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Occurrence of *Cryptosporidium* Oocysts on Vegetable Samples from a Vegetable Farm Irrigated with Treated Wastewater in Samaru, Zaria, Kaduna State

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ABSTRACT

Consumption of raw vegetables has been described as a major way of transmitting *Cryptosporidium* oocysts. This study assessed the occurrence of *Cryptosporidium* oocysts on vegetables sampled from wastewater irrigated farms at Ahmadu Bello University (ABU), Zaria, Nigeria. A total of 100 samples of five different vegetables including carrot, tomato, lettuce, cabbage and spinach were randomly examined for parasite eggs, cysts and oocysts using both the floatation techniques and the modified Zeihl Neelsen staining technique for *cryptosporidium* parvum detection. Each sample was washed with sterile distilled water and the wash was allowed to sediment at room temperature for 24 hours. 10 ml of sediment were centrifuged at 3000 rpm for 5 minutes. Slides were observed using a light microscopy and an oil immersion objective lens. Oocysts were confirmed under a higher power. The results confirmed an overall 31% occurrence of *Cryptosporidium* oocysts on the samples. Occurrence of *Cryptosporidium* oocysts on vegetables was cabbage 30%, carrot 25%, lettuce 30%, spinach 55% and tomato 15%. Distribution rate of *Cryptosporidium* oocysts was higher in spinach, 55% than in cabbage and lettuce 30% and the least contaminated was tomato 15%. Nine parasitic eggs and cysts were identified in all the samples which included *strongytodies* spp, *Ascaris* spp, *E.hyistolytica*, mite, Cyclospora, Nematode larvae. Proper cooking of vegetables before consumption, washing of raw vegetables with clean sterile water to avoid water-source contamination and proper handling of vegetables to avoid faecal contamination are some of the recommended ways for the control of the contamination of vegetables by the oocysts. *Cryptosporidium* oocysts contamination of raw vegetables irrigated with wastewater in Zaria may pose a health risk to consumers of such products.

Key words: *Cryptosporidium*, oocysts, cysts, raw vegetables, wastewater.

INTRODUCTION

Cryptosporidium is a single-celled obligate intracellular parasitic protozoa, which has been described in sixteen different species of domestic and wild mammals, birds and reptiles [1]. It is an important cause of diarrhoeal illnesses worldwide especially in children [2]. The incubation period is usually about one week, with clinical signs of profuse, offensive, watery diarrhoea, which may be accompanied by abdominal pain, vomiting and fever [3]. In the immuno-compromised individuals, such as those with Acquired Immunodeficiency Syndrome (AIDS), cryptosporidiosis is a common and life threatening condition causing profuse and intractable diarrhoea leading to severe dehydration, malabsorption and wasting [3, 4]. The most vulnerable specie of animals to C*ryptosporidium* infection is cattle [5]. The method of transmission is by ingestion of oocysts that are fully sporulated and infective when they are passed in faeces [6]. Oocysts are discharged into water by various animal hosts [7]. The sporocysts are resistant to most chemical disinfectants, but are susceptible to drying and ultraviolet portion of the sunlight [8].

Cryptosporidiosis is a major public health problem in both developing and developed countries. In developing countries, the disease probably exerts most of its impact on paediatrics patients or on infants and young children resulting in stunted growth [9]. In developed countries, water-borne outbreaks of cryptosporidiosis have a significant economic impact [10, 11]. Foodborne outbreaks of cryptosporidiosis have been reported since the early 1980s and have been identified from several commodities, mostly fruits, vegetables, and shellfish [12]. Because these, products are usually eaten raw, there is the increasing concern that foods treated with contaminated water during processing may be a vehicle for transmission of C*ryptosporidium* [13].

Contamination of food or water by cattle manure has been identified as one of the causes of several foodborne and waterborne outbreaks of cryptosporidiosis in man [14]. Reports of isolation of C*ryptosporidium* oocysts from vegetables are as follows: cilantro and lettuce in Costa Rica [15], radfish, carrots, tomato and cucumber in Costa-Rica [16], lettuce, cilantro and parley in Peru [16] and lettuce and mung bean sprouts in Norway [17]. In the United States of America alone, the number of cases of cryptosporidiosis in thousands per million persons reported through foodNet was 10.9 in 2003, 13.2 in 2004 and 29.5 in 2005 [18-20].

