Food Control 31 (2013) 45–48

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Contamination of raw vegetables with *Cryptosporidium* oocysts in markets within Zaria metropolis, Kaduna State, Nigeria

B.V. Maikai*, E.B.T. Baba-Onoja, I.A. Elisha

Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru, Sokoto Road, Zaria, Nigeria

A R T I C L E I N F O

Article history:
Received 20 January 2012
Received in revised form
17 September 2012
Accepted 25 September 2012

Keywords:
Vegetables
Cryptosporidium
Nigeria

A B S T R A C T

Fresh vegetables are an important part of a healthy diet and can be agent of transmission of intestinal parasites. A survey was carried out using 200 fresh vegetable samples in Zaria metropolis, Kaduna State for detection of *Cryptosporidium* oocysts in vegetables using sucrose flotation medium of 1.21 specific gravity and modified Ziehl Neelsen staining technique. Seventy (35%) out of the 200 vegetables examined were positive for *Cryptosporidium* oocysts. Lettuce had the highest (48%) contamination rate followed by Fluted pumpkin (44%), spinach and Jute mallow (40%) each, waterleaf (36%), tomatoes (32%), carrot (24%) and cabbage (16%). Sabo market had the highest contamination rate of 21 (37.5%) followed by Samaru 22 (34.4%) and Community market 27 (33.8%). Contamination was highest in leafy vegetables (37.3%), followed by fruit vegetables (32%) and root vegetables (24%). Thirty (30%) out of 100 vegetables consumed raw and 40 (40%) out of those cooked before consumption were positive for *Cryptosporidium* oocysts. There were no significant associations between occurrence of *Cryptosporidium* oocysts and the types of vegetables sold ($\chi^2 = 8.70$; $p = 0.2747$), markets in which the vegetables were sold ($\chi^2 = 0.22$; $p = 0.8959$), nature of vegetables ($\chi^2 = 1.79$; $p = 0.4091$) and way in which the vegetables are consumed (odds ratio (OR) 0.64; 95% confidence interval (CI) on OR: $0.34 < OR < 1.20$). This study has shown that vegetables sold in markets within Zaria metropolis, Kaduna State, Nigeria, are contaminated with *Cryptosporidium* oocysts. This is of public health significance.

1. Introduction

Cryptosporidiosis has been incriminated as the most frequent microbial cause of diarrhoea accompanied by pain and abdominal colic and a noticeable loss of weight. Cattle have been seen to be an important source of zoonotic cryptosporidiosis since 1980's (Guerrant, 1997). Cryptosporidiosis is typically an acute short-term infection but can become severe and non-resolving in children and immune-compromised individuals. In humans, it remains in the lower intestine and may remain for up to five weeks (Carreno, Martin, & Banta, 1999).

Cryptosporidium infection is transmitted by the faecal–oral route and results from the ingestion of *Cryptosporidium* oocysts through faecally contaminated water or food or through direct person-to-person or animal-to-person contact (Yoder & Beach, 2007).

Cryptosporidiosis is a major public health problem in both developing and developed countries. In developing countries, the disease results in stunted growth (Checkley et al., 1997, 1998; Molbak et al., 1997). *Cryptosporidium* has also become a well-known cause of opportunistic infections among AIDS patients and of gastrointestinal disease outbreaks (Adal, Sterling, & Guerrant, 1995). Foodborne outbreaks of cryptosporidiosis have been reported since 1980s and have been identified from several commodities, mostly fruits, vegetables, shellfish (McEvoy, Moriaty, Duffy, & Sheridan, 2003). These products are usually eaten raw and there is a concern that food may be a vehicle of transmission of *Cryptosporidium* (McEvoy et al., 2003). Evidence suggests that fresh produce consumption is a risk factor, at least in outbreak situations. However, others have observed that consumption of fresh produce may reduce the risk for cryptosporidiosis. Several outbreaks studied in the United Kingdom also revealed a protective association with the regular consumption of raw vegetables (Casemore, Wright, & Coop, 1997). *Cryptosporidium* has been isolated in foods such as chicken salad, apple cider, green onions, raw milk, Lettuce (Robertson & Gjerde, 2001), Mung bean Sprout (Robertson, Greig, Gjerde, & Fazil, 2005), Carrot, Tomatoes and Cucumber (Monge & Arias, 1996).

