ISSN No:-2456-2165

# Parasitic Infestation of Leafy Vegetables in Selected Caloocan City Markets

A Thesis Presented to the Faculty of the Natural Science Department
College of Arts and Sciences Manila Central University

In Partial Fulfillment of the Requirements for the
DegreeBachelor of Science in Biology

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March, 2013

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# APPROVAL SHEET

In partial fulfillment of the requirements for the degree Bachelor of Science in Biology, this thesis entitled **“Parasitic Infestation of Leafy Vegetables in Selected Caloocan City Markets”** prepared and presented by Harish Khadka, is hereby recommended for approval.

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# ACKNOWLEDGEMENT

The author would like to express his sincere gratitude and deep appreciation to thefollowing people who contributed directly or indirectly to the completion of this thesis:

First to the God, for the wisdom, strength, and patience He has given throughout the completion of this thesis.

His thesis adviser: Prof. Marilyn T. Malison, for the countless effort, kindness, and help formaking this thesis possible, particularly in the identification of intestinal parasites and editing the manuscript. Her advice, support and patience are deeply appreciated. Indeedwithout her guidance, the author would not be able to put the idea together.

Supportive thesis critic: Prof. Shiela Grace A. Martin for the help during microscopic examination of the intestinal parasites. She really gave time and effort despite of her busyschedule.

Bioresearch professor: Prof. Venus A. Solar for the techniques she has given in writing the manuscript.

Statistician: Prof. Ramil V. Flores for the help in validating the results through statistical analysis.

Prof. Rosemarie L. Balatbat, MS in Public Health - University of the Philippines, Manila: for authenticating the photographed intestinal parasites.

His thesis critics: Prof. Shiela Grace A. Martin, Prof. Nieves L. Capili, and Prof. Suzzeth M. Untalan, for their constructive criticisms, suggestions, and combined knowledge to improve the study. The author would like to thank them for their time and effort during thethesis defense.

Laboratory technicians: Angel and Tonette for preparing the reagents and lending the apparatus and glass wares needed for the thesis.

Lastly, his parents: for their moral and financial support throughout the completion of thisthesis despite of being far from his place.

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# ABSTRACT

The study focused on the identification and prevalence of intestinal parasites in leafy vegetables sold in selected public and private markets in Caloocan City.

Five kinds of leafy vegetables were chosen and collected as samples. The leafy vegetable samples included the kangkong (*Ipomea aquatica*), spring onions (*Allium fistolosum*), spinach (*Spinacea oleracea*), celery (*Apium graveolens*), and leek (*Allium porrum*).The collection of samples was done once a month from December, 2012 to January, 2013. The leafy vegetable samples obtained from the selected public and private markets were washed in distilled water and normal saline solution. Concentration method was used to isolate the intestinal parasites from the examined leafy vegetable samples. Light microscopy using LPO and HPO was used to examine and identify the intestinal parasites.

Fifteen medically important parasites were identified from the examined sediments of leafy vegetable samples. Pseudoparasites, fungal spores, pollen, mushroom spores, free-living ciliates, insects, and free-living organisms were also noted. The identified parasites were Ascaris, Strongyloides, Acanthocephalan, Secernentea, Strongylus, Hookworm, Toxocara, Fasciolid.egg, Enterobius vermicularis, Phasmid nematode, Ascarid, Entamoeba, Gregarina sp., Coccidian oocysts and Cyclophyllidean egg. *B*ased on the test on two proportions, it was found out that there is no significant difference in the prevalence of intestinal parasites in the selected public and private markets. After statistical analysis, the computed data indicated that there was a significant difference in the number of intestinal parasites present in the leafy vegetable samples washed in distilled water and normal saline solution.

# CHAPTER ONE

# INTRODUCTION

*A. Background of the Study*

Vegetables are cultivated for their edible parts or generally considered as the edible parts of a plant such as the root of beet, leaf of spinach, cauliflower, orthe flower buds of broccoli.

Some vegetables can be consumed raw, some may be eaten blanched, while others must be cooked in order to be edible. A number of processed food items are available in the market which contain leafy vegetables as the most common vegetable ingredients.

Vegetables are usually grown in the area where the soil is fertilized. Duringcultivation, animal manure is used as fertilizer and rain water or irrigation is used for watering them. In the process of irrigation, river water or pond water are used which are highly polluted with rotten wastes, excretions of animals and humans. Irrigation of polluted water in vegetables directly involved human health risk not only for consumers, but also for farmers who has direct contact with contaminated soil and waste water. The polluted water used to irrigate vegetables usuallycontains eggs of human intestinal parasites. Thus, eggs of human intestinalparasites have often contaminated vegetables sold in the market (Uga, 2009).

Vegetables are one of the basic foods of humans almost everywhere. Thus, due to the demand of vegetables, farmers used organic fertilizer to easily cultivatethem and with this practice, human health risk is also increasing in the same way.The consumption of raw or blanched leafy vegetables may lead to parasitic infections.

Local and international studies have shown that parasite infection may be acquired by eating raw and minimally-processed vegetables contaminated with eggs of intestinal parasites and protozoan cysts. Parasite infection caused by helminthes and protozoans constitute a major medical problem not only in the Philippines but also in various countries. One culprit would be the propensity of the Filipinos to eat raw vegetables mainly salads (Cauyan and Usero, 1994).

There are several inescapable factors of human activities which are often unsatisfactory in terms of standards of personal hygiene. Those who prepare the vegetables for the table must observe strict personal hygiene, as they can serve ascarriers of the eggs (Cauyan et al.,1994). Many vendors do not bother to obtain health clearances and often use water from questionable sources to keep their vegetables crisp and fresh. Paper money circulation in public markets has been moderately contaminated with parasitic ova and cysts. Money and vegetables are handled interchangeably during the course of daily transactions in the public markets (De Leon et. al.,1992). The major constituting factor for the contamination of vegetables that causes numerous cases of parasite infection and food-borne disease outbreaks in developing countries is continued use of untreated wastewater and manure as fertilizers for the vegetable production. Thus, public health workers involved in the control of parasitic and vector borne diseases require methods of characterizing the organisms which, although morphologically identical, differ quite markedly in their epidemiological significance. Information on these differences will have fundamental significance for the design of control strategies.

These collected information motivated the researcher to determine and identify the intestinal parasites that may infest fresh leafy vegetables. This study will provide epidemiological and parasitological information on vegetables and its degree of safety. Likewise, the result of this study may help in the establishment ofpreventive and precautionary measures to ensure safety of public health.

*B. Objectives of the Study*

This study entitled "Parasitic Infestation of Leafy Vegetables in SelectedCaloocan City Markets aims to:

- Identify the intestinal parasites that could be isolated from the leafy vegetablessamples.
- Determine the prevalence of intestinal parasites in the leafy vegetable samplesin two selected markets.
- Determine which method of washing is effective in isolating the intestinalparasites.
- Provide precautionary measures to prevent the occurrence of intestinalparasites in leafy vegetables

*C. Significance of the Study*

The health of the public is always of paramount concern in any kind of business ventures especially those involving food stuff for human consumption. Vegetables which are sources of vitamins and minerals are mostly leafy vegetables, hence, utmost care must be practiced when they are handled and processed for human consumption. Identification of the parasites that are putatively occurring on leafy vegetables can help nutritionists and consumers to determine the health risk of consuming these vegetables. Identifying the intestinalparasites can lead one to know the type of disease they may potentially acquire from eating miss-handled leafy vegetables.

The result of the study will provide epidemiological data on the parasites onleafy vegetables and the latter's degree of safety. Likewise, prognosis and preventive measures to take should be elevated to the consciousness of the consumers and the food servers to maintain safety of the public health.

*D. Scope and Limitations of the Study*

The study is limited to collecting samples of leafy vegetables sold in public and private markets in Caloocan City. The leafy vegetables used in the study are those vegetables that are eaten raw, blanched, and are added as garnishing in food viands. Five kinds of leafy vegetables were bought and collected by random sampling. The leafy vegetables included are kangkong (*Ipomea aquatica*), spring onions (*Allium fistolosum*), spinach (*Spinacea oleracea*), celery (*Apium graveolens*), and leek (*Allium porrum*). Leafy vegetable samples for each type were randomly collected from the public and private markets. Sampling was done once a month in two (2) months' period, from December 2012 to January 2013. Concentration method was used to isolate the intestinal parasites from the leafy vegetable samples. Light microscopy was utilized in identifying the intestinalparasites up to the species whenever possible.

- **Definition of Terms:** (from Medical Parasitology, 6$^{th}$ edition, 2012 by Ruth Leventhal and Russel Cheadle)
- **Bursa**– fan-shaped cartilage expansion at the posterior end of some male nematodes (e.g. hookworms), that holds onto the female during copulation
- **Cyst** – the immotile stage protected by a resistant cyst wall formed by the parasite. In this stage, the protozoa are readily transmitted to a new host
- **Corticated** – possessing an outer, mamillated, albuminous coating, as on the eggs of *Ascaris lumbricoides*
- **Filariform larva** – infective, non-feeding, sheated, third-stage larva
- **Infective Stage** – life cycle stage at which the parasite is capable of entering andcontinuing development within the host
- **Coccidian oocyst** - coccidian parasites that are transmitted between hosts by theingestion of food or water contaminated with oocysts, followed by the release of infectious sporozoites and invasion of the gastro-intestinal tract. In the external environment, sporozoites are protected from desiccation and chemical disinfectionby the oocyst wall.
- **Pseudoparasite** - A false parasite; may be either a commensal or a temporary parasite (the latter being an organism accidentally ingested and surviving briefly inthe intestine).
- **Rhabditiform larva** – non-infective, feeding, first stage larva; the larva have an hourglass-shaped esophagus.
- **Trophozoite –** the motile stage of protozoa in which they feed, multiply and maintain the colony within the host.

# CHAPTER TWO

# REVIEW OF RELATED LITERATURE

Vegetables are well recognized for their great nutritional value as a vital source of vitamins, minerals and great source of dietary fibre. In addition, vegetables also supply fair amounts of carbohydrates, proteins and energy.

Vegetables sold in the market or grown in garden are usually served uncooked, raw or fresh. Fresh vegetables are taken as an important healthy diet for our body. The number of reported cases of food borne illness linked to fresh vegetables has increased in these recent years. The major route in the transmission of parasitic contamination is theconsumption of raw vegetables.

There are several inescapable factors of human activities which are often unsatisfactory in terms of standards of personal hygiene. Those who prepare the vegetables for the table must observe strict personal hygiene, as they can serve as carriers of the eggs (Cauyan et al.,1994). Many vendors do not bother to obtain health clearances and often use water from questionable sources to keep their vegetables crisp and fresh. Paper money circulation in public markets has been moderately contaminated with parasitic ova and cysts. Money and vegetables are handled interchangeably during the course of daily transactions in the public markets (De Leon et. al.,1992).