Nigeria is generally at the meso-endemic level of the parasitic pathogens in paediatric patients presenting with diarrhoea [21]. The deplorable hygienic condition of the environment as a result of indiscriminate defaecation by both humans and animals can contaminate water sources [13]. Farm produce become contaminated when water containing *Cryptosporidium* oocysts are used on them. This study therefore focuses on detecting the occurrence of *Cryptosporidium* oocysts on the irrigated vegetables obtained from ABU wastewater irrigated farm site at Samaru Zaria, Kaduna State in Nigeria.

MATERIALS AND METHODS

Collection of Vegetable Samples

Five types of vegetable samples were collected for the research between December to February, 2015. The samples were identified by Mallam Namadi Sanusi of the Herbarium Unit of the Biological Science Department, Ahmadu Bello University, Zaria, Kaduna State with the following botanical, vernacularnames and voucher numbers: Cabbage (*Brassica oleracea var capitata* 09128) , locally called *cabbagee* in Hausa; Tomato (*Lycopersicum esculentum*1116) locally called *Tumatir* (Hausa); Lettuce (*Lactuca sativa* 1934*)*, called *Ganyen salad* (Hausa); Carrot (*Daucus carota var sativa* 0288) locally called *Caras* (Hausa) and Spinach (*Amaranthus hybridus* 0631) also called *Aleiho* (Hausa). The irrigated vegetables were randomly collected from ABU irrigated farms in Samaru Zaria, Kaduna State. Five irrigated farm lands were used and twenty samples were collected from each farm.

The samples were collected during the morning hours (8.00 a.m and 10 a.m) from each irrigated farmland to minimize undue exposure to contamination in the farm land and desiccation of the vegetables by sunlight. The selected vegetables are locally consumed raw in the form of salad and other varieties of diet. In all, one hundred samples were collected during the dry season. The samples were transported to the Helminthology Research laboratory in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, in sealed, well-labeled new and clean polythene bags and preserved in the refrigerator at between 4 and 8°C until the samples were processed for the detection of the parasites.

Floatation Technique for Recovering of other Parasites on Vegetable Samples

A modified method [22] was used as follows: Each vegetable sample was weighed (250 g) and washed in tap water (1500 ml) in a plastic container. The wash was left in the plastic container for 10 hr to sediment. The supernatant was discarded and the residue was transferred into the centrifuge tube and spun at 1500 rpm for five minutes. The supernatant was discarded and the residue was agitated gently in sucrose floatation medium of 1.21 specific gravity and then sieved through a guaze placed on a funnel in a test-tube. Each test-tube was topped to the brim with the floatation medium to form a convex meniscus. A cover slip was placed on the test-tube for 3 minutes and this was removed and placed on a clean glass slide and viewed under the microscope using x10 and x40 objective lens for the presence of *Cryptosporidium* Oocysts and other parasite eggs.

Laboratory Detection of *Cryptosporidium* Oocysts

Each sample was treated to the protocol described by Ortega et al [16]. One hundred grams of both the soil and vegetables samples were weighed and washed with distilled water by manual agitation for 10 minutes. The resulting washings were concentrated by centrifugation at 1500 rpm for five minutes.

Using modified Acid-fast Ziehl-Neelsen staining technique as described by Casemore [23], a thin smear of the sediment was made on a clean microscope glass slide. The slide was air-dried, fixed into methanol for 2-3 minutes. The slide was flooded with cold carbol-fuschsin for 15 minutes. The slide was then rinsed thoroughly in tap water and decolourised in 1% HCl in methanol for 10 to 15 minutes. Slide was again washed with tap water, counter stained with 0.25% Methylene Blue for 30 seconds, then rinsed with tap water to remove the excess stain and air-dried. The slides were then examined under the microscope using x10 and x40 objective lens.

