

PHYILLOPLANE PARASITES ON COMMON EDIBLE PLANTS OF EASTERN

NIGERIA: EASY LINK TO INTESTINAL PARASITES TRANSMISSION.

ABSTRACT:

Aims: In recent years, particularly in developing countries, there has been an unprecedented number of reported cases of parasitic illnesses linked to the high demand for fast foods, majority of which may be contaminated, improperly cooked or washed. This study was carried out to determine the parasitic load on some selected plant leaves, identify the possible parasites which could be found on these leaves, and determine which particular plant leaves harbour more parasites.

Study Design: The study was laboratory-based observational investigation.

Place and Duration of the Study: The research was done in the laboratory of Biological Sciences Department of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria between June 2014 and January 2015.

Methodology: The leaves of *Thaumatococcus daniellii*, *Alchornea cordifolia* and *Musa sapientum* were screened for parasites using standard microscopy methods.

Results: Out of the 30 samples of the plant leaves screened, 22(73.3%) were positive for intestinal parasites. The parasites encountered include some species of protozoa (*Entamoeba coli*), cestode (*Hymenolepis nana*), nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercularis*). *T. daniellii* leaves had the highest parasitic contamination of five species of parasites while *M. sapientum*, and *A. cordifolia* showed the less parasitic contamination of three and two parasites respectively.

Conclusion: From the findings of this study, it is obvious that plant leaves consumed by people are quite often contaminated with intestinal parasites. This is an indication that

humans are at risk of infection especially as some plant leaves are naturally present in diet of people of all classes. Hence, the findings underscore the public health implication of using improperly washed or undercooked leaves of these plants with vegetable farmers, sellers and consumers, being at high risk of infection with strongyloidiasis, ascariasis, amoebiasis and others. The need to soak plant leaves for 10 minutes in saturated salt solution or to steam adequately before using them to prepare or wrap food is recommended. There is also need for government and non-governmental organizations to provide portable water and toilet facilities in order to discourage indiscriminate defecation in the environment.

34

Keywords: Phylloplane, parasites, contamination, *Thaumatococcus daniellii*, *Alchornea cordifolia*, *Musa sapientum*

37

38 INTRODUCTION

In recent years, there has been an increase in the number of reported cases of food borne illness linked to consumption of fresh plant leaves [1,2]. Studies from different parts of the world implicate vegetables as agent for transmission of parasite ova, larvae, oocysts and cysts [3,4]. The consumption of raw plant leaves or their use in wrapping food items plays a major epidemiological role in the transmission of parasitic food-borne diseases [5]. Contamination may occur on the field during growth, harvesting, transporting, processing, distribution or marketing or in the home by food handlers. Once harvested, some of these leaves are not washed before use or are washed with contaminated water which serves as a source of contamination [6]. Intestinal parasites such as *Cryptosporidium* spp., *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, hookworms, *Enterobius vermicularis*, *Trichuris trichiura*, *Toxocara* spp., *Hymenolepis* spp., *Taenia* spp., *Fasciola* spp., and members of the family *Trichostrongylidae* could infect humans as a result of consumption of

51 contaminated, uncooked or improperly washed plant leaves or vegetables. Lack of efficacious
52 sanitizers that can remove or kill pathogens on raw plant leaves has contributed to their
53 ability to gain access to locations within structures and tissues that may harbour these
54 pathogens [7]. Epidemiological studies have also indicated that parasitic diseases are endemic
55 in areas where waste water is used to irrigate plants and vegetables [8]. In areas where
56 helminth infections are prevalent in the population and where waste water is used for
57 irrigation of farms, consumption of raw vegetables can serve as agent of transmission [9]. In
58 rural communities of Nigeria, defecation on open fields is still practiced due to inadequacy of
59 human waste disposal systems and the belief of uninformed residents that such practice help
60 fertilize their farms [10]. In developing countries, due to inadequate and lack of systems for
61 routine diagnosis, monitoring and reporting for many of the food borne pathogens, most
62 outbreaks caused by contaminated plant leaves go undetected and the incidence of this
63 occurrence in food is under-estimated. In recent years, in developing countries, there has been
64 an increase in the number of reported cases of parasitic illnesses linked to a high demand for
65 food wrapped in some plants' leaves. It therefore becomes imperative to investigate the
66 phylloplane parasites assemblage of some of these leaves used in wrapping food in order to
67 contribute to information required for the control of parasitic infections, especially those that
68 make up part of the neglected tropical diseases.

69

70 2. MATERIALS AND METHODS

71 2.1 Study Area

72 The study was carried out in Ebonyi State, South-east Nigeria, with vegetation of the mosaic
73 of the savanna and semi-tropical forest that is dense and remains evergreen throughout the
74 rainy season. Majority of the inhabitants of this area are peasant farmers and petty traders of

low economic status. It is a common practice that majority of the farmers use human and animal faeces as manure to augment and reduce the cost effective commercially processed fertilizer.