Local and international studies have shown that parasite infection may be acquired by eating raw and minimally-processed vegetables contaminated with eggs of intestinal parasites and protozoan cysts. Parasite infection caused by helminthes and protozoans constitute a major medical problem not only in the Philippines, but also in various parts ofthe world. One culprit would be the propensity of the Filipinos to eat raw vegetables mainly salads (Cauyan and Usero, 1994).

Several studies proved that eating raw and minimally-processed vegetables can be a potential source of helminthic and protozoan parasites. In 2009, Marilyn T. Malison and Glenn L. Siasu conducted a study on the prevalence of intestinal parasites in selected vegetables at major public markets in Metro Manila, Philippines. Results of the study showed that out of 200 vegetable samples randomly collected from the vendors of Divisoria and Balintawak Public Markets, 119 (59.5%) of the vegetable samples were contaminated with intestinal parasites. The most common parasite found was Ascaris lumbricoides. The result of the study also showed that there is no significant difference inthe prevalence of intestinal parasites between the two public markets, and concluded that vegetables can be a potential source of parasitic infection.

In 2011, Siasu et.al conducted a follow up and related study on assessing the parasitic infestation of vegetables in selected markets in Metro Manila, Philippines. Results of the study showed that in all vegetables examined, 36 of 80 (45.0 %) were infested with parasitic organisms. The vegetables obtained from Muntinlupa City showed 17 of 40 (42.5%) to have parasitic infestation, while the vegetables from Quezon City showed 19 of 40 (47.5%) to have parasitic infestation. Significant differences on the parasitic organisms existed between the public and private markets and between the twolocations ($p<0.05$). Hence, the findings indicated that vegetables can be potential source of parasitic infection, and thus there is a need to be secure that foodstuffs sold in the market today are safe and fit for human consumption.

Same study was done in Southwestern Saudi Arabia to determine the prevalence of parasites in commonly used leafy vegetables. Among the common leafy vegetables studied include green onion, radish, watercress, lettuce and leek in the month of September 2004 to May 2005. Concentration method using tap water and Tris-buffer- saline (TBS) as washings were utilized. The use of TBS for the extraction, significantly increased the isolation rate (27%) of the parasites compared with the use of tap water (7.8%) ($z=4.72$ $p<0.001$). The prevalence of the parasites was 28% in green onion, 25% in radish, 17% in watercress, 17% in lettuce, and 13% in leek. The parasites were most common in the months of September to December. Among the most common isolated parasites were *Ancylostoma duodenale*, *Entamoeba coli, Ascaris lumbricoides and Blastocystis hominis and the least common was Iodamoeba butschlii*. (Al-Binali et al., 2006).

Another study in Iran, a country in the Middle East showed that due to the more prevalence of endemic parasitological infectious diseases in Iran mostly contaminated from green vegetables, a parasitical study was carried out on 263 vegetables samples from 44 farms in the suburbs of Tehran city, and also 166 vegetable samples from 20 green grocery markets in the same city. Out of 263 samples of farms, 147 cases (16.5%)of human pathogenic parasites were isolated. The technique such as Baermann funnel, centrifuging of plants and soil temporal precipitation procedure were used in this study. The common parasites seen were *Ascaris lumbricodes, Trichuris trichiura, Trichostronglylus sp. Toxocara sp*. Larvae of nematodes, protozoan cysts like Amoeba sp. *Giardia lamblia* and some flagellates. Leek and parsley were with the highest percentof contamination and tarragon with lowest (Gharavi et al., 2002).

Also in Southeast Asia, the examination was done to detect the contamination of helminthic eggs on vegetables purchased at suburban market in Hanoi, Vietnam. Out of 317 examined vegetables, 82 (26%) were contaminated with parasite eggs. Out of 15 varieties, 13 were contaminated except horseradish and cucumber. The contamination was highest in leafy vegetables (31%) followed by root vegetables (17%) and fruitvegetables (3%).

In the survey, 453 eggs and five species of parasites were found: Ascaris sp, Trichuris sp.,Toxocara sp.,Taenia sp. and Ascaridia galli. Results showed that out of 149 villagers interviewed, 121 (81%) usually use both animal as well as human feces as fertilizer. The number of eggs recovered from vegetables was higher in the dry season (355 eggs) than in the rainy season (98 eggs). Thus, it was revealed that vegetables purchased in the market of suburban Hanoi (Vietnam) were highly contaminated with parasite eggs, and that vegetables seem to play an important role in the transmission of soil-transmitted helminthes infection in the country. (Uga, 2009)

In 2011, Garedaghi et al. conducted a study on parasitic contamination of fresh vegetables consumed in Tabriz, Iran. A total of 100 samples of different vegetables were randomly selected from the markets and gardens (markets:50 and gardens:50), and examined by the concentration method to determine the parasitological contamination of vegetables sold at markets and gathered from gardens. The contamination by different intestinal parasites was seen to be 40% (20/50) in market vegetables and 76% (38/50) ingarden vegetables. Prevalence of pathogenic parasites in vegetables of markets and gardens were 20% and 25% respectively. The parasites detected were Giardia cysts (71%), Dicrocoelium eggs (41%), Fasciola eggs (3%) and Ascaris eggs (1%). Hence, vegetables can play an important role in the transmission of intestinal parasites.

In 2009, another study was conducted to determine the parasitologicalcontamination of salad vegetables in Tripoli-Libya. A total of 126 samples of salad vegetables obtained from wholesale and retail markets were examined for helminth eggs and Giardia sp. cysts using concentration method. Results of the study showed that of the36 tomatoes, 36 cucumbers, 27 lettuce, and 27 cress samples examined, Ascaris sp. eggs were detected in 19%, 75%, 96% and 96%, respectively; eggs of Toxocara cati in 11%, 14%, 48% and 41%; eggs of Toxocara canis in 3%, 8%, 37% and 33%; and eggs of Taenia/Echinococcus sp. in 6%, 25%, 33% and 30%, respectively. Cysts of Giardia sp. were detected in 3%, 19%, 4% and 11%, respectively. The study concluded that raw salad vegetables sold in Tripoli-Libya may pose a health risk to consumers of such products. (Abougrain et al. 2009)

From various studies it is concluded that intestinal parasites are common in vegetablesin developing countries. To support this, a study was done in Jos, Nigeria in 2007 by J. G. Damen et. al. This study attempted to determine the level of intestinal parasitic contamination on vegetables sold in Jos. Sample of 200 each of Tomatoes (Lycopersium sativus), Letus (Loctus satival) Carrot (Davcus carota L) Cabbage (Brassica Denceal) and Green leafy vegetables were analyzed using standardized Centrifugal-floatation technique methods. Out of the 1250 samples of vegetables examined, 450 (36.0%) were positive for intestinal parasites, cabbage recorded the highest prevalence of 64% while tomatoes had the least prevalence of 20%.

Further the study concluded that vegetables in Jos are heavily contaminated with intestinal parasites and there is need for public enlightenment campaign on the dangerof consuming inadequately washed and prepared vegetables (J. G. Damen et. al., 2007).

Not only one stage but various stages of parasite lifecycle are found in vegetables in which most common is egg stage. A similar study was done in Benin City, EDO State, Nigeria to access Geohelminths eggs contamination of vegetables sold in major markets.The test tube flotation method was used to examine contamination of Geohelminths eggson vegetables. A total of twelve species of vegetables were examined. Geohelminth eggs contamination on vegetables was highest in rainy season, 336 (61.5%) than in the dry season, 210 (38.5%) ($P<0.05$). Seven species of helminthes eggs were detected during the survey: Ascaris lumbricoides (33.5%); Hookworm, (26.0%) Trichuris trichiura (15.0%); Toxocara canis (14.1%); Strongyloides stercoralis, (7.3%); Taenia sp. (2.9%) and Enterobius vermicularis (1.28%). The result obtained showed that vegetables might act as a veritable source of transmission of helminthiasis, if not properly washed before consumption (Edosomwan, E.U. et. al. 2011).

A similar survey was carried out to determine geohelminthes ova and larvae contamination of some fruits and vegetables sold in markets in Owerri, Nigeria between September and October, 2009. A total of 220 vegetable samples and 180 fruit samples were bought directly from the rural farmers and checked for helminth ova/ larvae by formalin ether concentration technique. All fruits and vegetables were contaminated. However, the prevalence of contamination was more in vegetables (65.8%) than fruits (34.2%), ($p> 0.05;x2=4.55$). Helminthes ova/larvae found were Ascaris lumbricoides, Hookworm, Trichuris trichiura and strongyloides stercoralis with A. lumbricoides as the most common helminth ova encountered (54.4%). The most contaminated Vegetables were Talinum triangulare (water leaf) and Telferiria occidentalis (fluted pumpkin) was themost contaminated while fruits were Solanum marcrocarpon (garden egg fruit) Psidium guajava (guava) harboured the least helminth ova and larvae. The contamination could be attributed to the use of manure-rich night soils and poor animal husbandry. Intervention by way of protective wears, thorough washing of samples with water containing salts prior to eating and compositing of human/animal faeces before use as manure, was recommended (Amaechi, A.A. et. al., 2009).

Among vegetables also the most common is leafy vegetables which can beeaten raw, blanched or cooked. They are considered as a important part of daily diet for our better health. In 2010, Wafa A. I. Al. Megrin did a research to evaluate some of the leafy vegetables sold in public markets to check whether they harbor different parasite stages. Out of 470 samples 76 (16%) contained parasite stages. It was found 27.8% (17/61) in lettuce, 22.8% (13/57) in watercress, 20.6% (7/34) in leek, 19.1% (9/47) in green onion, 17.4% (15/87) in parsley, 15.4% (4/26) in spinach, 13.6%(3/22)in basil, 11.5% (3/26) in coriander, 9.4% (3/32) in radish, 5.3% (1/19) in dill and 4.7% (2/42) in mint. No parasites were detected in 17 samples collected from cabbage.

Detected intestinal parasites were Entamoeba coli (35.5%), Giardia lambia (31.6%), Dicrocoelium sp. (28.9%), Ascaris sp. (26.3%), Tanea sp. (19.7%), Blastocystis hominis (17.1%), Fasciola sp. (13.5%), Hymenolepis sp. (14.5%), Ancylostoma sp.(11.8%), Taxoplasma gondii (6.6%) and Trichostrongylusm sp. (2.6%). The result showed a significant seasonal variation ($p<0.05$), with highest prevalence in spring (23.1%), followed by summer (17.9%), autumn (10.6%) and winter (9.9%) (Al- Megrin, Wafa A.I. 2010).