Detection of *Cryptosporidium* Parvum

The slides were viewed for *Cryptosporidium* oocysts using a light microscope viewed at magnification of x10 and x40 with the aid of oil immersion objective lens. Positive slides used were photomicrographs of oocysts of *Cryptosporidium parvum*

Statistical Analysis

Data obtained were analyzed using Statistical package for social sciences (SPSS) version 17. Chi-square and correlation analysis were calculated to check for association between the occurrences of *Cryptosporidium* on the samples.

RESULTS AND DISCUSSION

Oocysts were confirmed under a higher power (x100). Oocysts appeared as red or pink coloured round/oval bodies against a pale green background. An overall occurrence of 31% *Cryptosporidium* oocysts parasites on irrigated vegetables was established in this study as shows in Table 1. Spinach had the highest occurrence (55%), followed by cabbage and lettuce (30%) each while carrot and tomato had the lowest occurrence of 25% and 15% each. The difference (Appendix 1b) in all the irrigated vegetable samples was found to be statistically significant ($p < 0.05$), spinach being significantly higher than other varieties of irrigated vegetables sampled.

Table 1: Percentage occurrence of *Cryptosporidium* Oocysts on Irrigated Vegetables from ABU Irrigated Farms

Vegetable Samples	No of Samples	No(+ve)	(%) Occurrence
Tomatoes	20	3	15%
Spinach	20	11	55%
Lettuce	20	6	30%
Cabbage	20	6	30%
Carrot	20	5	25%
Total	100	31	31%

No (+ve) is number of positive sample while (%) is percentage occurrence of *cryptosporidium* oocysts

Out of a total of 100 vegetables examined, 44(4.9%) were positive for parasite. Out of the 5 varieties as shown in Table 2, all were found to be positive for parasite egg/cyst. Contamination was highest in carrot (7.1%), followed by spinach (5.7%), cabbage (5.0%). In the study 9 species of parasites were discovered as shown in Table 2. Regarding the frequency of parasite encountered, *Cryptosporidium* had the most occurrences (30.0%), followed by *Cyclospora* spp, Nematode larvae (4.0%), Hookworm (3.0%), *Ascaris* spp (2.0%). *Balantidium coli*, *E.hyistolytica*, and *Strongytodies* spp had the same percentage occurrence (1.0%) and they were the least identified. Though the parasitic load showed no significant difference, only Nematode larvae showed significant difference ($P<0.05$)

Table 2: Parasites recovered in irrigated vegetable in respect to vegetables (number positive/percentage)

Vegetable soil	Tomato	Carrot	Spinach	Cabbage	Lecttus	Total	χ^2	p-value
No of sample	20	11	29	20	20	100		
Parasites			No Positive (%)					
Hookwarm	0(0.0)	0(0.0)	2(6.9)	0(0.0)	1(5.0)	3(3.0)	3.365^a	0.499
Strongytodies spp	0(0.0)	0(0.0)	1(3.4)	0(0.0)	0(0.0)	1(1.0)	2.473^b	0.649
Nematode larvae	2(10.0)	2(18.2)	0(0.0)	0(0.0)	0(0.0)	4(4.0)	10.511^c	**0.033**
Ascaris spp	0(0.0)	0(0.0)	0(0.0)	1(5.0)	1(5.0)	2(2.0)	3.061^d	0.548
Mite	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	1(1.0)	4.040^b	0.401
B.Coli	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	1(1.0)	4.040^b	0.401

E. hyistolytica	0(0.0)	0(0.0)	0(0.0)	1(5.0)	0(0.0)	1(1.0)	4.040[b]	0.401
C.parvum	3(15.0)	5(45.0)	11(37.9)	5(25.0)	6(30.0)	30(30.0)	4.501[e]	0.342
Cyclospora	0(0.0)	0(0.0)	1(3.4)	0(0.0)	0(0.0)	1(1.0)	2.473[b]	0.649
Parasitic load	5(2.7)	7(7.1)	15(5.7)	9(5.0)	8(4.4)	44(4.9)	3.233[f]	0.520

P- Value in the same column are not significantly different (P<0.05). *=P<0.05

Figure 1 shows the distribution of *Cryptosporidium* oocysts among the five different irrigated vegetable sampled. *Cryptosporidium* oocysts were found to be highly distributed in spinach followed by tomato with the least distribution.

