



## STUDY OF PARASITES OF HUMAN IMPORTANCE FROM SALADS IN EATERIES IN A METROPOLITAN CITY

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### ABSTRACT

Salads are consumed in Indian and other countries there are some conventional methods to detect the parasites, and their culture method are also there. Although there are many different methods which are used to detect parasite in salads, newer molecular methods and culture method are most useful. Study of parasites in salads is very important from public health viewpoint because many diseases can spread due to eating contaminated salads. In this study we will assess the load of parasites in common ready to eat salads.

**KEYWORDS:** Public health, parasites, salads.

### INTRODUCTION

Salads are popular in Indian cuisine. They are easy to prepare and have a high nutritive value. They are of many different types like green salads, Russian salads, chicken salad and Caesar salads. However they may be contaminated with parasites, which may lead to diarrhea and other ailments.

The word 'salad' is derived from the Old French word 'salade', which comes from the old Provencal word 'Salada' which in turn, originates from the Latin word 'sal' meaning 'salt'. It is a dish of mixed ingredients, usually vegetables, that can be served cold or warm. Salads can include raw or cooked vegetable, fruit, nuts, cheese, meat, eggs or grains. They are often served with a dressing or small pieces of food.

#### Nutritive value of salad

- I. Salad contain many vitamins, minerals and are low in calories and Sodium.
- II. Vitamins C – Salads are rich in Vitamin C. It helps maintain or support the immune system, keep gums strong and is a strong antioxidant.
- III. Vitamin K - Vitamin K., found in salads is very crucial for blood clotting.
- IV. Fiber - Helps support healthy bowel movements and keeps one feeling satiated.

V. Potassium - Potassium modulates electrolyte balance and blood pressure via its effects on distal cell voltage and chloride.

VI. Folate helps in cell growth and function, protein synthesis, prevents birth defects in early pregnancy.

VII. Manganese - A mineral that our body needs to stay healthy.

#### Bacterial and parasite infection spreading from salads

With a surge in the supply and consumption of salad in developing countries, there has been an increasing threat of microbial contamination. Street-vended food remains the top-most source of foodborne illness due to high prevalence of different microorganisms. For instance, purchased and examined vegetables from the streets of Kolkata harbored a significant level of *Bacillus cereus*, which may cause foodborne illnesses. Interestingly, around 1990, about 12% of food-related diseases were specifically attributed to fruit and vegetable consumption. Recently foodborne outbreaks in Ghana were attributed to the presence of *Vibrio spp.*, *Salmonella spp.*, and other microbes in vegetables and salads. In the subsequent year (2019), diarrheal diseases were the second top cause of death in low-income countries.

Parasites in salads are important but neglected health risk-wise; salads are often consumed raw. Several types

of parasites can be found in them, originating from contaminated water, soil, or improper handling. These parasites can cause a number of gastrointestinal ailments and, in some cases, severe infections if not adequately controlled.

### COMMON PARASITES IN SALADS

#### Protozoa

*Giardia intestinalis* and *Entamoeba histolytica* are commonest protozoa that contaminate leafy greens through contaminated water or human faeces. *Giardia* infection leads to symptoms such as diarrhea, cramps, and fatigue. *Entamoeba histolytica* can cause amebiasis, leading to symptoms like stomach pain, diarrhea, and in severe cases, liver abscesses.

#### Nematodes (Roundworms)

Various species of roundworms can also be found in raw vegetables. *Ascaris lumbricoides*, the giant roundworm, can contaminate soil and is often transmitted to salads that are not properly washed. These parasites can cause ascariasis, with symptoms like mild abdominal discomfort to severe organ damage.

#### Trematodes (Flukes)

*Fasciola hepatica*, commonly known as liver fluke, is of concern in contaminated salad greens. These parasites are found in freshwater plants and can cause fascioliasis, a disease affecting the liver, bile ducts, and gall bladder, resulting in pain, jaundice, and digestive issues.

#### Helminths (Tapeworms)

Though uncommon, tapeworms, like *Taenia solium*, can sometimes contaminate salads through improper handling or exposure to infected animal waste. Tapeworm infections manifest as abdominal pain, nausea, and weight loss.

