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Full Length Research Paper

Assessment of the parasite load of lettuce, tomato and cucumber from some large vegetables production sites in Mali

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Fresh vegetables are an important part of a healthy diet. Consumed raw, they could be agents of transmission of intestinal parasites. The frequency and variability of parasites eggs on thirty-two samples each of lettuce, tomato and cucumber form irrigated vegetable farms in Bamako, Kati, Baguineda, Samanko, Sikasso and Niono were evaluated. The parasite load was assessed using Uga et al. method, which consist the counting of parasite eggs and cysts in 100 g of vegetable. The overall prevalence of parasitic contamination of vegetables was 20.83% of which 41.66% for lettuce and 16.66% for tomato. Cucumber was free of parasites eggs. Parasites found on the vegetables were *Entamoeba coli* and *Trichomonas intestinalis* (24.19% each), *Ascaris lumbricoides* (13.25%), *Giardia intestinalis* (12.9%), *Balantidium coli* (11.29%), *Entamoeba histolitica* (7.26%), *Fasciola hepatica* (3.23%), *Trichinella spiralis* (1.61%), *Ancylostoma duodenale* and *Schistosoma intercalatum* (1.04% each). The frequency of parasites on lettuce was 83.33% each in Bamako and Niono; 50% in Kati; 16.66% each in Baguineda and Samanko and 0% in Sikasso. Consumption of vegetables contaminated by parasites can cause a health risk for consumers.

Key words: Parasitic contamination, lettuce, tomato, cucumber, health risk.

INTRODUCTION

Vegetables are an excellent source of vitamins, dietary fiber and minerals. Their consumption reduces the risk of cardiovascular diseases, strokes and cancers (Anand et al., 2015; Aune et al., 2017). Despite the importance and increase of the consumption of vegetables around the world, their production faces challenges, in particular the low availability of cultivable land in the urban and peri-urban areas of cities, the use of untreated irrigation water and unconventional organic manure for the production (Samaké et al., 2011; Uyttendaele et al., 2015; Atidégla et al., 2016; Khan et al., 2018; Iwu and Okoh, 2019; Banach et al., 2020). These agricultural inputs are

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sometimes sources of parasitic contamination on vegetables (Abe et al., 2016; Ghimire et al., 2020). The accumulation of parasites in soil and the water used to irrigate crops, raises concerns about contamination of the food chain. Literature reported the link between foodborne illness and the consumption of fresh vegetables (Olyaei and Hajivandi, 2013; CallejÓn et al., 2015; Alhabbal, 2016). Contamination is linked to the use of untreated wastewater in vegetable production. Among pathogens causing diseases, intestinal parasitic infection is responsible for infecting more than two billion individuals worldwide (Tigabu et al., 2019). And some species of protozoan parasites could cause serious health problems and economic issues (Yusof, 2017).

Campos et al. (2018) have found helminth eggs in agricultural irrigation water (0.1–3 total helminth eggs/L) in Colombia. In addition, Kafle et al. (2014) obtained eggs of *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Giardia* in vegetables soil in Nepal. Also, studies done by various authors demonstrated the contamination of vegetables by many parasites such as *Entamoeba* spp. (*histolytica*, *dispar* and *coli*), *A. lumbricoides*, *Giardia lambliae*, *Trichuris trichura*, *Fasciola* spp., *Echonococcus* spp., Toxocora spp., *S. stercoralis* (Alemu et al., 2020; Idahosa, 2011; Sia et al., 2012; Eraky et al., 2014; Mohamed et al., 2016; Rahmati et al., 2017). The use of contaminated soil has been found to be source of parasitic contamination of vegetables (Kłapeć and Borecka, 2012; Amoah, 2014). Untreated irrigation water has been reported to be responsible for vegetables contamination in West and East Africa (Cobbina et al., 2013; Amoah, 2014; Akinde et al., 2016; Woldetsadik et al., 2017; Amoah et al., 2018; Kayombo and Mayo, 2018). Untreated irrigation water is used in agriculture because of its advantages, as they are rich in nutrients. It increases yield and reduces production costs by saving fertilizers (Valipour and Singh, 2016; Libutti et al., 2018; Arena et al., 2020; Ungureanu et al., 2020).Literature reported that long-term irrigation with wastewater induces changes in chemical and biological properties of soils. This could present a potential risk of contamination of vegetables by biological and chemical agents (Edokpayi et al., 2016; Alengebawy et al., 2021). Transmission of helminths from the digestive tract is linked to the consumption of raw vegetables exposed to wastewater (Adegoke et al., 2018; Amoah et al., 2018). WHO has reported that lettuce eaten raw, irrigated with untreated sewage could cause transmission of parasitic nematode infections (WHO, 2006). No study has been done in Mali to determine the parasitic quality of vegetables.The economy of Mali is not strong to support the construction of irrigation channels as recommended by WHO standard (WHO, 2006) and Government cannot also stop farmers from using the water because the livelihoods of many people depend on it. Since the treatment of untreated water is not a realistic option and banning its use will threaten many livelihoods and urban vegetable supply in Mali. This study therefore aimed at determining the parasitic contaminants of lettuce, tomato and cucumber from some vegetable production sites in Mali, in order to assess the health risk related to their consumption.