Among the infections Intestinal parasitic infections are most common worldwide and according to various epidemiological studies, its prevalence has been found high in developing countries. A study was carried in Turkey with the objective to determine Intestinal parasites prevalence and related factors in school children. The objectives of this study were to determine the prevalence of intestinal parasitic infections in Aydin among 7–14 years old school children and to identify associated socio-demographic and environmental factors, behavioral habits and also related complaints. A Multistage sampling was used in the selection of the study sample. A questionnaire, cellulose adhesive and a stool specimen examination were done. Out of total 456 stool specimenscollected, 145 students (31.8%) were found to be infected with one or more intestinal parasites, 29 (6.4%) with more than one parasite, 26 (5.7%) with two parasites and 3 (0.7%) with three parasites. The three most common parasites detected were E. vermicularis, G. intestinalis and E. coli. Intestinal parasite prevalence was found higher inrural area in less educated society. (Okyay, P. et. al., 2004).

As concluded in different studies, fresh vegetables play a vital role in transmissionof parasites in human body. A similar studywas carried out in Urmia City, Iran in 2004 to do the survey of the parasite transmission role of fresh vegetables. A total of 640 samples from 11 species of vegetables (2,221.93 g) used for raw consumption was analyzed for the presence of helminths (eggs, parasite larvae, and free-living larvae) and protozoa (oocysts and cysts), for one year. Helminth contamination (2,776, 2,840, and 1,680, respectively) was detected in all vegetable samples (celery, radish, lettuce, leek, spinach,parsley, dill, green onion, cress, fenugreek, and coriander leaves). Oocysts and protozoan cysts were not detected. Prevalence of eggs was found more during the rainy season (summer and fall) and in cress (12.6%). Detected common parasite genera were Trichuris, Trichostrongylus, Ascaris, Schistosoma, Taenia, and Hymenolepis nana (Yakhchali, M. et.al, 2004).

As we all know salads are basic requirements in our daily food. Salads are madeof vegetables that are usually eaten raw. But they are considered as the most important mode of transmission of parasites in our body which may lead to parasitic infections. In 2011, a similar study related to occurrence of parasitic contamination in raw vegetables that are particularly used in salads was carried out in Izatnagar.

A total of 515 vegetable samples were collected from different vendors of wholesale and retail markets of Bareilly. It was found that 6.79% of vegetables were positive for parasitic infestation (ova, cyst, larvae, etc.). Nematode larvae (3.30%) were the most predominant, followed by ova of Ascaris spp.(1.35%), Taenia spp. (0.97%), Toxocara spp. (0.77%), oocyst of Coccidia (0.77%), ova of Strongyle spp.(0.58%), ova of Trichuris spp. (0.38%), ova of Hymenolepis spp. (0.38%), cysts of Giardia spp. (0.38%) and Entamoeba spp. (0.11%). Out of all the vegetables examined, coriander was the most commonly contaminated (15.3%), followed by lettuce (15%), green onion leaves (13.06%), mint (12.5%), cabbage (10.52%), radish (9.3%), carrot (7.8%), turnip (5.26%) and water chestnut (1.93%). Consumption of raw vegetables with the high percentage ofviable-stage parasites may pose a potential risk of their transmission to consumers (Samanta, S. et. al., 2011).

One of the main routes of parasitic contamination in vegetables is the minimally processed way of preparing dishes. In minimally processed vegetable dishes, the most common is salad. In 2011, Mohammad Khiyami et. al. did a survey to access food borne pathogen contamination in minimally processed vegetable salads in Riyadh, Saudi Arabia. According to the capital city of Saudi Arabia, eating green salad was a daily habit of about 50% individuals in the country. Hence microbial quality of minimally processed vegetable salads (Tabbouleh, Fattoush, Hummus, Mutabbel and Caesar) being served in restaurants and homes in Riyadh were evaluated to ascertain that they were safe for human consumption and free from potential food borne pathogens. The samples were assessed for the presence of total aerobic bacterial plate count, total coliforms, Escherichia coli, Salmonella, and Shigella. The total aerobic plate count for salad prepared in the restaurants was around 2 – 4.5 x105 CFU/g and homemade was 2-8 x 104 CFU/g. The total coliform counts in restaurants salad were around 2-8 x 104 CFU/g as compared to 2-4.8 x 103 CFU/g of homemade salads. All salads, except Caesar, recorded E. coli and Enterobacter aerogenes, while Shigella sp and Salmonella sp were present in few samples. The results of the present study warranted an urgent need to hadstrict control measures to eliminate food borne pathogen contamination. This was the firstreport on microbial quality of the said minimally processed vegetable salads in Saudi Arabia (Khiyami, M. et. al., 2011).

Although there are various studies and researches on isolation of parasites from fruits and vegetables, there is a rapid growth of the organic food industry and the increasing popularity of organic fruits and vegetables due to which the health benefits andrisks of organic produce are issues of significant importance (Chen, Michael C 2005). In 2005 Chen, Michael C did a review which will compare organic and conventional produce in terms of nutritional value, pesticide contamination, and microbiological safety. According to current literature it showed that organic produce tends to contain higher levels of vitamin C and lower levels of nitrates and contains fewer and lower levels of pesticides than conventional produce.

Organic farming methods could potentially lead to microbiological contamination, but the literature has shown that organic produce does not carry any higher risk of significant microbiological contamination than conventional produce. These findings in the current literature seemed to suggest that organic produce could potentially be more beneficial, but certainly not more harmful, than conventional produce for the health ofthe consumer (Chen, M.C., 2005).

Further studies on parasitic contamination of fresh leafy vegetables sold in local markets in the tropics still need to be done.

# CHAPTER THREE

# METHODS

*A. Establishment of the Sampling Area*

The collection sites chosen by the researcher were the Monumento Public Market and Robinson's Supermarket both located in Caloocan City. The Monumento Public Market is located at the edge of the highways and public footways, where different typesof vehicles particularly buses passed by and emit toxic and harmful pollutants, whereas Robinson's Supermarket is a private market and is located in the well managed building with better management and sanitation. The management of both markets is totally different from each other.

The public market is found to be totally exposed to open environment and exhibit poor environmental sanitation and management of wastes. The stalls of the public marketwere not well constructed to prevent dirt or pollution from contaminating the foodstuffs sold in there. Big umbrellas or any similar materials were only used by the vendors as roofto prevent vegetables from the direct heat of sunlight, and the vegetables sold here are just placed in "bilaos" or "kariton". Some vendors even used old sacks as placemats on the muddy and dirty roads where they placed the vegetables to be sold. On the other hand, Robinson's Supermarket is found in a good constructed building to prevent vegetables from exposure to foreign matters. The stalls here are well managed with vegetables and fruits placed in different sections with proper sanitation and handling of fresh produce. Thus, factors like exposure of fresh vegetables to environmental pollutionlike garbage and wastes, foreign matters, presence and role flies, pollution contributed byvehicles, improper handling, and the like created the risk to vegetables of being contaminated with intestinal parasites.

*B. Collection of the Samples*

Five kinds of leafy vegetables were bought in Monumento Public Market and Robinson's Supermarket in Caloocan City. The fresh leafy vegetable samples used in thestudy are those eaten raw, blanched, or added as garnishing in food viands. The leafy vegetable samples included the kangkong (*Ipomea aquatica*), spring onions (*Allium fistolosum*), spinach (*Spinacea oleracea*), celery (*Apium graveolens*), and leek (*Allium porrum*). Sampling was done once a month in two (2) months period, from December 2012 to January 2013. A 200g for each of the vegetable samples were bought from the public and private markets by random sampling. A total of 20 fresh leafy vegetables randomly selected from the public and private markets were placed in plastic bags,transported to the laboratory, and examined by the concentration method.

*C. Processing and Examination of the Samples*

Each type of 200g leafy vegetable samples brought to the laboratory was divided equally into 100g each. 100g of each type of vegetable samples were thoroughly washedwith distilled water and the other 100g were washed with physiological normal saline solution (0.85% NaCl solution) to compare which of the solutions is effective in removing or isolating the parasites from the leafy vegetable samples. The washings were collectedin wide-mouth containers and were allowed to undergo sedimentation at room temperature for 6 hours. The supernatant was discarded and the filtrate was centrifuged for 10 minutes at 3,200 rpm. The resulting supernatant fluid was again discarded to collect the sediments for slide preparation. The sediment was examined in Lugol-stained slides through light microscopy. The sediments in each test tube were consumed during slide preparation for the microscopic examination of the intestinal parasites.

*D. Identification of the Parasites*

Low Power Objectives and High Power Objectives were used in observing the sediments of leafy vegetable samples. A parasitology atlas and a dichotomous key basedon the specimen's morphology, size, shape and color were used to identify the parasites.Experts in the field of Parasitology also authenticated the pictures of the intestinalparasites taken by the researcher. Further, data were noted and were tabulated.

*E. Determination of the Prevalence of the Parasites*

The given formula was used to determine the prevalence of parasites in the leafy vegetable samples examined:

$$\text{Prevalence (\%)} = \frac{\text{no. of leafy vegetables infected}}{\text{no. of leafy vegetables examined}} \times 100\text{total}$$

*F. Statistical Analysis*

Z test or Test on Two Proportions was used as the statistical instrument to determine if there is a significant difference on the prevalence of intestinal parasites in theleafy vegetable samples and in the two selected markets. The formula for Test on Two Proportions is given below:

$$Z = \frac{\Pi_1 - \Pi_2}{\sqrt{pq\left[\left(\frac{1}{n_1}\right) - \left(\frac{1}{n_2}\right)\right]}}$$