The primary cause of parasitic contamination in salads is improper agricultural practice. Contaminated water used for irrigation is a very important risk factor, as many parasites thrive well in water. Poor hygiene during harvesting, handling, and storage, and using untreated manure as fertilizer introduces parasitic worms into salad greens.

#### Risks to Human Health

Ingesting raw or improperly washed vegetables harboring parasites can lead to a plethora of gastrointestinal symptoms, and in more severe cases, organ damage. Vulnerable populations include young children, the elderly, pregnant women, and immunocompromised individuals. Infections may cause significant complications in these groups and require prompt medical intervention.

#### Prevention and Safety Measures

1. Thorough Washing: Washing may not eliminate all parasites; it can help remove dirt and reduce the

parasite load. Using vinegar or salt-water soak for a few minutes may also eliminate parasites.

2. Proper Handling and Storage: It is essential to handle salad ingredients properly throughout the supply chain. Washing hands thoroughly before handling fresh produce, using clean utensils, and storing salads at the correct temperatures minimizes the risk of contamination.
3. Source Control: Ensuring that produce comes from reputable sources adhering to strict hygiene and agricultural standards is important. Organic produce are less likely to be treated with chemicals, but this does not eliminate the risk of parasitic contamination.
4. Proper Cooking: Although salads are typically consumed raw, cooking leafy greens and other vegetables can effectively kill parasites. For those concerned about parasitic infections, lightly steaming or blanching vegetables can grant an added layer of protection.

Despite the convenience and health benefits of raw salads, individuals must ensure that the produce they consume is properly cleaned and sourced from safe environments. Parasites in salads have been looked for by microscopy, culture and molecular methods. Here we have looked for them by microscopic examination and culture. Figures 1 and 2 below show containers for salad collection.



Figure 1: Clean container with lid used for collecting salad samples.



Figure 2: Second container with lid used for collecting salad samples.

Salads are good sources of vitamins, minerals and dietary fiber. However they can also harbour many parasites. Perim *et al* have found that many parasites can be found in salads, like *Giardia duodenalis*; *Ascaris lumbricoides*; *Moniezia spp.* (11.7%); *Entamoeba coli*; *Toxocara spp.*; *Taenia spp.* (4.2%); *Fasciola hepática*; *Enterobius vermicularis*; *Trichuris trichiura* and *Toxoplasma gondii* in salads.<sup>[1]</sup> Salad samples were collected and parasitological assessment was performed using the Willis, Hoffman, Faust and Ziehl Neelsen techniques as well as culture for isolating free-living amoebae. Fifty one<sup>[51]</sup> samples were analyzed, out of which 16 (31.4%) were positive. The parasites detected were: *Acanthamoeba spp.* in 12 (23.5%); free-living larvae, *Schistosoma mansoni* and *Entamoeba coli* in 1 (2.0%); *Endolimax nana* in 2 (3.9%). The PCR technique determined that 17.6% of the samples showed *Toxoplasma gondii* DNA. Traore *et al* found that *Giardia* and *Entamoeba histolytica*; *Trichomonas intestinalis*; *Ascaris lumbricoides*; *Balantidium coli*; *Fasciola hepatica*; *Entamoeba coli* and other parasites have also been found in tomato, cucumber, lettuce and other salads.<sup>[2]</sup> They found that *Entamoeba coli* and *Trichomonas intestinalis* had the highest prevalence with 24.19% each. These were followed by *Ascaris lumbricoides* (13.25%), *Giardia intestinalis* (12.9%), *Balantidium coli* (11.29%), *Entamoeba histolytica* (7.26%), *Fasciola hepatica* (3.23%), *Trichinella spiralis* (1.61%), *Ancylostoma duodenale* and *Schistosoma intercalatum* (1.04% each).<sup>[2]</sup> *Balantidium coli* (25.6%) and *Entamoeba spp.* (21.7%) have been found in salads in Cameroon, Africa.<sup>[3]</sup> In 2018 *Cyclospora* oocysts were found in salad vegetables in The USA.<sup>[4]</sup> When travelling to tropical and subtropical areas, people should avoid consuming uncooked foods, like salads and vegetables. Hand washing with soap and water is recommended. *Giardia* and *Blastocystis* were mostly found in salad vegetables in Damascus, Syria. Lettuce was found to be most vulnerable to parasitic contamination.<sup>[5]</sup> In a study from Spain, salad vegetables were found to be contaminated with *Acanthamoeba spp.*, *Blastocystis spp.*, *Entamoeba coli* and *Endolimax nana*.<sup>[6]</sup> Along with parasites, bacteria and fungi are also important in salads, like *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Enterobacter spp.*, *Klebsiella spp.*, *Vibrio spp.*, *Aspergillus spp.*, and *Shigella spp.*<sup>[7]</sup> Using single extractant, researchers were able to recover oocysts of *Giardia spp.* and *Cryptosporidium* in salads contaminated with dirty water.<sup>[8]</sup>