MATERIALS AND METHODS

Study sites

The study was done from June to December 2015 in Bamako (12° 39′ 00″ N, 8° 00′ 00″ W), Kati (12° 45′ 00″ N, 8° 04′ 00″ W), Baguineda (12° 37′ 00″ N, 7° 47′ 00″ W), Samanko (12°32'1" N, 8°5'3" W), Sikasso (11° 19′ 00″ N, 5° 40′ 00″ W) and Niono (14° 15′ 09″ N, 5° 59′ 34″ W). Kati, Baguineda and Samanko are peri-urban areas of Bamako, whilst Sikasso is the third region and Niono is part of the fourth region in Mali. These sites were chosen based on their accessibility and the presence of active farmers. The choice of these sites was also due to the fact that they are bulk vegetables production sites where producers use untreated water for irrigation and undecomposed organic manures for their cultivation. After the selection of sites, farmers who agreed to collaborate were selected for this study.

Sampling

Samples of lettuce, tomato and cucumber were taken randomly from farms irrigated with untreated irrigation water in Bamako, Kati, Baguineda, Samanko, Sikasso and Niono. Six samples each of lettuce, tomato and cucumber were taken randomly per site, for all of the sites (except Bamako, where only lettuce samples were taken) selected for the study. Thirty-six heads of lettuce, thirty tomatoes and cucumbers fruits each were taken from different farms. The various samples were carefully labeled, put in a sterile plastic bag, placed in polythene bags and transported on ice to the laboratory where they were analyzed immediately or stored at 4°C within 24 h before analysis. Lettuce leaves were removed from each head and combined to obtain a composite sample. Each sample of tomato and cucumber was carefully cut using sterile scissors and mixed to obtain a composite sample. Parasitic analysis was performed according to the method of Uga et al. (2009). Helminth eggs population on lettuce leaves, tomato and cucumber surface were determined by washing 100 g of each in one liter of sterile physiological saline solution (0.85% NaCl). The washing solution was allowed to settle for 10 h in one liter flask. The suspension was decanted, and the pellet transferred into 15 ml centrifuge tubes and centrifuged at 2164 rpm for 15 mn. The flask was rinsed three times with distilled water, and the solutions transferred into centrifuge tubes. All pellets contained in different tubes were added together into one tube and centrifuged again for fifteen minutes at 2164 rpm. The suspension was decanted and all deposits were added together into one tube and centrifuged again at the same speed and for the same period. Then, much of the supernatant was removed by micropipette by leaving one millilitre of the pellet. The pellet (for each sample) was observed examined in lugol on a slide under the microscope (x100), and the eggs were counted on three pellets and averaged.

RESULTS

The composition of parasites

Lettuce and tomato samples in Bamako, Baguineda, Kati, Samanko and Niono were contaminated by parasites

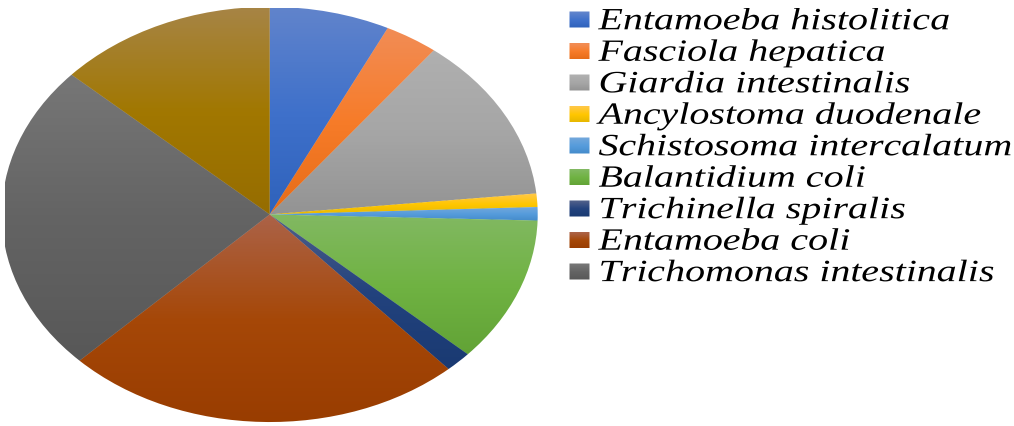

Figure 1. Percentage of parasitic contamination.