Nigeria is said to be at the meso-endemic level of the parasitic pathogens in children presenting with diarrhoea (Belding, 1999, pp. 62–80). The poor hygienic condition of the environment which is due to defaecation by both humans and animals can contaminate

* Corresponding author. Tel.: +234 802 847 2921.
E-mail address: Beatt18@yahoo.com (B.V. Maikai).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2012.09.032

water sources. When such contaminated water is used to irrigate vegetable farms, the produce can become contaminated as well (McEvoy et al., 2003). Nigeria is also an agrarian society and livestock are raised predominantly on free range which likely results in the contamination of pasture and food crops like vegetables. Zaria has some ready to eat leafy vegetables on sale at Sabo market, Samaru and Community market and the determination of *Cryptosporidium* oocysts in such vegetables is important in order to ensure the safety of the general public from foodborne diseases and this will go a long way in enlightening the public on the need to properly wash or cook vegetables before consumption.

2. Materials and methods

2.1. Study area

The research study was carried out in Zaria metropolis, Kaduna State, Nigeria. It has a total land space of 300 km^2 and population of 408,198 people (2006 census) (Federal Republic of Nigeria Official Gazette, 2007, p. 187). It is typically a Hausa settlement mixed with other tribes and is located between latitude 10°11′ N and Longitude 70°88′ E in the Northern Guinea savannah. About 80 percent of the Kaduna state's population is engaged in farming producing both food and cash crops. Retail outlets within Ahmadu Bello University Community market, Samaru and Sabon-gari markets were randomly selected for the study.

2.2. Collection of vegetable samples and parasitologic analysis

Two hundred samples of the following vegetables were used during the study:

Common name	Botanical name	Nature of vegetable
Spinach	*Spinacia oleracea*	Leafy
Fluted pumpkin (Ugwu)	*Telfairia occidentalis*	Leafy
Jute mallow (Ayoyo)	*Corchorus olitorius*	Leafy
Tomato	*Lycopersicon esculentum*	Fruit
Lettuce	*Lactuca sativa*	Leafy
Carrot	*Daucus carota var sativa*	Root
Waterleaf	*Talinum triangulare*	Leafy
Cabbage	*Brassica oleracea* var capitata	Leafy

Prior to sample collection, the traders were interviewed on sources of vegetables, source of water for washing vegetables and if the vegetables were gotten fresh from the farms. The samples were collected into clean polythene bags during rainy months May to August, 2011. They were transported to Public Health laboratory of Ahmadu Bello University, Zaria. A modification of the method used by Abougrain, Nahaisi, Madi, Saied, and Ghenghesh (2010) was used as follows: Two hundred and fifty gram of each sample collected was weighed into plastic bags and washed with 250 ml physiological saline solution (0.95% NaCl) and the wash was left for 10 h for sedimentation to take place. The supernatant was discarded and the residue was transferred into a centrifuge tube and spun for 5 min at 1500 rpm. The supernatant was decanted and the residue was agitated gently in sucrose flotation medium of 1.21 specific gravity. Each test tube was then topped to the brim with the flotation medium to form a meniscus. A cover slip was placed on the test tubes for about 3 min and this was removed and placed on a clean glass slide and viewed under the microscope for the presence of parasitic oocysts. Oocysts-positive slides were allowed to air dry and stained using modified Ziehl–Neelsen technique (WHO, 1991, pp. 16–18) by fixing the air-dried slides in methanol for 2–3 min. The slide was flooded with cold carbol fuschin for 5–10 min and then with 1% hydrochloric-acid ethanol until colour ceased to flow out and rinsed in tap water. It was then counterstained with 0.25% methylene blue for 30 s, rinsed in tap water again and air-dried.

2.3. Identification of Cryptosporidium oocysts

The slide was examined using a light microscope at ×40 magnification with oil immersion. The cryptosporidial oocysts appeared as bright rose pink spherules on a pale green background. Positives slides used were photomicrographs of oocysts of *Cryptosporidium parvum* from Department of Parasitic Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, USA (www.dpd.cdc.gov/dpdx/html/imagelibrary/a-f/cryptosporidiosis/body_Cryptosporidiosis_il.htm).

2.4. Data analysis

With the use of EPI INFO 2002 (Dean, 1994), Chi square test was used to test for association between *Cryptosporidium* oocysts and factors such as type, nature, consumption and source of vegetables. OR values greater than unity denote association and less than unity denote that the factor may have a protective effect. $p \leq 0.05$ was defined as significant. Prevalence was estimated by dividing the number of positive samples by the total number of samples.