*G. Research Paradigm*

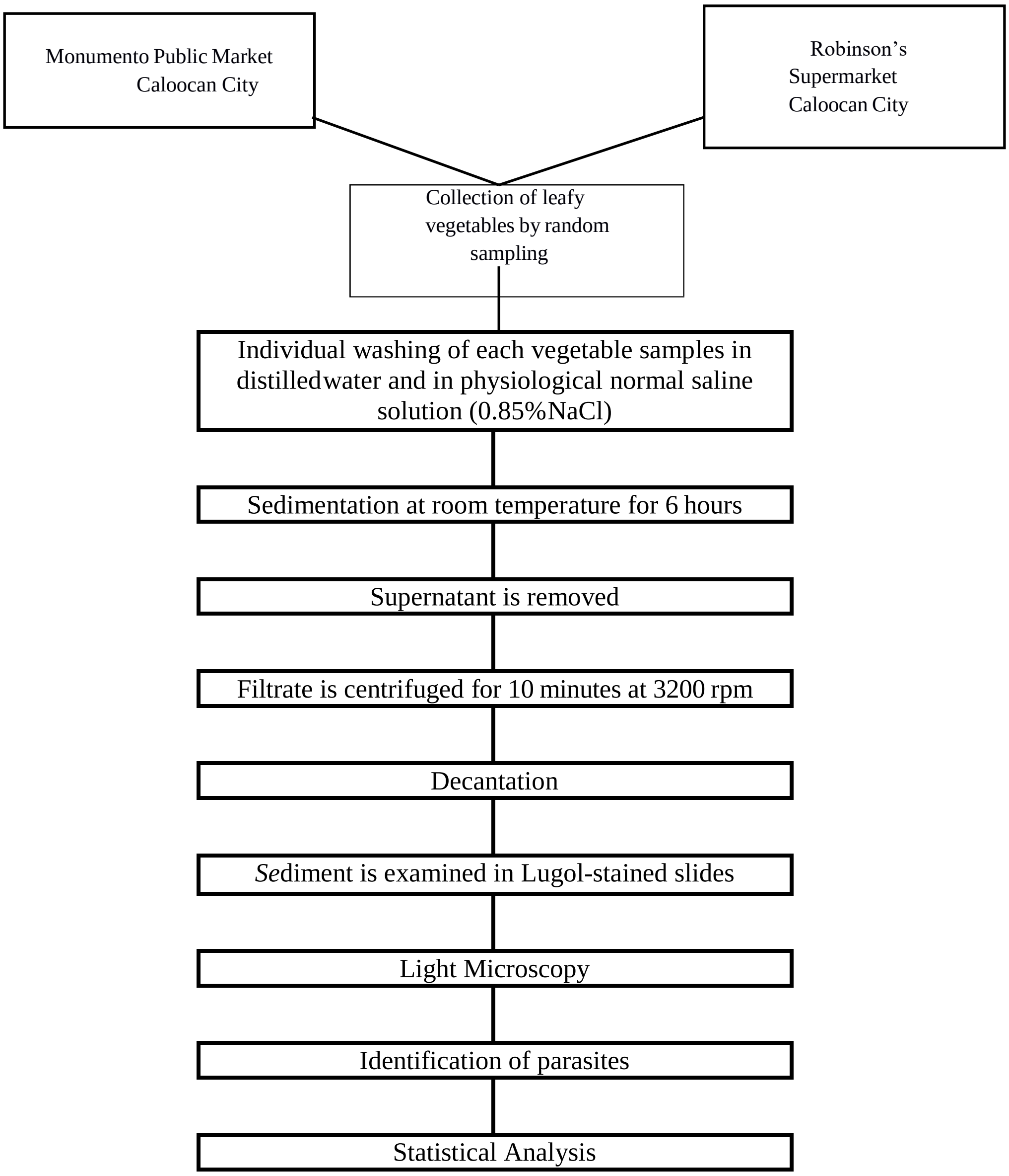


Fig. 1: Research Paradigm

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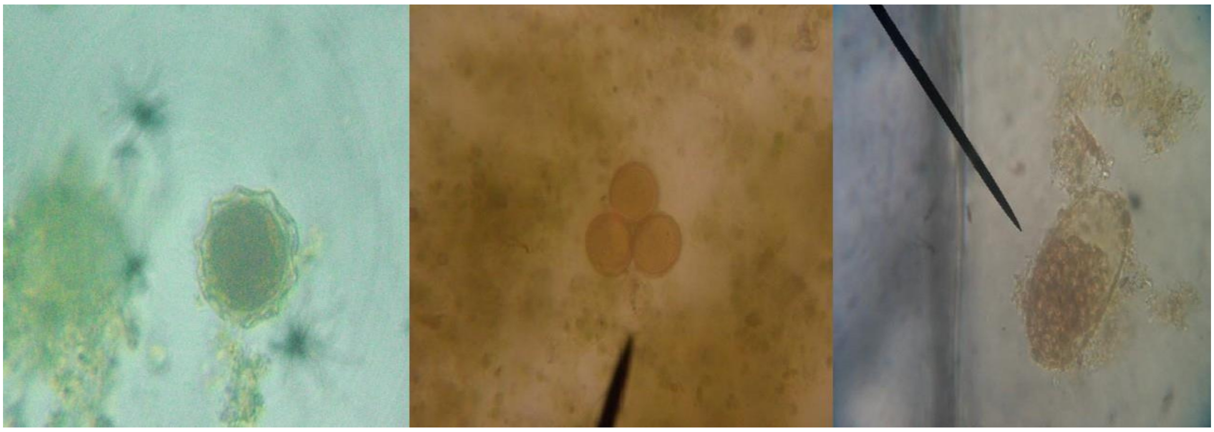
# CHAPTER FOUR

# RESULTS AND DISCUSSION

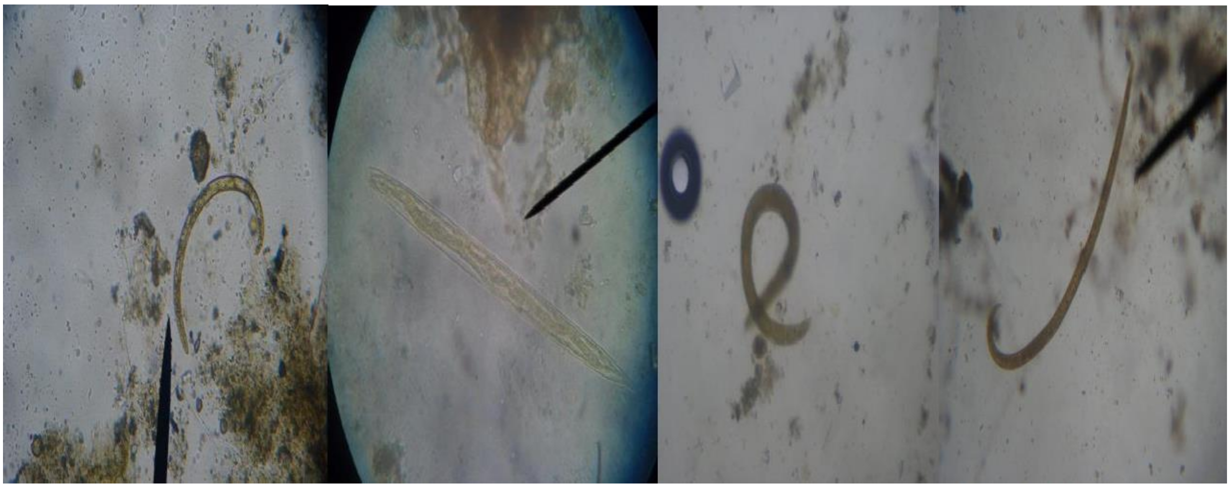
Using concentration method and light microscopy to isolate and examined the intestinal parasites from the sediments of the leafy vegetable samples, the examined leafy vegetable samples from selected public and private markets in Caloocan City were found to be positive for the presence of intestinal parasites. Fifteen medically important parasites were identified from the examined sediments of leafy vegetable samples. Pseudoparasites, fungal spores, pollen, ciliates, insects, and free-living organisms were also noted. The identified parasites were *Ascaris, Strongyloides, Acanthocephalan, Secernentea, Strongylus, Hookworm, Toxocara, Fasciolid.egg, Enterobius vermicularis, Phasmid nematode, Ascarid, Entamoeba, Gregarina sp., Coccidian oocysts and Cyclophyllidean egg.*



**Fertilized, corticated egg** **Fertilized, decorticated egg** **Unfertilized egg**

Plate 1 : *Ascaris*

*Ascaris* fertilized, corticated egg has lipoidal vitelline layer and mammilated albuminous layer; in fertilized, decorticated egg, the mammmilated albuminous layer is absent;unfertilized egg is elongated with heavy albuminous mammilated coat.



**Rhabditiform larva** **Filariform larva** **L1 stage** **Male *Strongyloides***

Plate 2: *Strongyloides*

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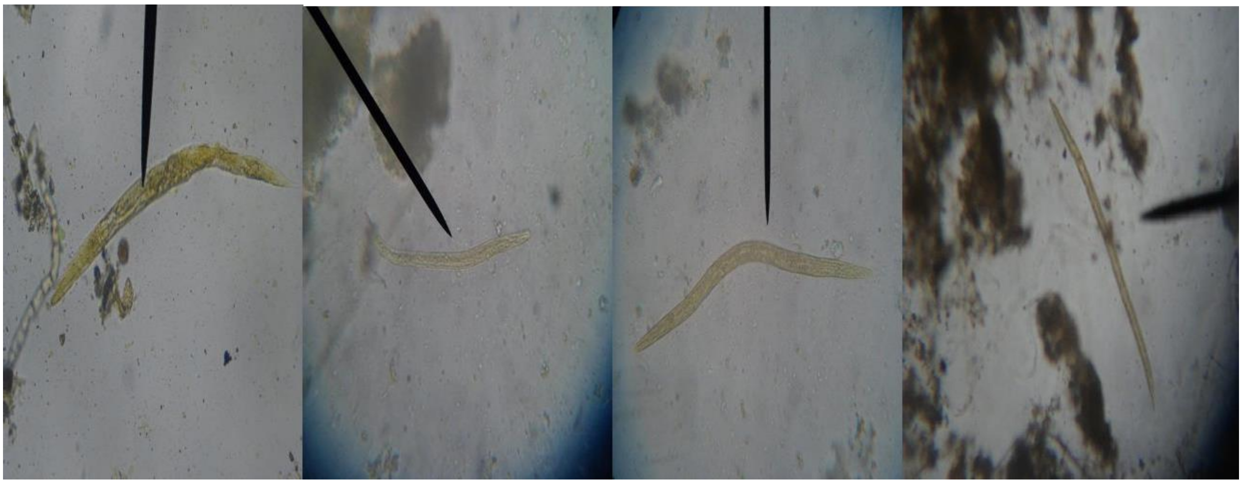
In rhabditiform larva, the esophageal bulb is evident at the junction of the esophagus and intestine; infective, third-stage filariform larva (L3) is up to 600 µm long and has a notched tail; L1 stage is 180-380 µm long, with a short buccal canal, a rhabditoid esophagus and a prominent genital primordium; males possess two copulatory spicules in addition to a gubernaculum.