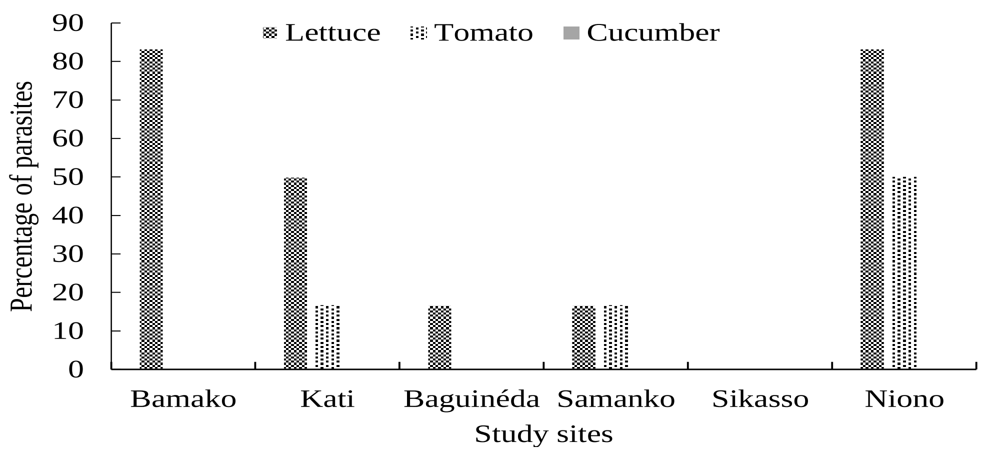

Figure 2. Prevalence of parasitic contamination of lettuce, tomato and cucumber per site.

vegetative form and parasites eggs. Between parasites, protozoan like 30 *Entamoeba coli* and *Trichomonas intestinalis* had the highest rates with 24.19% each. These were followed by 16.43 *A. lumbricoides* (13.25%), 16 *Giardia intestinalis* (12.9%), 14 *Balantidium coli* (11.29%), 9 *Entamoeba histolitica* (7.26%), 4 *Fasciola hepatica* (3.23%), 2 *Trichinella spiralis* (1.61%), 1.29 *Ancylostoma duodenale* and *Schistosoma intercalatum* (1.04% each) (Figure 1). The host of these parasites are humans, so their presence on lettuce and tomato samples could pose a health risk to population. Lettuce, most vulnerable vegetable to parasites was contaminated by 30 *Entamoeba coli*, 16 *G. intestinalis*, 11 *B. coli,* 5 *E. histolitica*, 4 *F. hepatica*, 2 *T. spiralis*, 1.29 *A. duodenale* and *S. intercalatum*. Only 30 *T. intestinalis,* 3 *B. coli* and 4 *E. histolitica* have been found on tomato samples.

Parasite load per site and per vegetable

The parasitic load of lettuce, tomato and cucumber were determined in Bamako, Baguineda, Kati, Samanko and Niono. Cucumber samples were free of parasites at all sites and no parasite eggs were found on vegetables from Sikasso. In total, 20 (20.83%) of all vegetable samples were contaminated by parasites eggs, for which 15 (14.40%) lettuce and 5 (4.80%) tomato samples. All the 6 lettuce samples at Bamako were contaminated by parasites; 3 samples of lettuce and 1 of tomato in Kati; 1 sample each of lettuce and tomato in Samanko; 1 lettuce sample in Baguineda; four samples of lettuce and 3 of tomato in Niono. Among vegetables, lettuce was the most contaminated by parasites eggs with a percentage of 41.66, following by tomato (16.66). No parasite was found on cucumber (Figure 2). Lettuce in Bamako and Niono had the highest percentage of contamination (83.33). High percentage of contamination of tomato (50.00) was obtained in Niono.

Lettuce samples were contaminated with 30 *E. coli* (30 at Niono), *Ascaris lumricoides* (16.43 for which 12 at Bamako and 4.43 at Niono), *Giardia intestinalis* (16 at Baguineda), *B. coli* (11 at Niono), *E. histolitica* (5 at

Bamako), *F. hepatica* (4 for which 2 in Bamako and Kati, each), *T. spiralis* (2 at Bamako), *A. duodenale* (1.29 at Bamako) and *S. intercalatum* (1.29 at Kati). Only *T. intestinalis* (30 at Niono), *B. coli* (3 at Kati) and *E. histolitica* (4 for which 2 at Samanko and 2 at Niono) have been found on tomato samples.