In parts of North India salad contaminated with Tapeworms has been related to younger age.<sup>[9]</sup> In a study from South India, *Strongyloides spp.*, *Entamoeba histolytica* and *Giardia spp.* were found in about 6-26% of salad samples like beetroot.<sup>[10]</sup>

However, more studies are needed regarding parasitic contamination of salad in India, especially Eastern India. Most of the parasite in salad come from contaminated water that is used to wash these salads.

Amebiasis, or amoebic dysentery, is a gastrointestinal illness that develops when an organism called a parasite (*Entamoeba histolytica*) enters our intestines.

### Symptoms

- 1] Diarrhea (often with mucus or blood)
- 2] Stomach cramps or pain
- 3] Fatigue
- 4] Fever
- 5] Weight loss

Symptoms of Giardiasis are watery, sometimes foul-smelling diarrhea that may alternate with soft, greasy stools. Fatigue, Stomach cramps, bloating and weight loss with steatorrhea and creatorrhea may be present.

Signs and symptoms of *Giardia* infection may last 2 to 6 weeks, but in some people they last longer or recur.

Cryptosporidiosis is an illness from the parasite *Cryptosporidium spp.* Symptoms begin two to 10 days after exposure to *Cryptosporidium*. The protozoan *Cryptosporidium* can infect humans and a wide range of host animals. *Cryptosporidium parvum* (formerly known as *C. parvum* genotype II) and *C. hominis* (formerly known as *C. parvum* genotype I) are the leading causes of human cryptosporidiosis. *C. meleagridis*, *C. felis*, *C. canis*, *C. ubiquitum*, *C. cuniculus*, *C. viatorum*, Chipmunk genotype I, *Cryptosporidium* mink genotype, and *C. muris* can also infect humans.

Symptoms include watery diarrhea (sometimes severe), abdominal pain or cramping, nausea, vomiting, anorexia and low-grade fever.

There are many methods to study the presence of parasites in salads like washing with or without detergents. Tween 20 should be useful in this regard. Hence we here planned to study the effect of Normal saline vis-à-vis normal saline with Tween 20 in eluting parasites from the salads.

Several protozoan species in the genus *Entamoeba* colonize humans, but not all are pathogenic. *Entamoeba histolytica* is well recognized as a pathogenic amoeba, associated with intestinal and extraintestinal infections. Other morphologically-identical *Entamoeba spp.*, including *E. dispar*, *E. moshkovskii*, and *E. bangladeshi*, are generally not associated with disease although investigations into pathogenic potential are ongoing. Cysts and trophozoites are passed in feces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection with *Entamoeba histolytica* (and *E. dispar*) occurs via ingestion of mature cysts from fecally contaminated food, water, or hands.

Giardiasis usually represents a zoonosis with cross-infectivity between animals and humans. *Giardia intestinalis* has been isolated from the stools of beavers,

dogs, cats, and primates. Beavers may be an important reservoirs for *G. intestinalis*. Other *Giardia* species include *G. muris* in rodents; *G. agilis* in amphibians; *G. psittaci* and *G. ardeae* in birds; and *G. microti* in voles and muskrats.

*Giardia* species are endemic in areas of the world with poor sanitation. In developing countries, the disease is an important cause of morbidity. Water-borne and food-borne outbreaks are common. Since ingestion of as few as 10 *Giardia* cysts may be sufficient to cause infection, giardiasis is common in daycare center attendees and institutionalized patients in developed countries. *G. intestinalis* is a particularly significant pathogen for people with malnutrition, immunodeficiencies, or cystic fibrosis.