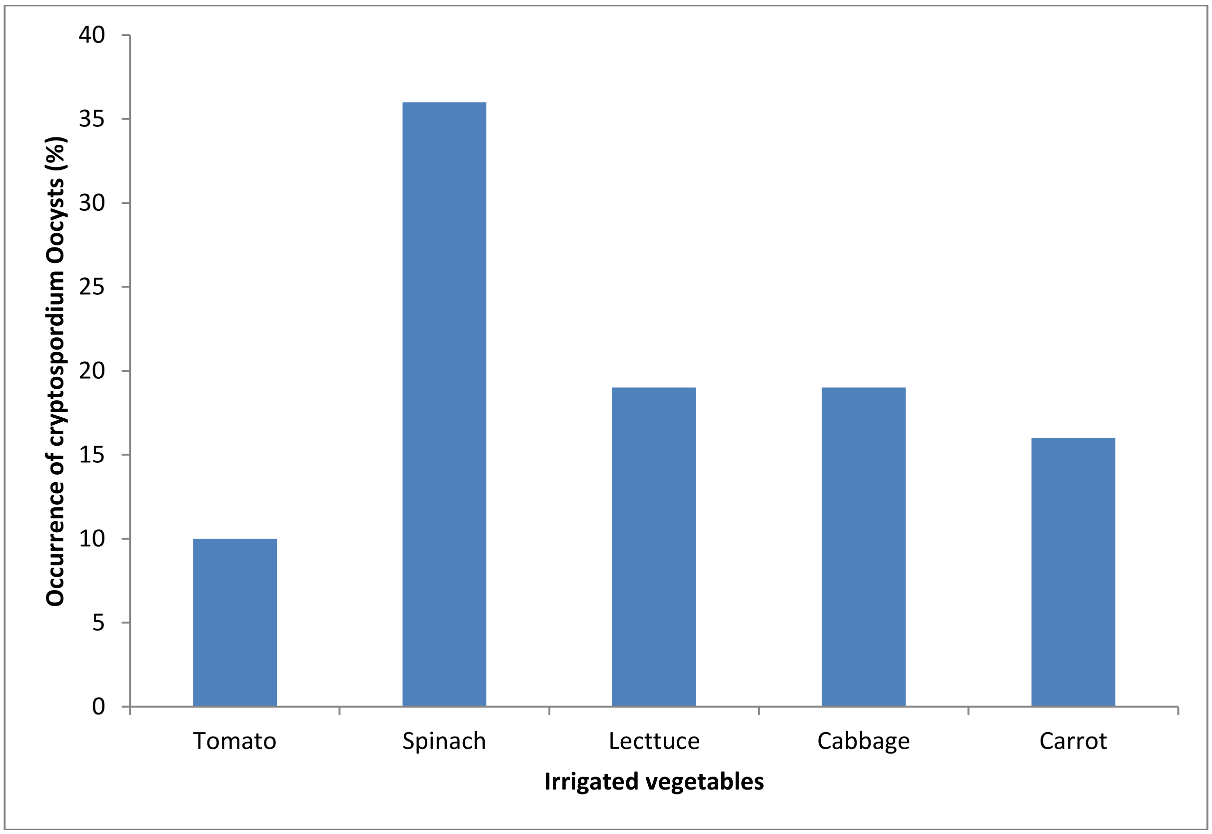

FIGURE 1: Distribution of *Cryptosporidium* oocysts on irrigated vegetable samples from ABU irrigated farm

In this study, 100 vegetable samples including carrots, spinach, lettuce, cabbage and tomato from ABU wastewater irrigated farms were examined. *Cryptosporidium* was found present on samples across the five vegetables of the study.