**L1 stage** **L2 stage** **Filariform larva** **Rhabditiform larva**

Plate 3: Hookworm

L1 stage larvae that hatch from eggs are 250-300µm wide. They have a long buccal canal and an inconspicuous genital primordium; a rhabditiform L2 stage, will feed for approximately 7 days and then molt into the third stage larvae, or L3; infective, third-stage (L3), filariform larvae are 500-600 µm long. They have a pointed tail and a striated seath; rhabditiform larvae that hatch fromeggs are 250-300 µm long and approximately 15-20 µm wide. They have a long buccal canal and an ninconspicuous genital primordium.

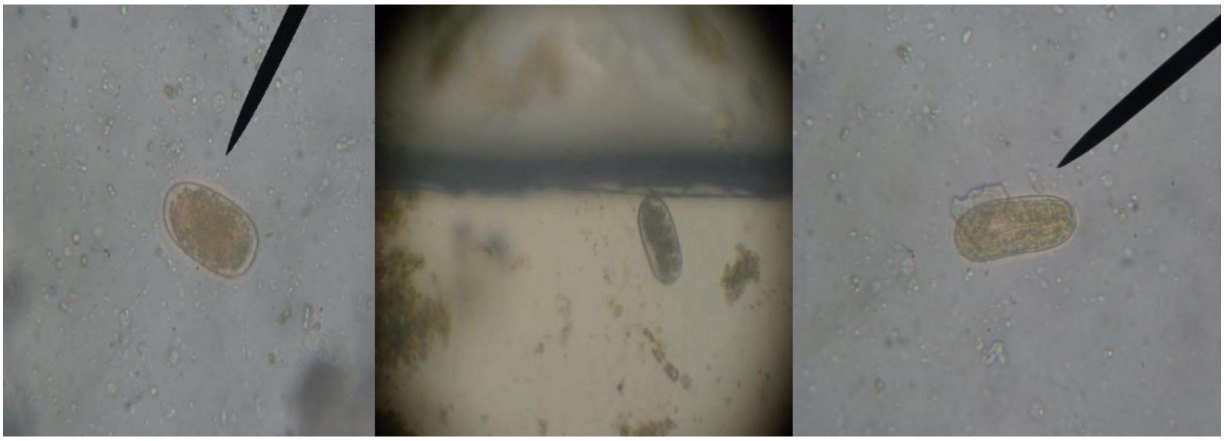
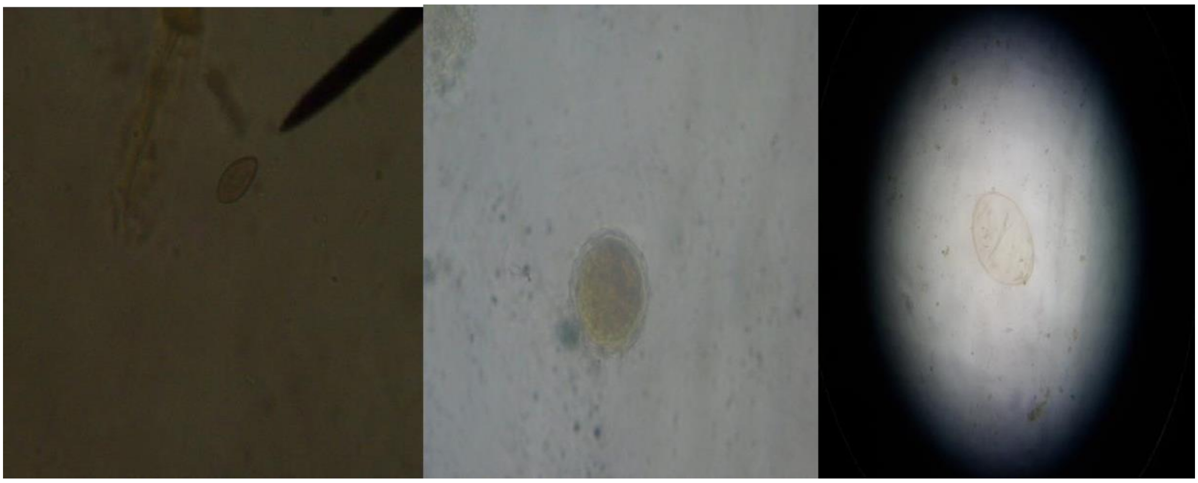


Plate 4: Hookworm eggs

**Hookworm immature eggs** usually contain an immature embryo in the 4-8 cell stage division and measures about 30 µm x 50 µm, while **mature eggs** contain a developing rhabditiform larva ;eggs hatch in 1 to 2 days to liberate rhabditiform larva which then further mature in the soil to become infective filariform larva.



***Enterobius vermicularis*** ***Toxocara egg*** ***Fasciolid egg***

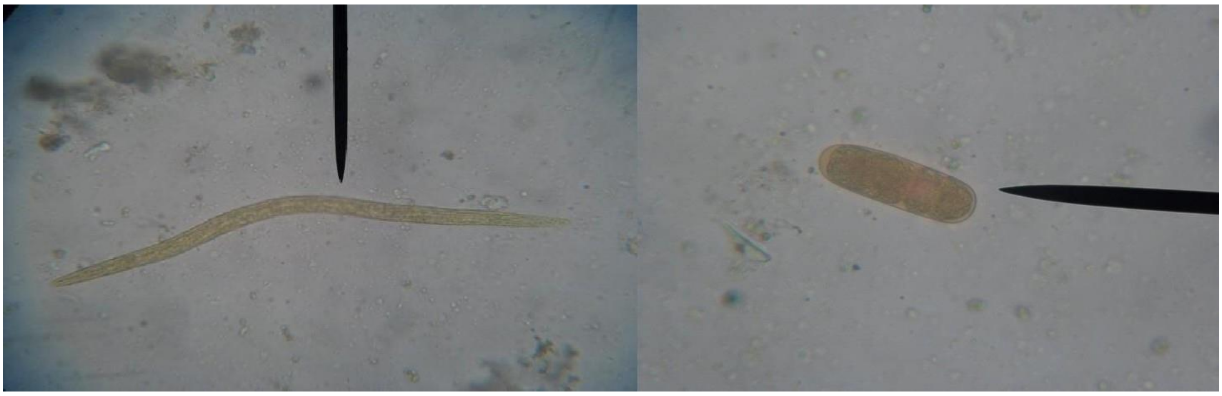
*Plate 5:* E. vermicularis, Toxocara, & Fasciolid egg

- ***Enterobius vermicularis egg*** measures 50-60 µm by 20-30 µm , are elongate-oval and slightly flattened on one side. They are usually partially-embryonated when shed.
- ***Toxocara eggs*** are spherical, brown and pitted and measures 75-90 µm and contaminated infective, larvated eggs are usually found in fecal soil.
- ***Fasciolid eggs*** are broadly ellipsoidal, operculated, and measure 130-150 µm by 60-90 µm.

***Acanthocephalan*** ***Secernentea*** ***Phasmid nematode***

Plate 6: Acanthocephalans, Secernentea, & Phasmid nematode

- ***Acanthocephalans*** are worms of the phylum Acanthocephala that live parasitically in the intestines of vertebrates and are characterized by a cylindrical, retractile proboscis that bears many rows of hooked spines. They are also called spiny-headed worm.
- **Secernenteans** possess primary excretory system that consists of intracellular tubular canals joined anteriorly and ventrally in an excretory sinus, into which two ventralexcretory gland cells may also open.
- **Phasmid nematode** is characterized by unsegmented & elongated body; has a pseudocoele that contains the digestive,excretory, nervous & reproductive systems & caudal chemoreceptors.



***Strongylus larva*** ***L1 stage*** ***Strongylus egg***
Plate 7: *Strongylus*

- ***Strongylus*** are parasitic worms with a pair of elongated buccal glands and including as gastrointestinal parasite of a horse; eggs measure 90 x 50 microns; ovoid, thin-shelled, embryos in morulate stage.

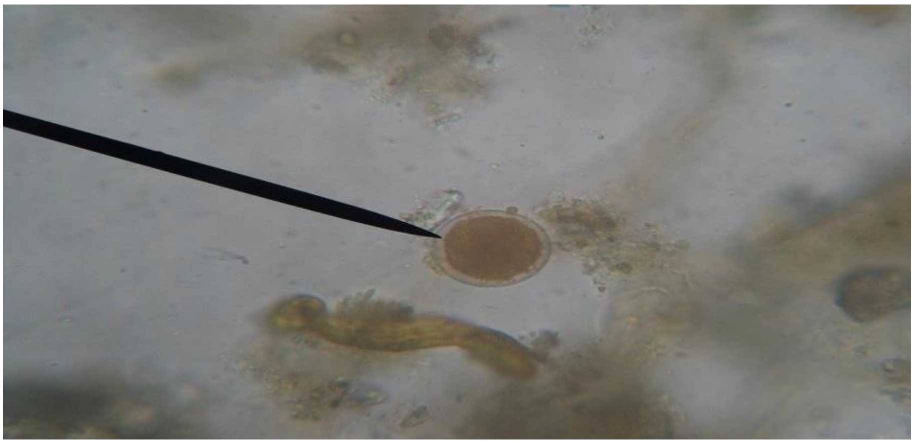


Plate 8: Ascarid egg

broad, oval in shape; brown in color; average size is 45 x 70 µm ; the outer coarsely mammilated covering is absent in decorticated eggs

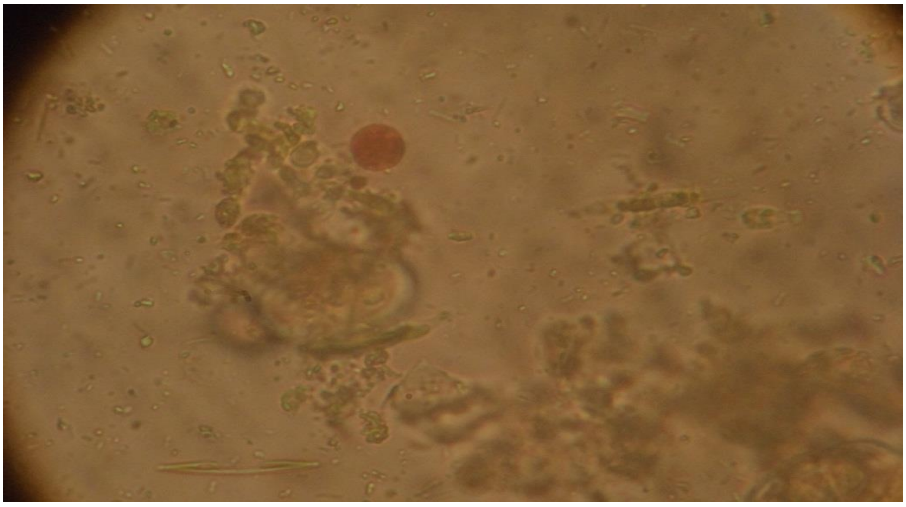


Plate 9:*Entamoeba cyst*

-mature cysts have four nuclei with centrally placed endosomes. Cyst size averages 12um but ranges from 10-20 um in diameter.