3. Results

Of the 200 vegetables examined, 70 (35%) were positive for *Cryptosporidium* oocysts. Eight varieties of vegetables were examined and all were positive for *Cryptosporidium* oocysts. Lettuce had the highest (48%) contamination rate followed by Fluted pumpkin (44%), spinach and Jute mallow (40%) each, waterleaf (36%), tomatoes (32%), carrot (24%) and cabbage (16%). There was no statistical significance ($\chi^2 = 8.70$; $p = 0.2747$) between occurrence of *Cryptosporidium* oocysts and types of vegetables examined (Table 1).

Table 2 shows the occurrence of *Cryptosporidium* oocysts in the different markets. Twenty one (37.5%) of the vegetables examined from Sabo market was positive for *Cryptosporidium* oocysts followed by Samaru 22 (34.4%) and Community market 27 (33.8%). There was no statistical significance ($\chi^2 = 0.22$; $p = 0.8959$) between occurrence of *Cryptosporidium* oocysts and the markets in which the vegetables were sold.

Contamination was highest in leafy vegetables (37.3%), followed by fruit vegetables (32%) and root vegetables (24%). There was no statistical significance ($\chi^2 = 1.79$; $p = 0.4091$) between occurrence of *Cryptosporidium* oocysts and the nature of vegetables examined (Table 3).

Thirty (30%) out of the 100 vegetables consumed raw and 40 (40%) out of those cooked before consumption were positive for *Cryptosporidium* oocysts. There was no significant association (odds ratio (OR) 0.64; 95% confidence interval (CI) on OR: $0.34 < \text{OR} < 1.20$) between the occurrence of *Cryptosporidium* oocysts and way in which the vegetables are consumed (Table 4).

Responses from the retailers show that about 30% of the vegetables were gotten straight from farm while 70% were from middle men who got them from farms and sold to retailers in the market before being sold to consumers. Likewise, vegetables were either washed in the farms and when brought to the market water from well and tap are used to constantly sprinkle on them to prevent from drying or they are washed in the market when brought in using well or tap water. About 60% of the water used on the

Table 1
Contamination of raw vegetables with *Cryptosporidium* oocysts in markets within Zaria metropolis, Kaduna State, Nigeria.

Vegetables		No. of vegetables examined	Positive samples		Chi square (χ^2)	Degree of freedom (df)	*p*-Value
Common name	Botanical name		No.	%			
Spinach	*Spinacia oleracea*	25	10	40			
Fluted pumpkin (Ugwu)	*Telfairia occidentalis*	25	11	44			
Jute mallow (Ayoyo)	*Corchorus olitorius*	25	10	40			
Tomato	*Lycopersicon esculentum*	25	8	32	8.70	7	0.2747
Lettuce	*Lactuca sativa*	25	12	48			
Carrot	*Daucus carota var sativa*	25	6	24			
Waterleaf	*Talinum triangulare*	25	9	36			
Cabbage	*Brassica oleracea* var capitata	25	4	16			
Total		200	70	35			

vegetables is from well, 20% from stream when they are washed in the farm, and 10% from tap water. Water is always sprinkled on them so they appear fresh and attract consumers. The water is exposed to unhygienic market environment with no cover on the containers. Containers used in collecting the water are usually dirty and the water is usually re-used for the whole day no matter how dirty it appears. Vegetables from the ground are dipped into the water and same water re-used on all other vegetables either to wash or sprinkle.

4. Discussion

The 35% contamination rate of *Cryptosporidium* oocysts in this study is relatively high compared to the work of others (Monge, Chinchilla, & Reyes, 1996; Ortega et al., 1997). This may be due to the use of dirty water in washing the vegetables because as a routine, traders usually sprinkle vegetables with water to preventing them from drying. Water has been recognised as an important vehicle for the transmission of *Cryptosporidium* (Rose & Smith, 1990). The ubiquitous nature of this protozoan parasite and its potential for waterborne transmission is further facilitated by the perpetual infectivity of the oocysts, their small size (3.5–6.0 μm) and their low sedimentation rate (0.5 μm/s) (Rose & Smith, 1990). Surface water may become contaminated through the entry of human or animal faeces by either a direct or indirect route. Direct contamination of water may be due to the entry of faeces from agricultural run-off from adjacent farm animals or indirectly through accidental contamination from human sewage (Peng et al., 1997). Contact of the vegetables with soil may also play significant role in the contamination of the vegetables with *Cryptosporidium* oocysts as the vegetables were kept in dirty environment and in contact with soil. Also fertilization of horticultural crops, including vegetables, with manure from cattle and sheep, containing viable oocysts of *Cryptosporidium*, represents a significant risk to the contamination of vegetables (Moore et al., 2007).

