance travel from the eastern parts of Nigeria to Makurdi. Also, there are possibilities of contamination from dust in the air which eventually gets to the table for the consumers.

Although, no significant association existed between vegetable parasite contamination and market location,

parasitic contamination was notably higher in the Railway market at 76.25%. Other studies also supported the same finding of no significant relationship between market location and parasite contamination of vegetables (Usman & Sahura, 2023). The increased contamination at Railway market could potentially be linked to several factors, including the market's proximity to the pig abattoir, the way vegetables were displayed on tables, and their exposure to dust and flies. Flies are significant vectors for transmitting various parasites. Parasite prevalence recorded in this study highlights the potential health risks associated with consuming contaminated vegetables. Contaminated water sources, hygiene practices, and local environmental factors may all contribute to the presence of these parasites. Open defecation is practiced by residents in the study area. It is essential for public health and food safety measures to address these health concerns to reduce the risk of parasitic infections associated with vegetable consumption. Public enlightenment of concerned stakeholders (farmers, vegetable sellers, residents of Makurdi) is a fundamental key in addressing these issues.

In conclusion, the present study demonstrated that green, leafy and spicy vegetables sold in Markudi were contaminated with zoonotic parasites. A significant association existed between vegetable type and parasite contamination while none was observed between market location and vegetable contamination. The study recommends public enlightenment of vegetable sellers in Makurdi on the importance of hand hygiene and the safe handling of vegetables to prevent public exposure to pathogenic microorganisms. Also, consumers of vegetables need to be educated on the importance of proper washing of vegetables to prevent exposure to zoonotic parasites.

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