2.2 Sample Collection

Leaves of different species of plants were collected in three major zones of Ebonyi State, South-east of Nigeria. The plants screened were *Thaumatococcus daniellii*, *Alchonea cordifolia* and *Musa sapientum*. These plant species were selected for the study due to the extensive use of their leaves for wrapping food stuffs. They were randomly collected in batches of 10 per every location. A total of 30 leaves were investigated. The leaves were identified by the plant taxonomist in the Department of Applied Biology, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria and the voucher specimen are deposited in the Ebonyi State University Herbarium.

2.3 Analysis of the Sample

Thirty leaves of the plant species were examined for intestinal parasite profile as previously described[11]. Samples were washed with distilled water and the suspension was strained through a sterile sieve to remove undesirable materials. The filtrates were centrifuged at 3000 rpm for five minutes and the supernatant discarded while the deposits were placed on clean glass slides and covered with cover slips. This was followed by examination under a light microscope using 10x and 40x objectives fixed with digital camera to identify the cysts and eggs of these organisms as previously described[12]. Different parasite eggs, larvae or cysts present in the samples were counted and results documented as previously described [3].

Statistical Analysis

Data collected were analyzed in simple percentage prevalence.

3. RESULTS

Out of the 30 samples of the three plant leaves analyzed, 22 (73.3%) were positive for intestinal parasites. The incidental parasites are some species of protozoa (*Entamoeba coli*), cestode (*Hymenolepis nana*) and nematodes (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Strongyloides stercularis*).

Among the different locations surveyed, nine (90%) contamination rate occurred in plants from Izzi, 7 (70%) in those from Ezza North while the least 6 (60%) occurred in plants collected from Ebonyi (Table1). *Thaumatococcus daniellii* had highest parasitic contamination with five species of parasites followed by *Alchonea cordifolia* which had three species, while *Musa sapientum* showed the least parasitic contamination with two parasite species (Table 2). The rates of contamination of the three plants were 90%, 60% and 70% respectively (Table 2).

Table 1. The intensity of parasitic contamination of plants at different locations.

Area of collection (LGA)	Number vegetables screened	of Number contaminated	Percentage contamination
Ebonyi	10	6	60.0%
Ezza North	10	7	70.0%
Izzi	10	9	90.0%
Total	30	22	73.3%

Table 2. The contamination and infection rate of different plants' leaves

Parasites	TH	MS	AL
-----------	----	----	----

1	Hookworm	-	+	+
2	<i>Ascaris lumbricoides</i>	+	-	+
3	<i>Strongyloides stercoralis</i>	+	+	+
4	<i>Trichuris trichiura</i>	+	-	-
5	<i>Hymenolepis nana</i>	+	-	-
6	<i>Entamoeba coli</i>	+	-	-

Types of plant leaves	NE	NP	PS (%)
<i>Thaumatococcus daniellii</i>	10	9	90
<i>Musa sapientum</i>	10	6	60
<i>Alchornea cordifolia</i>	10	7	70
Total	30	22	73.3

115 TH=*Thaumatococcus daniellii*, MS= *Musa sapientum*, AL= *Alchornea cordifolia*, NE =
 116 Number examined, NP = Number positive, PS = positive specificity
 117 + = Presence of a parasite, - = Absence of a parasite.

118 4 Discussion

119 The presence of intestinal parasites in plant leaves sampled is suggestive of faecal
 120 contamination of those leaves within their habitats. Routine diagnosis has implicated plant
 121 leaves as source of transmission of parasites. This result is in conformity with other
 122 previously reported works[13, 14 15, 16, 17,10] which noted that factors such as hygiene of
 123 consumers and farmers, plant leaf anatomy cum nature of infective forms of the parasites
 124 have contributed to the prevalence of parasitic infection. Other factors such as the
 125 behavioural attitude of farmers in application of untreated human and animal dung as

manure, and the use of irrigation source which receives raw affluent from human or animal wastes have also been implicated. These are also in tandem with previous reports work [18]. High levels of environmental sanitation, proper sewage disposal and personal hygiene tend to be more observed in Ebonyi Local Government area due to its proximity to the capital city of Ebonyi State compared to Ezza North and Ezzi that are far away. The prevalence of parasites according to different plant leaves showed that *T. daniellii* had the highest number of contamination of parasite of different species. This is due to the fact that *T. daniellii* are majorly found in humid areas where most of the contaminated faecal materials are dumped through erosion current [19]. It might also be due to its popular use as sweetener since its aril contains a non-toxic, intensely sweet protein, thaumatin, which is at least 3000 times as sweet as sucrose [20]. The low prevalence of parasites on banana leaves might be due to the reason that plantation sites are not majorly found in water logged areas.

5 Conclusion

Plant leaves consumed by people or used for wrapping foods are quite often contaminated with intestinal parasites. *Thaumatococcus daniellii* which is usually used in wrapping uncooked foods had the highest rate of contamination. This is an indication that humans are always at risk of infection especially as some plant leaves are naturally present in diet of people of all classes. These findings underscore the public health implication of vegetable farmers, sellers and consumers, being at high risk of infection with strongyloidiasis, ascariasis, amoebiasis and others.