DISCUSSION

In this study, 20.83% of parasitic contamination were obtained amongst all (96) vegetable samples. This is in agreement with the findings of Abougrain et al. (2010) who found 58% of vegetables contamination by helminth eggs and cysts of *Giardia* spp among 126 samples. Their results and ours are similar in term of the presence of parasites, but different regarding the percentage and type of parasites. Their high rate of contamination (58%) compared to us (20.83%) could be due to the fact that they used standard methods, by which the parasites were clearly identifiable.

Lettuce from Bamako and Niono (with the parasitic prevalence of 83.33% of each) were the most contaminated compared to other sites. This high percentage of contamination in Bamako could be due to the fact that vegetable producers use poor quality water and unconventional compost for their production because of the scarcity of cultivable land (Dia, 2017; Traoré, 2020). This can be the basis of several intestinal diseases caused by parasites (Adegoke et al., 2018; Shah et al., 2019).

However, lettuce, tomato and cucumber from Sikasso were free of parasites eggs. Sikasso is an agricultural area par excellence, farmers have enough land and water source. Also, vegetable farmers receive a lot of training in good agricultural practices (composting methods and the use of irrigation water) and irrigation water and soil may not be contaminated compared to other sites. These could be the reason for the absence of parasite eggs in these samples.

In the present study, parasitic analysis of vegetables showed the presence of *E. coli* and *T. intestinalis* (24.19% each), *A. lumbricoides* (13.25%), *G. intestinalis* (12.9%), *B. coli* (11.29%) *E. histolitica* (7.26%), *Fluke* (3.23%), and *Trichina* spp. (1.61%). Helminth eggs of *A. duodenale* and *S. intercalatum* (1.04% each) were relatively low. Of three types of vegetables examined, lettuce is the most parasitic-infected vegetable (41.66%), followed by tomato (16.66%) and cucumber (0%). This confirms work done by Amaechi et al. (2016), who found *A. lumbricoides*, *Entamoeba* spp., *F. hepatica*, *T. trichura* on lettuce and tomato. But, found that 40% of cucumber were contaminated, which is different from this study. This is also in line with the result of (Amoah et al., 2014), who obtained eggs of *A. lumbricoides*, *Hymenolepis diminuta*, *T. trichura*, *F. hepatica* and *Strongyloides* larvae in lettuce samples. This study is however, not consistent with that done by (Chau et al., 2014) who found eggs of *Fasciola* spp. (83.33%), *Ascaris* spp. (85.19%) and *Trichuris* spp. (64.81%), *Clonorchis sinensis* (16.67%); *Cryptosporidium, Isospora* and *Cyclospora* protozoan oocysts in vegetables.

The contamination of vegetables by parasites eggs in other countries have also been reported in Nigeria (Idahosa, 2011), Egypt (Eraky et al., 2014), Ethiopia (Woldetsadik et al., 2017), Ghana (Amoah et al., 2018), Philippines (Sia, 2012), and Sudan (Mohamed et al., 2016). As in the present study, *Ascaris* spp. have been the common parasites identified in vegetables from these countries at different rates. *Ascaris* spp. are soil transmitted helminths and their eggs remain persistent in soils for years (Echazú et al., 2015). This may explain his presence in those vegetables. The reasons for parasitic contamination on vegetables were probably from contaminated fertilizers on soil (Uga et al., 2009; Kłapeć and Borecka, 2012), and wastewater (Keraita et al., 2014). The differences observed in the levels of different pathogenic intestinal parasites of fresh vegetables reported in this work and other studies were expected. Several factors such as the use of untreated supplies of sewage and water, manure as a fertilizer for crop production, various agronomic practices, methods used for detection of intestinal parasites, the type and number of samples examined (Park et al., 2015; Adegoke et al., 2016).

Consumption of vegetables particularly raw plays an important role in the transmission of parasitic contaminations (Odeyemi, 2016; Punsawad et al., 2019) and is important source of foodborne epidemics in developing countries. A high incidence of human intestinal parasites has been found in communities that consume raw vegetables, particularly when vegetables are grown on farmland fertilized with untreated human and animal waste (Ishaku et al., 2013). The contamination took place before harvest, through contaminated manure, sewage sludge, untreated irrigation water or runoff water from livestock farms or directly from animals wild and domestic.

Conclusion

Vegetable crops analyzed in this study were contaminated by parasites (up to 20.83%). Vegetables (41.66% of lettuce and 16.66 of tomato) were contaminated with parasites, and the parasite load was higher in Bamako and Niono with a prevalence of 83.33% of each compared to other sites. Cucumber samples were free of parasites at all sites. Contamination of fresh vegetables produced in Mali by pathogenic intestinal parasites could constitute a risk of public health concern. The Malian government should sensitize and assist farmers to treat irrigation water and organic fertilizers for vegetable production. Sellers should wash their vegetables with clean water (and changed water after each use) before displaying them on the table. Consumers should also sanitize vegetables before consumption, particularly those eaten raw.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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