## MATERIALS AND METHODS

**Type of study:** It was a laboratory based observational study that was carried out in the bacteriology laboratory of the institute.

**Timeline of study:** It was completed in 6 months (December 2024 to May 2025)

**Sample size:** One hundred and thirty five (135) salad samples were collected from various zones of the city and selected for the study. Initially 150 samples were planned but due to logistics issue, 135 were processed. Sample size was calculated by method of convenience. Forty three (43) sample each were collected from Central, 49 from East including Salt Lake, and 37 from North zones of the city. Five (5) samples were obtained from South Zone of the city and 1 sample from West zone.

Permission was obtained from the Institute ethics committee before commencement of the study. Written informed consent was obtained from eateries before obtaining their samples.

### Sample Preparation and Microscopic Examination

Samples were collected in clean plastic containers with lid. After collection, the sample were divided into two equal parts. 2.9 gm sample and 10 ml normal saline were taken in one set and in another, 2.9 gram salad was mixed with 10 ml normal saline containing 0.01% Tween 20. One part was washed in normal saline for 5 minutes, while the second part were washed in normal saline containing 0.01% Tween 20. Both parts were centrifuged at 1500 RPM for 5 minutes. The resulting sediments were observed under 10X and 40X objectives of microscope for ova, parasites and cysts. The sediment from the second part was subjected to modified (cold) ZN stain and also subjected to culture.

### Procedure of Acid fast stain (Kinyoun -cold Method)

I. Smear was prepared and fixed. Smear was covered with Carbol fuchsin for 6 to 7 minutes.  
II. Then, gently the slide was rinsed with water. Then 4% sulfuric acid decolorizer was run over the slide for

approximately 3 minutes. Again, the slide was rinsed with water until the solution ran clear. After that, the smear was covered with the counterstain, 0.2% methylene blue, for 1 minute. Gently the slide was again rinsed with water. Lastly the slide was blotted dry and observed under OIF.

A new culture medium, a modification of the Boeck and Drbohlav's culture medium was used for culturing the samples.

### Formula of the new culture medium

#### Solid slant

1. 27 ml egg albumin
2. 7.5 ml ringer lactate
3. 30 mcg chloramphenicol. In later half of cultures chloramphenicol was omitted.

In a tube, 2.5 ml of this mixture were taken and inspissated. For overlay, 10 mg  $\text{NaHCO}_3$  and 1mg  $\text{MgCl}_2$  were added to 100 ml peptone water containing 1% human serum. So the final medium consisted of 2.5 ml solid medium with 5ml overlay. Figures 3 and 4 below show some salads collected.



Figure 3: Common Indian salad (green salad).



Figure 4: Common Indian salad (chickpea salad).

Simultaneously, wherever possible, the condition of the eatery and the hand hygiene of the food handlers were assessed. They were secretly informed about the

parasites found in the salads they serve, and advised regarding decontamination and prevention measures.

Ethical principles satisfied in this study

- Principle of beneficence
- Principle of non-maleficence
- Principle of autonomy

## RESULTS

Total no of samples studied: – 135 {From North Kolkata – 37 samples, from East Kolkata – 49 samples, from Central Kolkata – 43 samples and from other parts – 6 (South Kolkata- 5, West Kolkata -1)}.

Figure 5: Below shows maps of Kolkata and areas from where samples were obtained.