The high occurrence of *Cryptosporidium* oocysts in lettuce may be probably due to its broad leafy nature which may enhance it trapping higher number of oocysts from contaminated sources. Contamination of lettuce with food poisoning and foodborne pathogens may occur in the fields as a result of the introduction of the organisms from non-potable irrigation water or manure/compost, which still contains viable pathogens (Moore et al., 2007). There has been a number of lettuce associated foodborne pathogens (Moore et al., 2007) and the consumption of raw vegetables plays an important role in the transmission of parasitic contaminations (Anuar & Ramachandran, 1977).

Sabo market, which is a well patronized market and more centrally located has more wholesale and retail outlets. This may mean that there was a higher chance of buying contaminated vegetables from Sabo market, though this claim is not statistically significant. The central location of Sabo market and the increase in number of activities may enhance an easy introduction of infectious agents into vegetables sold in the market. Hence, the higher recovery rate of *Cryptosporidium* oocysts in Sabo than the rest of the markets.

The results in this study showed that contamination was highest in leafy than fruit and root vegetables. Some other studies have also demonstrated a higher contamination rate in leafy vegetables (Uga et al., 2009). The reason for such high rate may be due to the nature of the leaves which may tend to retain more trapped pathogens than fruit or root vegetables.

It is a well known fact that vegetables are either consumed raw or cooked (Moore et al., 2007; Ortega et al., 1997; Uga et al., 2009). Results from this study have also shown the contamination rates of vegetables normally eaten raw or cooked. Spinach (*Spinacia oleracea*), fluted pumpkin (*Telferia occidentalis*), jute mallow (*Conchorus olitoriusi*), and waterleaf (*Talinum triangulare*) are normally eaten cooked while Tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), Carrot (*Daucus carota var sativa*) and Cabbage (*Brassica oleracea* var capitata) are eaten raw. Though there was higher contamination in vegetables normally cooked before consumption, the fact still remains that all vegetables should be properly washed before consumption whether they are eaten raw or cooked to reduce the chances of ingestion of *Cryptosporidium* oocysts.

Table 2
Prevalence of *Cryptosporidium* oocysts on raw vegetables in markets within Zaria metropolis, Kaduna State, Nigeria.

Market	No. of vegetables examined	Positive samples		Chi square (χ^2)	Degree of freedom	*p*-Value
		No.	%			
Samaru	64	22	34.4			
Community	80	27	33.8	0.22	2	0.8959
Sabo	56	21	37.5			
Total	200	70	35.0			

Table 3
Contamination of types of raw vegetables with *Cryptosporidium* oocysts in markets within Zaria metropolis, Kaduna State, Nigeria.

Vegetable	No. of vegetables examined	Positive samples		Chi square (χ^2)	Degree of freedom	*p*-Value
		No.	%			
Leafy	150	56	37.3			
Fruit	25	8	32.0	1.79	2	0.4091
Root	25	6	24.0			
Total	200	70	35.0			

Table 4
Odds ratio (OR) and 95% confidence intervals on the prevalence of *Cryptosporidium* oocysts on raw or cooked vegetables within Zaria metropolis, Kaduna State, Nigeria.

Factor	No. of vegetables examined	Positive samples	Specific rate	Odds ratio (OR)	95% confidence intervals on OR
Consumption					
Raw	100	30	30	0.64	
Cooked[ref]	100	40	40	1.00	0.34–1.20

5. Conclusion

This study has shown that vegetables sold within major areas in Zaria metropolis, Kaduna State are contaminated with *Cryptosporidium* oocysts. The study also showed a higher prevalence of *Cryptosporidium* oocysts on leafy vegetables than fruit or root vegetables. Well populated and more patronized Sabo market was more contaminated with *Cryptosporidium* oocysts than the rest of the markets. Since vegetables are an important part of a healthy diet and can be consumed either raw or cooked, the findings from this research study is of great public health significance.

Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2012.09.032.

References

Abougrain, A. K., Nahaisi, H., Madi, N. S., Saied, M. M., & Ghenghesh, K. S. (2010). Parasitological contamination in salad vegetables in Tripoli-Libya. *Food Control, 21*, 760–762.

Adal, K. A., Sterling, C. R., & Guerrant, R. L. (1995). *Cryptosporidium* and related species. In M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, & R. L. Guerrant (Eds.), *Infections of the gastrointestinal tract* (pp. 1107–1128). New York, N.Y.: Raven Press, Ltd.

Anuar, A. K., & Ramachandran, C. P. (1977). A study on the prevalence of soil transmitted helminths among lettuce leaves sold in local markets in Penang, Malaysia. *Medical Journal of Malaysia, 31*, 262–265.