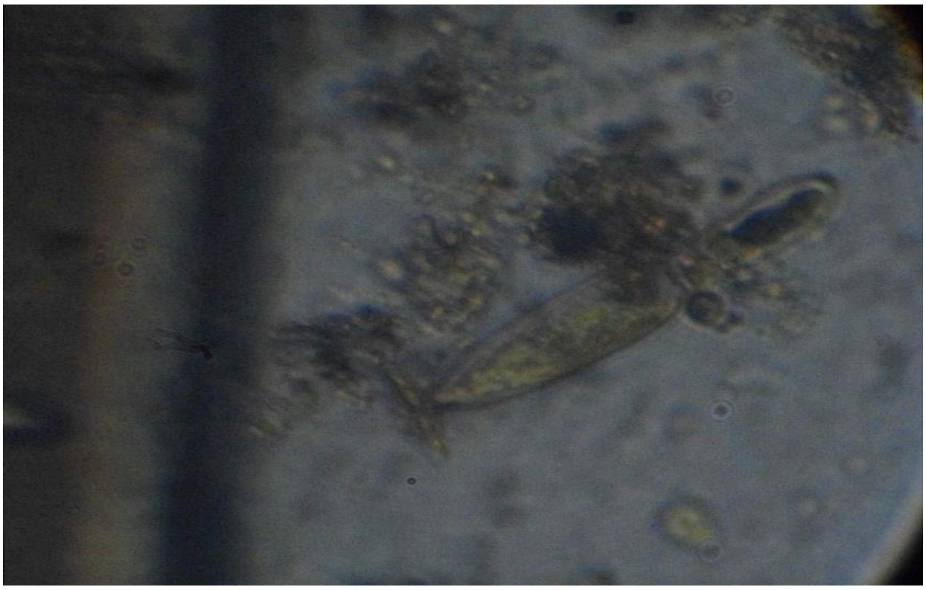


Plate 10: *Gregarina sp.*

- Two gregarines become attached to one another, forming a couple (syzygy) and are surrounded by a common cyst; Gregarina is a sporozoan protozoan that is parasitic within the digestive tracts of various invertebrates including arthropods and annelids.

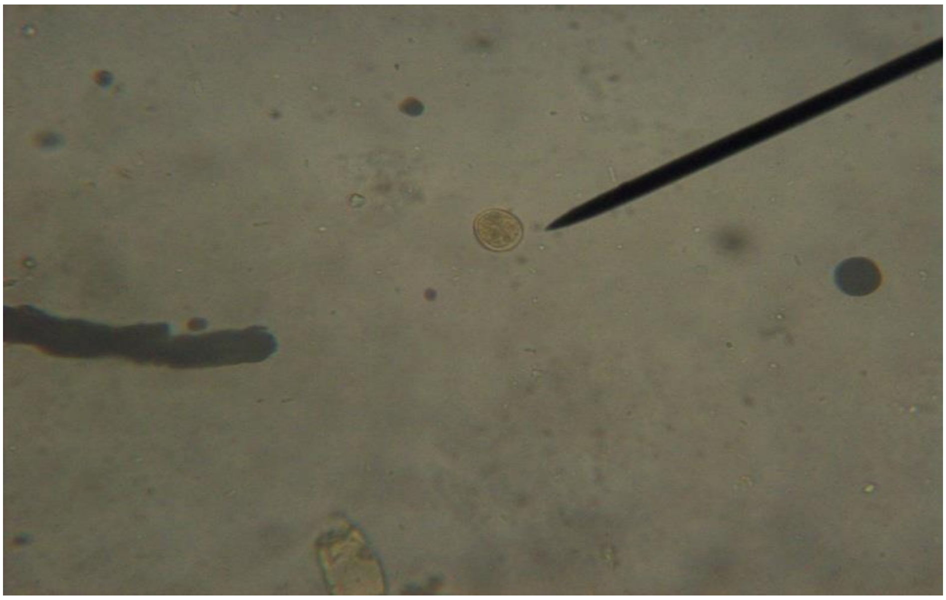


Plate 11: *Coccidian oocysts*

-are microscopic, spore-forming, single-celled obligate intracellular parasites belonging to the apicomplexan; coccidian parasites infect the intestinal tracts of animals, and are the largest group of apicomplexan protozoa.

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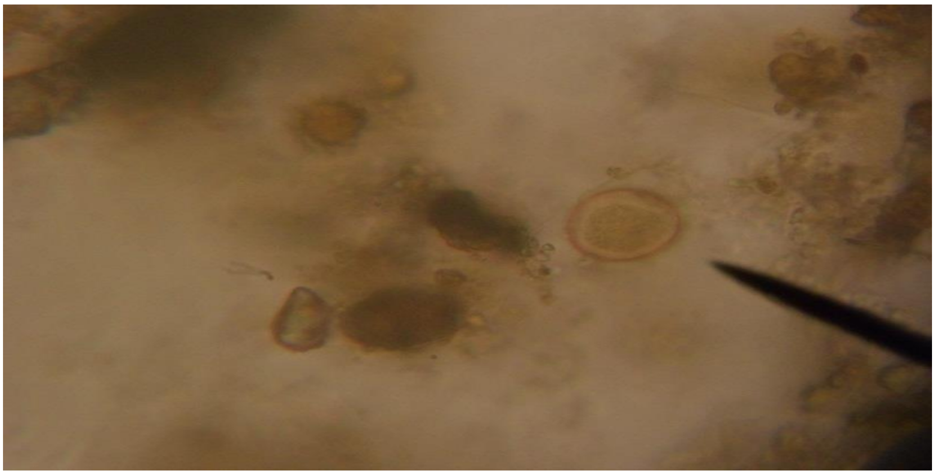

Plate 12: *Cyclophyllidean egg*

-the egg of the cyclophyllideans tapeworms has a very thick, resistant egg shell, with nooperculum.

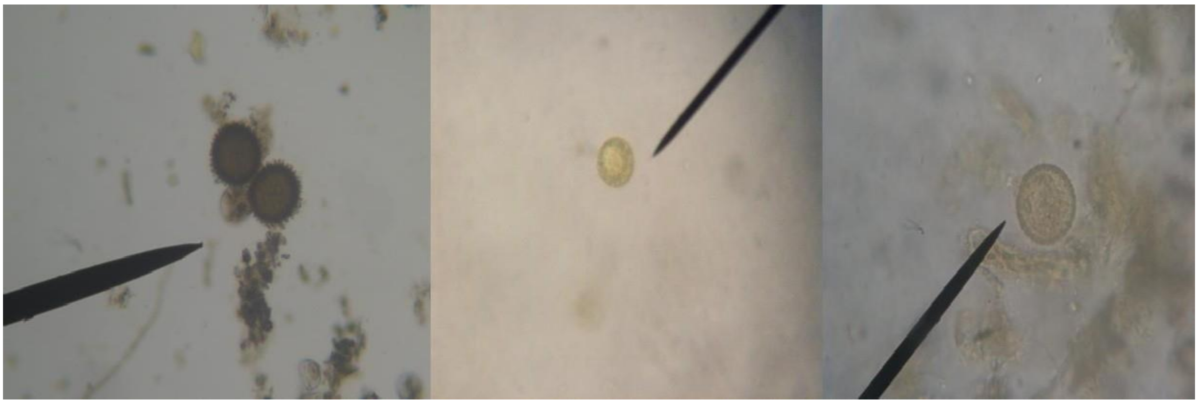

Plate 13: Pollen

Pollens of the Malvaceae is spherical, porate and echinate, with a remarkably thickinner wall (endexine). The spines are about twice as long as the wall is thick, and some are hollow. "Small spines" are less than twice the height of the wall, and "large spines" are bottle-shaped at the base and have a conical base or platform made of long columellae. Both lengths of the tapered "spines" have rounded tips.

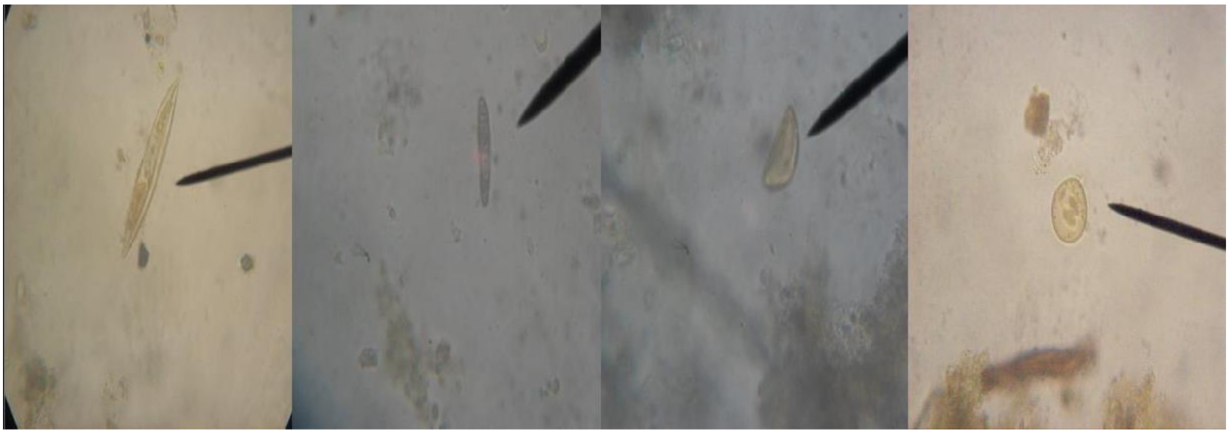

Plate 14: Fungal spores

-may be unicellular or multicellular; reproductive or distributional cells develop intodifferent phases of the complex life cycles of fungi