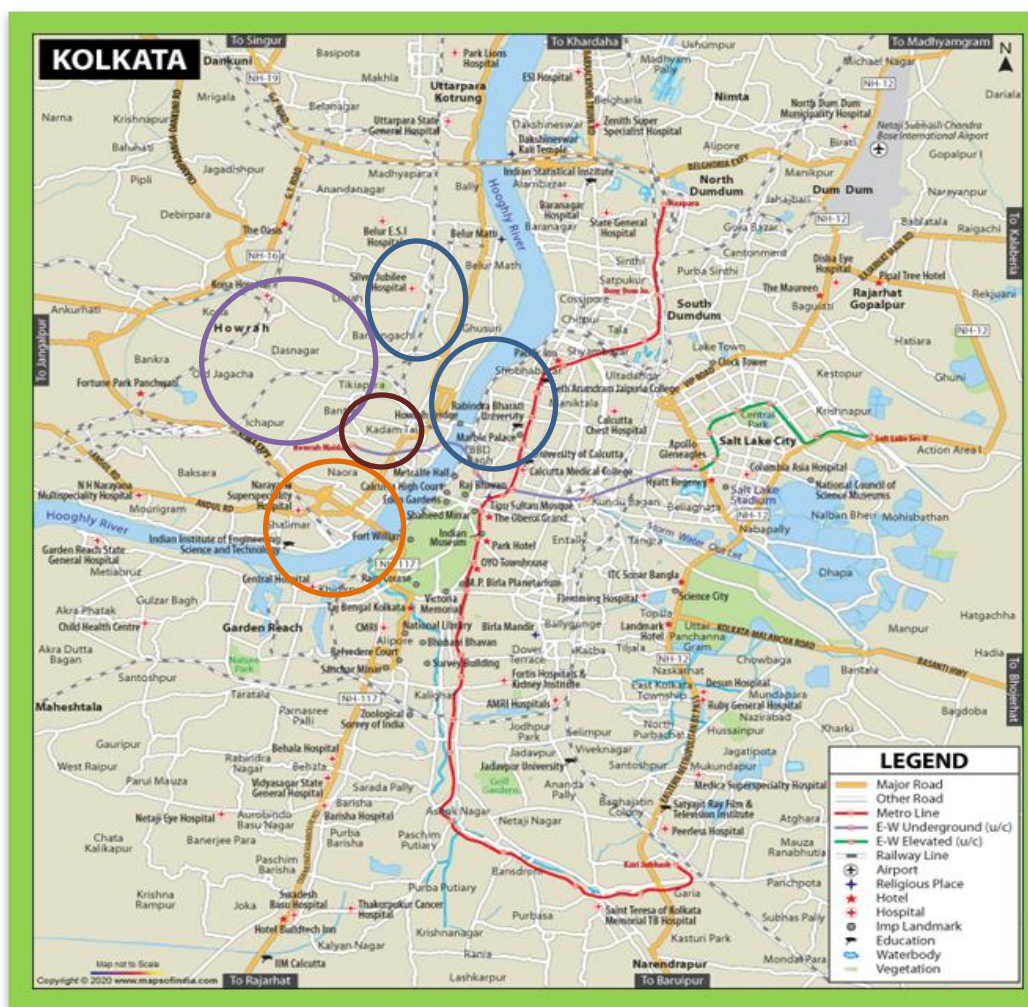


Figure 5: City of Kolkata with zones (North, South, East, Central and West marked in colored circles).

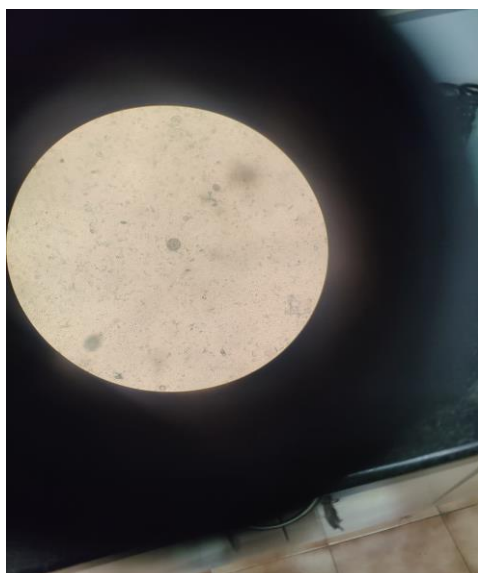
Total number of positive samples –36 (26.67%)  
 Positive samples from North Kolkata – 24.33 %  
 Positive samples from East Kolkata – 32.65 %  
 Positive samples from Central Kolkata –18.60 %  
 Other samples that were positive – 50 %