Recommendations

Plant leaves cannot be removed from human diet but can be excluded from the cycle of transmission and dispersion of parasites. This can be achieved by maintenance of simple personal and environmental hygiene by sellers and consumers. There is need to avoid the use of untreated human and animal wastes as fertilizer. There is also need to soak plant leaves for

10 minutes in vinegar or saturated salt solution for plasmolysis of parasites. Steaming leaves adequately before using them to prepare food (such as African salads) or wrapping of food should be practised. There is need to avoid indiscriminate defecation in the environment through provision of adequate sewage disposal facilities. The campaign to eradicate parasitic infections must also be intensified.

REFERENCES

- 1.Said D. Detection of parasites in commonly consumed raw vegetables. *Alerandria Journal of Medicine*. 2012; 48(4): 345 - 352.
- 2.Oyaei A, Hajivand L. Parasitological contamination of markets and farms in vegetable consumed in Southern Iran. *Global Veterinaria*. 2013; 10(3): 327 - 331.
3. Alade GO, Alade TO, Omobuwajo ORM. Intestinal Parasites Load and Microscopy of the Leaves of *Telfairia occidentalis* Hook. F (*Cucurbitaceae*) in Niger Delta Region of Nigeria. *World Applied Sciences Journal*. 2014; 32(2): 183 - 192.
- 4.Alhabbal TA. The prevalence of parasitic contamination on common sold vegetables in Alqualamoun Region. *International Journal of Pharmaceutical Science Review and Research*. 2015; 30(1): 94 - 97.
5. Al-megrm WAI. Prevalence of intestinal parasite in leafy vegetables in Riyadh, Saudi Arabia. *International Journal of Zoological Research*. 2010; 6: 190 - 195.
6. Sunil B, Thomas RD, Latha C, Shameem H. Assessment of parasitic contamination of raw vegetables in Mannuthy kerala State, India. *Veterinary World*. 2014; 7: 253 - -256.
7. Beuchat LR. Ecological factors influencing survival and growth of human pathogen on raw fruits and vegetables. *Microbes and Infection*. 2002; 4 413 - 423.

- 174 8. Damen JG, Banwat GB, Egah O, Allanona JA. Parasitic contamination of vegetables in
175 Jos, Nigeria. *Journal of Africa Medicine*. 2007; 6(3): 115 - 118.
- 176 9. World Health Organization. Parasite related diarrhoeas. Scientific Working Group Series,
177 number 500. World Health organization Geneva.
- 178 10. Ani OC, Urom EN. Parasitic contamination of salad vegetables sold in Abakaliki, Ebonyi
179 State, Nigeria. *Animal Research International*. 2015; 12(2): 2212 - 2217.
- 180 11 Nyarango RM, Aloo PA, Kabiru EW, Nyanchongi BO. The risk of pathogenic intestinal
181 parasite infections in Kisii Municipality, Kenya. *BMC Public Health*. 2008; 8: 237
- 182 12. Suzuki N. *Colour Atlas for Human Helminth Eggs*. APS & JOICFP, Hoken Kaikan
183 Group, Tokyo. 1981; 44 - 56.
- 184 13. Rajeswari B, Sinnaih B, Hussein H. Socioeconomic factors associated with intestinal
185 parasites among children living in Gombak Malaysia. *Asia Pacific Journal of Public*
186 *Health*. 1994; 7(1): 21- 25.
- 187 14. Meremikwu MM, Antia-Obong OE, Asindi AA, Ejezie GC. Prevalence and intensity of
188 intestinal helminthiasis in pre-school children of peasant farmers in Calabar, Nigeria.
189 *Nigeria Journal of Medical Laboratory Science*. 1995; 2: 40 - 44.
- 190 15. Al-Agha R, Teodorescu I. Intestinal parasites infections and anaemia in primary schools
191 children in G29 Gonerorates Palestine. *Roumanian Archives of Microbiology and*
192 *Immunology*. 2000; 59(1-2): 131- 14.
- 193 16. Adamu H, Endeshaw T, Teka T, Kife A, Petros B. Prevalence of intestinal parasites.
194 *Ethiopian Journal of Health Development*. 2006; 20(1): 39 - 47.

- 195 17. Noor-Azian MY, San YM, Gan CC, Yusri MY, NurulsyamzawatyY, Zuhaizam AH,
196 Maslawaty MN, Norparina I, Vythilingam I. Prevalence of intestinal protozoa in an
197 aborigine community in Pahang, Malaysia. *Tropical Biomedicine*. 2007; 24: 55 - 62.
- 198 18. Hedberg CW, Heyneman D, McDonald KL, Osterhaim MT. Changing epidemiology of
199 food borne disease. *International Journal of Parasitology*. 1994; 18: 167- 682.
- 200 19. Cheesbrough M. Parasitological test in: *District Laboratory Practice in Tropical*
201 *Countries part 1*. (first edition). Tropical Health Technology. 1998; 184-201.
- 202 20. Stivala A, Wybrow M, Wirth A, Whisstock JC, Stuckey PJ. Automatic generation of
203 protein structure cartoons with Pro-origami. *Bioinformatics*. 2011; 27 (23): 3315 -
204 3316.