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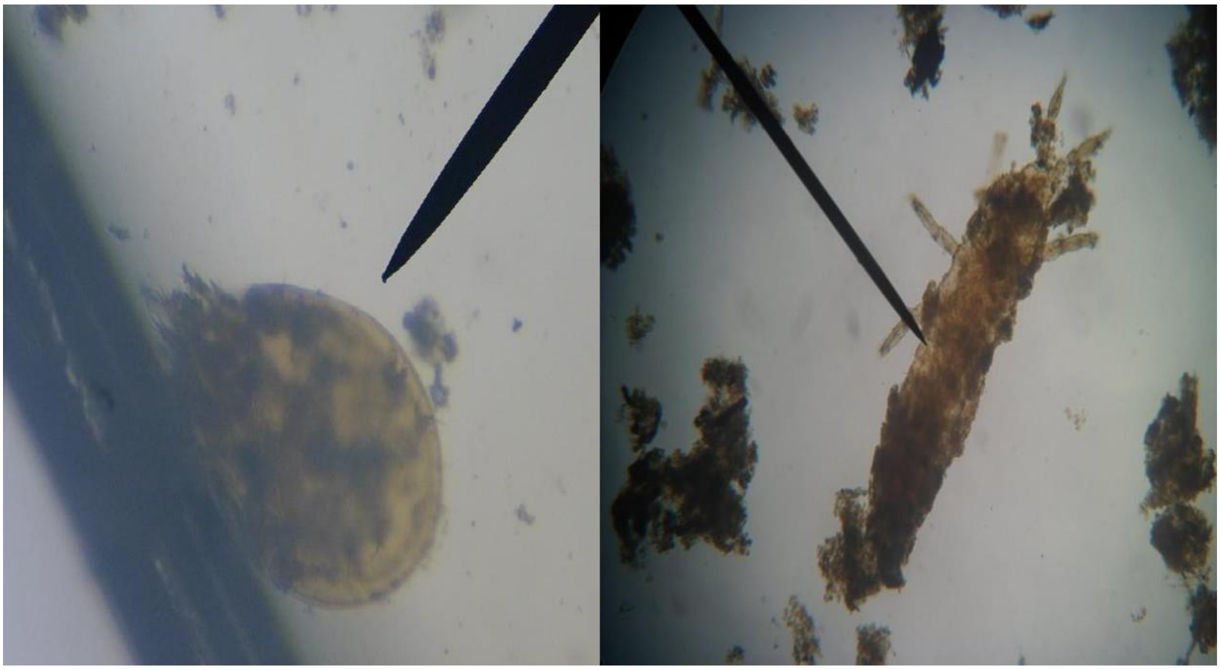

**Mite** **Thrips larva**

Plate 15: Insects

Insects are by far the largest group of arthropods which are considered as the most abundant group of eukaryotes on earth. They can live in every conceivable habitat on land and in fresh water and a few have even invaded the sea.

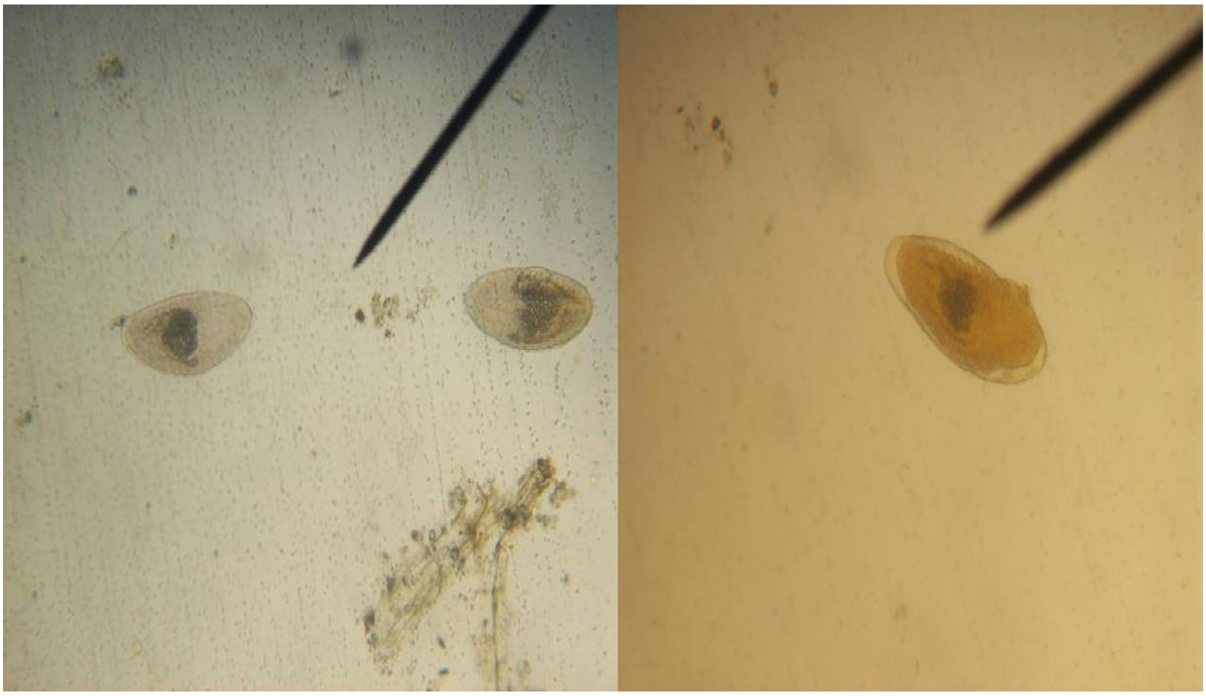

Plate 16: Ciliates

Microscopic, elongated cells that possess cilia as structures for motility.

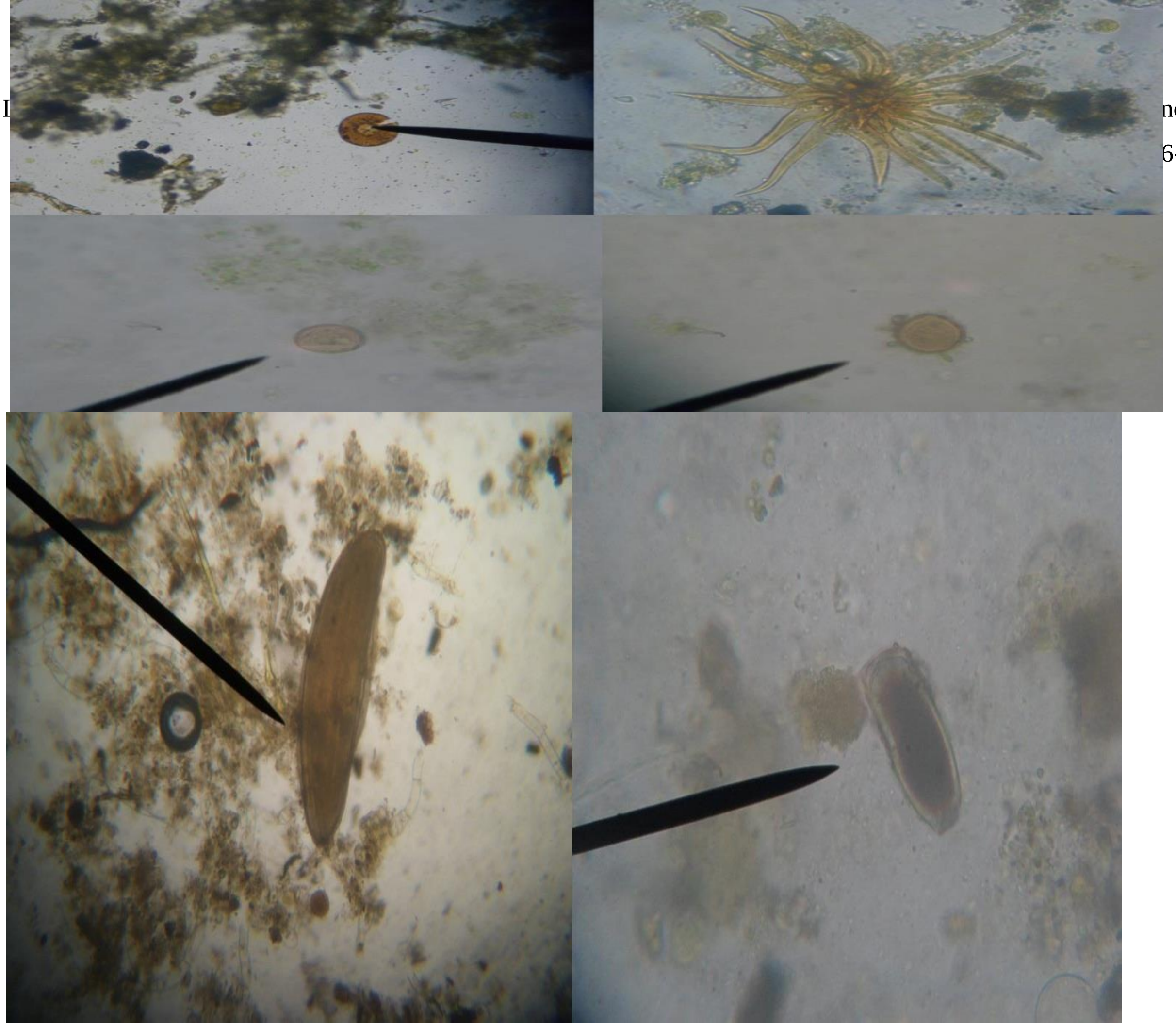

Plate 17: Pseudoparasites

--a false parasite; may be either a commensal or a temporary parasite (the latter being an organism accidentally ingested and surviving briefly in the intestine)

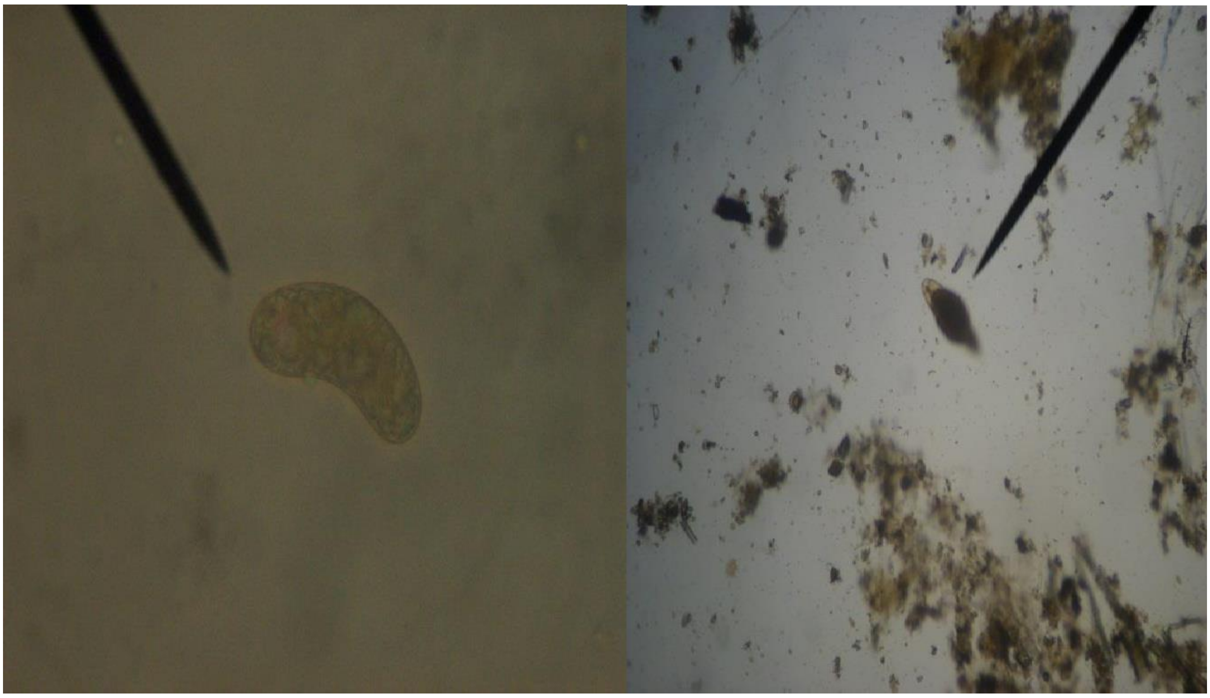

Plate 18 Free-living organisms

Organisms which are not directly dependent on another organisms for survival

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Table 1: Prevalence of Intestinal Parasites between two (2) Selected Markets