The most common parasites were *Entamoeba histolytica*. (36, 26.66%). Out of these 2 were trophozoites of *Entamoeba histolytica*/E. *dispar*. Both were from east Kolkata which showed trophozoites. Then *Entamoeba coli* (36, 26.66 %). *Blastocystis hominis* (8, 5.92 %). *Giardia* spp. (1, 0.74 %) Mixed parasites (11, 8.14 %). The table shows that the number of parasite and percentage of parasite:-

Table 1: Distribution of parasites in salads.

| Name of parasite            | Total number | Percentage (%) |
|-----------------------------|--------------|----------------|
| <i>Entamoeba coli</i>       | 36           | 26.66          |
| <i>Blastocystis hominis</i> | 8            | 5.92           |
| <i>Giardia</i>              | 1            | 0.74           |

Out of mixed parasites, 5 were a mixture of *Entamoeba histolytica*/E. *dispar* with *Blastocystis*. Four (4) samples showed mixture of *Entamoeba histolytica*/E. *dispar* and *Blastocystis hominis*. One (1) sample showed mixture of *Blastocystis hominis* and *Entamoeba coli*. One (1) sample showed mixture of *Entamoeba histolytica*/E. *dispar*, *Blastocystis hominis* and *Entamoeba coli* (all 3) in the same salad sample.



**Figure 6: *Entamoeba* cyst in salads in microscopy.**

ZN positive : - 1 (0.74 %) This was a sample from Central Kolkata, all the other tests were negative but the modified ZN stain came positive and showed oocysts of *Cryptosporidium* spp.

Culture positive: - 3 (1.48 %) There was a sample from Central Kolkata and another sample was from East Kolkata. The main ingredients of these samples were cucumber. Both samples were trophozoite form of *Entamoeba histolytica*.

Mean number of cysts of *E. histolytica*/*E. dispar*/*E. dispar* in positive samples: \_ 2.

Mean number of cysts of *Entamoeba coli* in positive samples: \_ 1.

Mean number of cysts of *Blastocystis hominis* in positive samples- 1

Figures 7,8, and 9 below highlight the poor condition of food preparation areas of the eateries while figure 10 shows how the medium was heated.



**Figure 8: Poor hygiene in kitchen.**



**Figure 9: Overcrowding of utensils and lack of cleanliness.**



**Figure 7: Poor hygiene of outlet.**



**Figure 10: Heating process of egg albumin-based medium.**

#### pH of samples

The pH of positive samples ranged from 5.5. to 6. For negative samples, range was 4.5 to 6. pH was measured by pH paper shown in Figure 1 below.



**Figure 11: Picture Showing pH value of 5.5.**

The number of total positive case in normal saline side – 27 (75 %)

The number of total positive case in Tween 20 side – 14 (38.89 %)

- Water supply of establishment --- Tap water from municipal supply in most outlets.
- Hygiene of establishment – Cleaned everyday, maintained food Waste Management
- Hand hygiene & related knowledge – Maintained properly and also used hand sanitizer

#### Distribution of salad samples (how many)

- Cucumber salad:- 60 (44.44 %)
- Green salad (cucumber +\_onion):- 11 (8.14 %)
- Cucumber +onion +carrot:- 15 (11.11 %)
- Tomato salad:- 14 (10.37 %)
- Onion salad:- 30 (22.22 %)
- Fruit salad:- 3 (2.22 %)
- Chicken salad:- 2 (1.48 %)
- Potato and cream salad:- 1. (0.74 %)

#### Positivity in individual salad types

Cucumber – 20 (14.5 % of all samples and 33.3% of cucumber samples)

Cucumber & Onion – 10 (7.40 % of all samples and 90.9% of cucumber+onion mixed samples) cucumber, onion & carrot –3 (2.2 %)