Belding, L. D. (1999). *Text book of parasitology*. Applet on Croft, New York.

Carreno, R. A., Martin, D. D., & Banta, J. R. (1999). Cryptosporidiosis is more closely related to the gregarines than coccidian as shown by phylogenetic analysis of apicomplexa parasites inferred using small-subunit ribosomal RNA gene sequences. *Parasitology Research, 85*(11), 899–904.

Casemore, D. P., Wright, S. E., & Coop, R. L. (1997). Cryptosporidiosis: human and animal epidemiology. In R. Fayer (Ed.), *Cryptosporidium and cryptosporidiosis* (pp. 65–92). New York, N.Y.: CRC Press, Inc.

Checkley, W., Epstein, L. D., Gilman, R. H., Black, R. E., Cabrera, L., & Sterling, C. R. (1998). Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *American Journal of Epidemiology, 148*, 497–506.

Checkley, W. R. D., Gilman, L. D., Epstein, M., Suarez, J. F., Diaz, L., Cambrera, R. E., et al. (1997). Asymptomatic and symptomatic cryptosporidiosis, their acute effect on weight gain in Peruvian children. *American Journal of Epidemiology, 145*, 156–163.

Dean, A. G. (1994). *A course in micro computer use for epidemiologist and others who count things using EPI INFO*. Atlanta, USA: Center for Disease Control and Prevention, Epidemiology Program Office.

Federal Republic of Nigeria Official Gazette. (2007). *Two thousand and six census figures for Kaduna State*. Lagos, Nigeria: Federal Government Printer.

Guerrant, R. L. (1997). An emerging highly infectious threat to our water supply. *Emerging Infectious Diseases, 3*, 51–57.

McEvoy, J. M., Moriaty, E. M., Duffy, G., & Sheridan, J. J. T. (2003). The National Food Centre, Ashtown, Dubling 15, Ireland.

Molbak, K., Anderson, M., Aaby, P., Hojlyng, N., Jacobsen, M., Sodmann, M., et al. (1997). *Cryptosporidium* infection in infancy as a cause of malnutrition: a community study from Guinea-Bisau, West Africa. *America Journal of Clinical Nutrition, 65*, 149–152.

Monge, R., & Arias, M. L. (1996). Presence of various pathogenic microorganisms in fresh vegetables in Costa Rica. *Archive of Latinoam Nutrition, 46*, 292–294.

Monge, R., Chinchilla, M., & Reyes, L. (1996). Seasonality of parasites and intestinal bacteria in vegetables that are consumed raw in Costa Rica. *Revista de Biologia Tropical, 44*, 369–375.

Moore, J. E., Millar, B. C., Kenny, F., Lowery, C. J., Xiao, L., Rao, J. R., et al. (2007). Detection of *Cryptosporidium parvum* in lettuce. *International Journal of Food Science and Technology, 42*, 385–393.

Ortega, Y. R., Roxas, C. R., Gilman, R. H., Miller, N. J., Cabrera, L., Taquiri, C., et al. (1997). Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *American Journal of Tropical Medicine and Hygiene, 5*, 683–686.

Peng, M. M., Xiao, L., Freeman, A. R., Arrowood, M. J., Escalante, A. A., Weltman, A. C., et al. (1997). Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. *Emerging Infectious Diseases, 3*, 567–573.

Robertson, L. J., & Gjerde, B. (2001). Occurrence of parasites on fruits and vegetables in Norway. *Journal of Food Protection, 64*, 1793–1798.

Robertson, L. J., Greig, J. D., Gjerde, B., & Fazil, A. (2005). The potential for acquiring cryptosporidiosis or giardiosis from consumption of Mung bean sprouts in Norway: a preliminary step-wise risk assessment. *International Journal of Food Microbiology, 98*(3), 291–300.

Rose, J. B., & Smith, H. V. (1990). Waterborne cryptosporidiosis. *Parasitology Today, 6*, 8–12.

Uga, S., Hoa, N. T. V., Noda, S., Moji, K., Cong, L., Aoki, Y. A., et al. (2009). Parasite egg contamination of vegetables from a suburban market in Hanoi, Vietnam. *Nepal Medical College Journal, 11*(2), 75–78.

World Health Organization. (1991). *Basic laboratory methods in medical parasitology*. Geneva: World Health Organization.

Yoder, J. S., & Beach, M. J. (2007). Centers for Disease Control and Prevention (CDC). Cryptosporidiosis surveillance – United States, 2003–2005. *MMWR Surveillance Summit, 56*(7), 1–10.