Reports on the occurrence of *Cryptosporidium* oocysts on wastewater irrigated vegetables in literature are few. In Nigeria, the frequency of occurrence of *Cryptosporidium* oocysts as shown in this study is higher than the 14.5% obtained by Ortega *et al* [16] on the vegetable markets of the peri-urban slums of Peru. The differences may be due to the unhygienic practices in the markets, level of health education of the marketers, on-the market product presentation and handling of the vegetables. The presence of Cryptosporidium oocysts on the vegetables, though relatively high as compared to the work of Ortega [16] is of great public health concern since this vegetables can be eaten raw or under-cooked. This work therefore demonstrates the presence of *Cryptosporidium* oocysts contaminating vegetables presented for sale in Kaduna metropolitan markets. Fresh vegetables can be agents for the transmission of protozoan oocysts [24]. Outbreaks of intestinal parasitic infections epidemiologically associated with raw vegetables have been reported from developed and developing countries [16].

The frequency of detection of *Cryptosporidium* oocysts on spinach, cabbage and lettuce was higher than in tomato and carrots. Spinach had the highest frequency followed by lettuce and cabbage. The overlapping nature of the leaves of cabbage that sieves contaminated water and the large contact area with the soil surface of the broad leaves of cabbage and lettuce [25] may in part explain their high level of contamination with the oocysts. Carrot had a moderate frequency of detection of *Cryptosporidium* parasites. As a root crop it is continuously exposed to soil contaminants [25]. This finding agrees with those of Robertson and Gjerde [17].

Generally these vegetables had high level of parasitic contamination from the wastewater irrigated farm during the dry season. The distribution rates of *Cryptosporidium* oocysts on spinach, cabbage and lettuce were the highest levels of contamination of the vegetables examined. Spinach obtained from the wastewater irrigated farm appeared most contaminated. Poor hygiene practices of irrigation farm from wastewater treatment plant may have exposed the vegetables to direct contact with the soil and its composite contaminants. Fertilization of these crops with animal wastes may also have contributed to their high contamination.

Parasite-wise, this study has shown *Cryptosporidium parvum* and Nematode larvae as having greater likelihood of contaminating lettuce, tomato, cabbage, carrot and spinach that are irrigated with wastewater around the dry season. This may be due to the poor hygienic practices of wastewater treatment and poor irrigation channel down to the farm point. The sources of contamination of vegetables generally include, use of untreated wastewater and water supplies contaminated with sewage for irrigation and post-harvest handling. Other factors include unhygienic conditions of preparation in food service or home and market settings due to pest infestations such as cockroaches, houseflies, mice and rats [26]. Food is generally contaminated during production, collection, transport and preparation or processing. The sources of zoonotic contamination are usually faeces and water [27].

Contaminated vegetable has long been proposed as a possible route for transmission of infectious organisms particularly *Cryptosporidium* and *Cyclospora* [28]. These parasites are considered as pathogenic agents for man and the consumption or manipulation of such contaminated vegetables is considered unsafe and might constitute a risk to the consumers [29]. Vegetables are often consumed raw or uncooked and are easily contaminated and thus provide organisms with an optimal environment for survival prior to host ingestion. The sources of zoonotic contamination are usually faeces and water [27].

CONCLUSION

This study has established the presence of *Cryptosporidium* oocysts on various vegetables in the study area. The levels of contamination with *Cryptosporidium* oocysts depend on the type of vegetable and the source of water use for irrigation. The highest detection rate of *Cryptosporidium* oocysts was on spinach while moderate contamination was detected on cabbage, lettuce and carrot from the ABU irrigated site. Tomato was the least contaminated. Thus the level of contamination of irrigated vegetables from ABU wastewater irrigated vegetable site in Samaru Zaria, Kaduna state, Nigeria is of great public health importance

The presence of *Cryptosporidium* oocysts on vegetables as contaminants means there is public exposure to the parasites through the consumption of raw vegetables. The study therefore established raw vegetables as a factor in the spread of cryptosporidiosis in Zaria state and possibly, Nigeria.

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