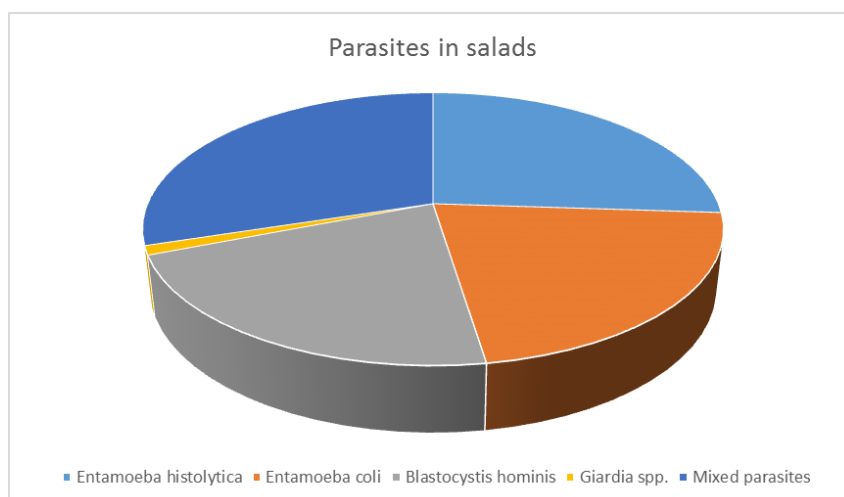
Tomato – 2 (1.48 % of all samples and 14.28% of tomato samples)

Onion:- 1 (0.74% of all samples and 3.33% of onion samples) (had *E. polecki* and *E. histolytica*/*E. dispar*/*E. dispar*)

Fruit salad:- nil.

While comparing positivity in cucumber only vs onion only, it was found that positivity in microscopy for parasites was significantly more in cucumber than onion.

While comparing positivity in cucumber only vs tomato only, it was found that positivity in microscopy for parasites was significantly more in cucumber than tomato. Figure 12 below shows parasitic distribution in salads.



**Figure 12: Pie diagram showing pattern of parasites in salad samples.**

We also found that normal saline is better than Tween - 20. This may be due to possible detrimental effect of Tween 20.

Culture positivity:- 3. (All showed *E.histolytica* as well as culture)

Culture positivity was much less because we prepare in the test tube and egg rich in many chemical and antimicrobials.

#### Positivity in microscopy according to seasonality

- Winter (January-February): 21 out of 60 (35%).
- Spring (March-April): 15 out of 60 (25%).
- Summer (May): 0 out of 15 (0%).

So positivity was statistically more in winter than summer and also, in spring than summer. However positivity in winter was not significantly more than positivity in spring.

We were able to assess the burden of parasite in different salad and also will assess the efficacy of the tween twenty in washing the surface of testing presence of parasites. We will also compare the efficacy of saline with Tween 20 versus only normal saline in eluting the parasites, their cysts and ova from the surface of the salad vegetables. The new culture medium also be evaluated for recovering these parasites.

#### DISCUSSION

Parasites in salads are of significant public health concern. Various protozoan cysts and helminth eggs can be found in salads, which can lead to illnesses such as giardiasis and cysticercosis. While the nutritional value of salads should not be overlooked, one better be cautious due to the risk of consuming parasitic eggs and cysts.

Parasites and their eggs in salads pose major public health hazards, causing a range of illnesses, particularly when consumed raw or undercooked. Salads, typically made from vegetables grown in soil or irrigated with contaminated water, are potential sources of parasitic transmission. Parasites such as *Giardia*, *Toxoplasma*, and *Cryptosporidium*, and eggs of helminths like *Ascaris* and *Trichuris*, can be commonly found in salad ingredients. These parasites typically exist as eggs or cysts in contaminated water, soil, or animal feces and can remain viable in the environment for long periods. When humans consume contaminated vegetables, they may develop gastrointestinal issues such as diarrhea, vomiting, or abdominal pain, and sometimes more serious systemic infections. In a study in Iran, the parasites detected in unwashed salad vegetables included *Ascaris lumbricoides* (8.17%), *Cryptosporidium* spp.