| SAMPLES | LOCATION OF PURCHASE | | | | |
|---|---|---|---|---|---|
| **Vegetables** | **Monumento Public Market** | | **Robinson's Supermarket** | | **Overall** |
| | DistilledH2O | NSS | DistilledH2O | NSS | |
| Spinach | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 8/8 (100%) |
| Celery | 1/2 (50%) | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 7/8 (87.5%) |
| Kangkong | 2/2 (100%) | 1/2 (50%) | 0/2 (0%) | 2/2 (100%) | 5/8 (62.5%) |
| Leek | 1/2 (50%) | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 7/8 (87.5%) |
| Spring Onions | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 2/2 (100%) | 8/8 (100%) |
| **Overall** | 8/10 (80%) | 9/10 (90%) | 8/10 (80%) | 10/10(100%) | 35/40(87.5%) |

Based on the data found on table 1, the overall prevalence rate of intestinal parasites from the examined leafy vegetable samples was 87.5% (35 out 40). Overall, spinach and spring onions were the most contaminated with parasites (100.0%) followed by celery and leek (87.5%), and the least contaminated is kangkong (62.5%).

The computed data above show that the leafy vegetables sold at the selected public and private markets were found to be highly contaminated with parasites. Several sources of contamination can be speculated upon. The high prevalence rate of contaminated samples from both markets is due to poor sanitation and management of wastes in the market's areas that may serve as an avenue through which parasite contamination can occur. Monumento Public Market is well-known for its garbage heaps which are found alongside the vegetable stalls. The presence and role flies in both areasshould not be overlooked (Monzon et al, 1991). The use of night soil in agricultural farms in the provinces of the north is still a common agricultural practice (De Leon et al., 1992). Another factor to be considered is the compromised sanitation practices of the sellers andvendors when handling fresh produce.

The result of this study is parallel to the study conducted by De Leon et al., (1992)wherein green leafy vegetables with more exposed surface area and with direct contact with the soil are more likely to be contaminated. This may also be attributed to the compromised methods of handling, processing, packaging and distribution of freshproduce (Beuchat et al., 1997).

Table 2. Determination of the significant difference in the prevalence of parasitesbetween two (2) selected markets using Test on Two Proportions

| Sample | X | N | Sample p |
|---|---|---|---|
| 1 | 17 | 20 | 0.85 |
| 2 | 18 | 20 | 0.90 |

Difference = p (1) - p (2) Estimate for difference: -0.05

95% CI for difference: (-0.166002, 0.106002)

Test for difference = 0 (vs not = 0): Z = -0.43 P-Value = 0.665

The computed value of the data above shows that there is no significant difference (-0.05) in the prevalence of parasites between the two (2) selected markets.

Ho: There is no significant difference in the prevalence of parasites between the twoselected markets.

H1 : There is a significant difference in the prevalence of parasites between the twoselected markets.

Alpha: 0.05

Critical Region: If computed P-Value < 0.05, reject Ho, and accept H1; otherwise do notreject Ho (if computed P-Value > 0.05)

Decision: Since computed P-Value (0.665) is greater than 0.05, do not reject Ho. There is nosignificant difference in the prevalence of parasites between two (2) selected markets.

Z computed = 0.5

Z tabular= 2.58 at 0.05 level of significance

Since, Z computed= 0.5< Ztabular = 2.58 accept all hypothesis. There is no significant difference between sample 1 and sample 2.

Table 3: Frequency distribution of parasites isolated from 35 positive leafyvegetable samples between two (2) selected markets

| Parasites | Monumento Public Market | Robinson's Supermarket | Overall Frequency |
|---|---|---|---|
| **Helminthes** | | | |
| Ascaris | | | |
| Strongyloides | | | |
| Hookworm | | | |
| E. vermicularis | | | |
| Toxocara | | | |
| Fasciolid egg | | | |
| Acanthocephalans | | | |
| Secernenteans | | | |
| Phasmid nematode | | | |
| Strongylus | | | |
| Ascarid egg | | | |
| **Protozoans** | | | |
| Entamoeba | | | |
| Gregarina sp. | | | |
| Coccidian oocyst | | | |
| Cyclophyllidean egg | | | |
| **Others** | | | |
| Pollen | | | |
| Fungal spores | | | |
| Insects | | | |
| Ciliates | | | |
| Pseudoparasites | | | |
| Free-livingorganisms | | | |

The table above shows the frequency distribution of parasites among the 35 positive vegetable samples between two (2) selected markets. Ascaris was the most common contaminant with a frequency of (73.1%), followed by Hookworm (27.7%), and unidentified parasite (24.4%). Gregarina spp. has the lowest prevalence rate with only (3.4%). Since both markets exhibit poor sanitation, there is no difference in the frequencyof Ascaris between the two public markets. This may be due to the fact that Ascaris eggs favor warm and moist climate and prevail in those areas with poor sanitation (Cauyan et al., 1994).

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The result above also shows the difference in the frequency of other parasites recovered from the positive samples, implying that there were parasites found on the vegetable samples sold at Monumento Public Market but were not found on the vegetable samples sold at Robinson's Supermarket. This was probably due to the different sourcesof vegetables sold at two public markets.

This limited but significant study suggests thorough washing of leafy vegetables toremove the parasites, hygienic practices and proper food handling should be observed, eradication of the use of night soil as fertilizers in order not to produce an avenue throughwhich parasites can contaminate the plants.

# CHAPTER FIVE

## SUMMARY, CONCLUSION AND RECOMMENDATIONS

The study focused on the identification and prevalence of intestinal parasites in leafy vegetables samples sold in selected public and private markets in Caloocan City.

Five kinds of leafy vegetables were chosen and collected as samples. The leafy vegetable samples included the kangkong (*Ipomea aquatica*), spring onions (*Allium fistolosum*), spinach (*Spinacea oleracea*), celery (*Apium graveolens*), and leek (*Allium porrum*).The collection of samples was done once a month from December, 2012 to January, 2013. The leafy vegetable samples obtained from the selected public and private markets were washed in distilled water and normal saline solution. Concentration method was used to isolate the intestinal parasites from the examined leafy vegetable samples. Light microscopy using LPO and HPO was used to examine and identify the intestinal parasites.

Fifteen medically important parasites were identified from the examined sediments of leafy vegetable samples. Pseudoparasites, fungal spores, pollen, mushroom spores, free-living ciliates, insects, and free-living organisms were also noted. The identified parasites were *Ascaris, Strongyloides, Acanthocephalan, Secernentea, Strongylus, Hookworm, Toxocara, Fasciolid.egg, Enterobius vermicularis, Phasmid nematode, Ascarid, Entamoeba, Gregarina sp., Coccidian oocysts and Cyclophyllidean egg.* Based on the test on two proportions, it was found out that there is no significant difference in the prevalence of intestinal parasites in the selected public and private markets. After statistical analysis, the computed data indicated that there is a significant difference in the number of intestinal parasites present in the leafy vegetable samples washed in distilled water and normal saline solution.

Parasites found in the examined sediments of leafy vegetable samples were both in their infective and non-infective stages. Despite of being non-infective, they are still a threat to human health.

The presence of intestinal parasites in the examined leafy vegetable samples may be attributed to geography, climate of place and human activities. The hot climate or summer season provides a suitable environment in the growth and development of intestinal parasites. Human activities such as the use of night soil, organic fertilizers and polluted water during cultivation of vegetables play a vital role in parasitic infestation. Poor sanitation, improper disposal of wastes, and mishandling of vegetables are also important factors to consider in the parasitic contamination of leafy vegetables.

Based on the test on two proportions, it was found out that there is no significant difference in the prevalence of intestinal parasites in the selected public and private markets. This was probably due to the use of night soil and organic fertilizer during cultivation of leafy vegetables. The data also showed that there is a significant difference in the number of intestinal parasites isolated from vegetables washed with distilled waterand normal saline solution. There were more intestinal parasites isolated from normal saline solution than in distilled water. This implies that normal saline solution is effective inisolating the intestinal parasites from the leafy vegetable samples.

After statistical analysis, computed data indicated that most of the vegetable samples were contaminated with intestinal parasites. These intestinal parasites can be athreat to human health if they are incidentally transmitted to the human body. Eating raw or minimally-processed leafy vegetables, poor personal hygiene of handlers of leafy vegetables, and poor sanitation can be modes of transmission of intestinal parasites to human body which are harmful to human health.

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The researcher then recommends that leafy vegetables be washed with normal saline solution because this effectively dislodges intestinal parasites from the leafy vegetables. Proper personal hygiene of handlers of leafy vegetables, proper disposal and environmental sanitation of wastes must always be observed to reduce the parasitic infestation of leafy vegetables sold at public and private markets. There is also a needto safeguard the general public on the risk brought about by the use of night soil as fertilizer in agricultural farms in raising the leafy vegetables, as it can be a potential source of parasitic contaminants.

The researcher would also like to recommend the following for the improvement and betterment of this research:

- Increase the sample size of the leafy vegetables to be collected.
- Do the sampling every month in one year period to identify which months have the highest prevalence of intestinal parasites.
- Use microscopes with good resolution and magnification to facilitate the examination and identification of intestinal parasites.

# APPENDICES

Fig. 1: Monumento Public Market


Fig. 2: Robinson’s Supermarket

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Fig. 3: Vegetable Samples from the Public Market

Fig. 4: Vegetable Samples from the Supermarket


Fig. 5: Washing of Vegetable Samples

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Fig. 6: Sedimentation

Fig. 7: Centrifugation


Fig. 8: Examination of Intestinal Parasites

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