(2.87%), *Enterobius vermicularis* (2.65%), *Taeniid* spp. (4.86%), *Strongyloides* spp. (1.1%), *Toxocara* spp. (3.97%), and *Entamoeba coli* (2.43%), while *A. lumbricoides* (3.57%), *Cryptosporidium* spp. (1.79%),

and *E. vermicularis* (1.12%) were detected in pre-washed ones.<sup>[11]</sup> In a study in Jordan, parasitic contamination was detected in 10 out of 180 (5.6%) vegetables, from which 10 different parasite species were identified. The parasitic load was approximately 100 - 300 eggs/cysts/larvae per 300 grams of vegetable. The Jordanian lettuce had the highest parasitic contamination (3 out of 20, 15.0%) with 4 species (i.e., *Hymenolepis diminuta*, *Entamoeba coli*, *Iodamoeba* spp., and *Anoplocephala* spp.), followed by Kuwaiti celery leaves (3 out of 20, 15.0%) with 3 species (i.e., *Heterophyes heterophyes*, *Strongyloides stercoralis* larva, and *Sarcocystis* spp.), while 2 out of 20 (10.0%) Kuwaiti arugula bunches had *Cryptosporidium* spp. and *Giardia intestinalis* cyst.<sup>[12]</sup> *Giardia lamblia* cysts were the most prevalent parasite (8.8%) followed by *Entamoeba* spp. cysts (6.8%), *Enterobius vermicularis* eggs (4.9%), various helminth larvae (3.6%), *Hymenolepis nana* eggs (2.8%), *Hymenolepis diminuta* eggs (2.1%), and *Ascaris lumbricoides* eggs (0.6%).<sup>[13]</sup>

In a study from Portugal, ready-to-eat salads showed DNA of *Giardia* and *Cryptosporidium* spp. by qPCR nested PCR.<sup>[14]</sup> In a study from Northern India, by immunofluorescence assay (IFA) combined with the polymerase chain reaction (PCR), the commonest parasites in unprocessed foods were *Entamoeba* spp., *Giardia* spp., and *Cryptosporidium* spp.<sup>[15]</sup> Tapeworms have been found in salads in Faridabad, North India as per newspaper reports.<sup>[16]</sup> Same findings have been observed in Mumbai, where tapeworms have been abundantly found in salads.<sup>[17]</sup> Protozoan parasites like *Giardia duodenalis*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* have been found in raw edible leaves tulsi and pudina.<sup>[18]</sup> A famous Indian tennis player also suffered few years back from eating salads. Salad ingredients like carrot, beetroot, tomato, are dangerous as they are eaten raw and may transmit parasites.<sup>[19]</sup> Cases of cyclosporiasis, an intestinal illness caused by a microscopic parasite that can spread by contaminated foods, have been increasing alarmingly across the United States and has been linked to bagged salad.<sup>[20]</sup>

Seasonality may play a role in parasitic contamination of salads. Parasites are found more in rainy season on salads, then in winter and least in summer, when the plants and vegetables dry up.<sup>[21]</sup> Contaminated manure has been reported to contaminate vegetables as pathogens seen in soil are reflected in the vegetables. *Strongyloides stercoralis*, *Taenia*, and *Entamoeba* spp. were observed in raw manure of animal origin and vegetables.<sup>[22]</sup> So, soil and water are potent reservoirs of parasites. This is of one health importance also.

The parasites we found may have come from soil or drinking water or even from the hands of the food handlers. Parasites in salads commonly come from soil or water. So at every step strict monitoring and vigil are important.

The risk is especially high for individuals with compromised immune systems. Proper washing and handling of vegetables, along with maintaining sanitation and hand hygiene, can help minimize the risk of parasitic infections. Furthermore, raising public awareness about safe food handling, particularly concerning the consumption of raw salads, is crucial in preventing these health risks. As far as we know, this type of study has not been carried out in this part of the country.

We only found one *Cryptosporidium* in modified ZN stain, so we can say that acid fast protozoa are rare in salads in this part of the country. Salads are mainly contaminated with water, soil, and unclean hands. They are frequently contaminated with parasites and cut or sliced cucumber is the riskiest vegetable salad in this regard. In our study area, as per our observation, *Entamoeba histolytica*/*E. dispar*, *Blastocystis hominis* and others like *Giardia* spp. and *Cryptosporidium* spp., are common while others like helminthic eggs and larvae are rare. More studies like this will elicit the potential risk of transmission of parasites from consumption of salads.

## CONCLUSION

Our study has helped understand the burden of pathogenic parasites present in various salads, and help in devising methods to decontaminate these salads or make them free of parasites. Another takeaway is the pH range in salads where parasites are commonly found. This information will be of great public health importance.

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