Autonomous University of San Luis Potosí
Faculty of Chemical Sciences

Laboratory of General Microbiology

Searching for intestinal parasites in vegetables

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Days: Tuesday-Thrusday Schedule: 08:00-09:00 hrs
Abril 5th of 2017

QUÍMICO
FARMACO
BIÓLOGO

Objective

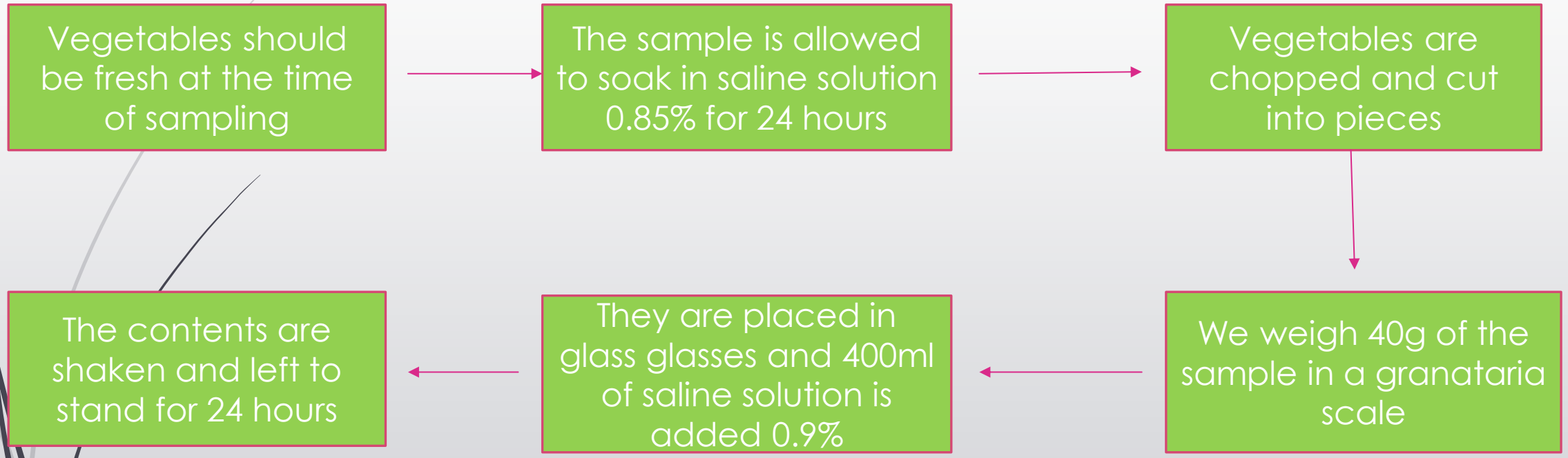
To perform the search of parasitic forms of protozoa and intestinal helminths in vegetables sold in home samples, using the saline centrifugation technique, microscopic observation with 10X and 40X objective, using lugol as a contrast dye

Introduction

- Protozoans are unicellular microorganisms that lack a cell wall. They usually lack color and are mobile. They are distinguished from prokaryotes by their larger size, algae lacking chloroplast and chlorophyll, yeasts and fungi by being mobile and mucosal fungi because of their inability to form fruiting bodies

- Because of their appreciable content of ascorbic acid, carotene and dietary fiber, vegetables are widely recommended as part of the daily diet. Celery, lettuce, cabbage, brussels sprouts and other vegetables that are generally eaten raw have been associated with outbreaks of diarrhea and even listeriosis. In addition, contamination with parasitic eggs such as Ascaris lumbricoides, Trichocephalus trichiurus, Entamoeba histolytica cysts, Giardia intestinalis and viruses such as hepatitis A has been found in this type of plant.

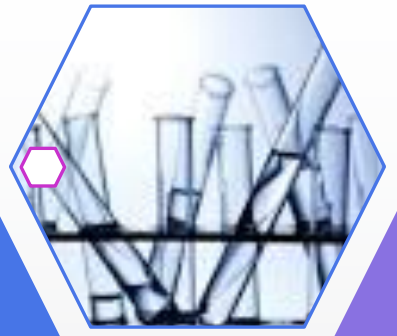
Collection and preservation of vegetables



Process

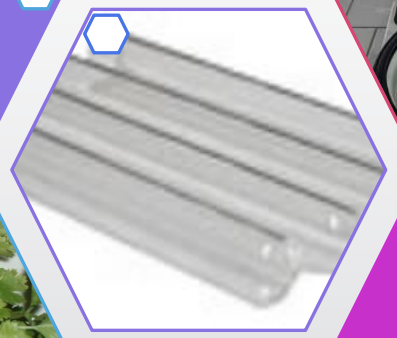
1. Samples will be removed.

2. Let the water rest for another hour.



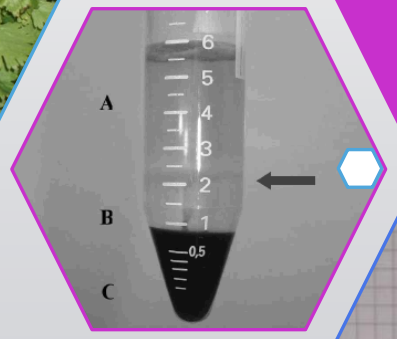
3. Decant the water 9/10 parts of the solution.

4. Place the sediment in test tubes.

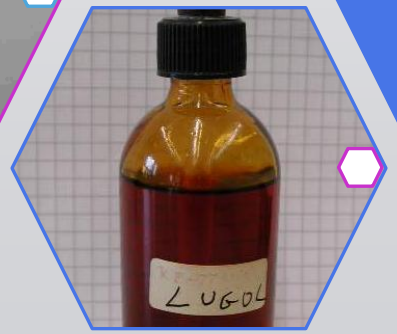


5. Centrifuge at 3000 rpm for 10 minutes.

6. Discard supernatant.



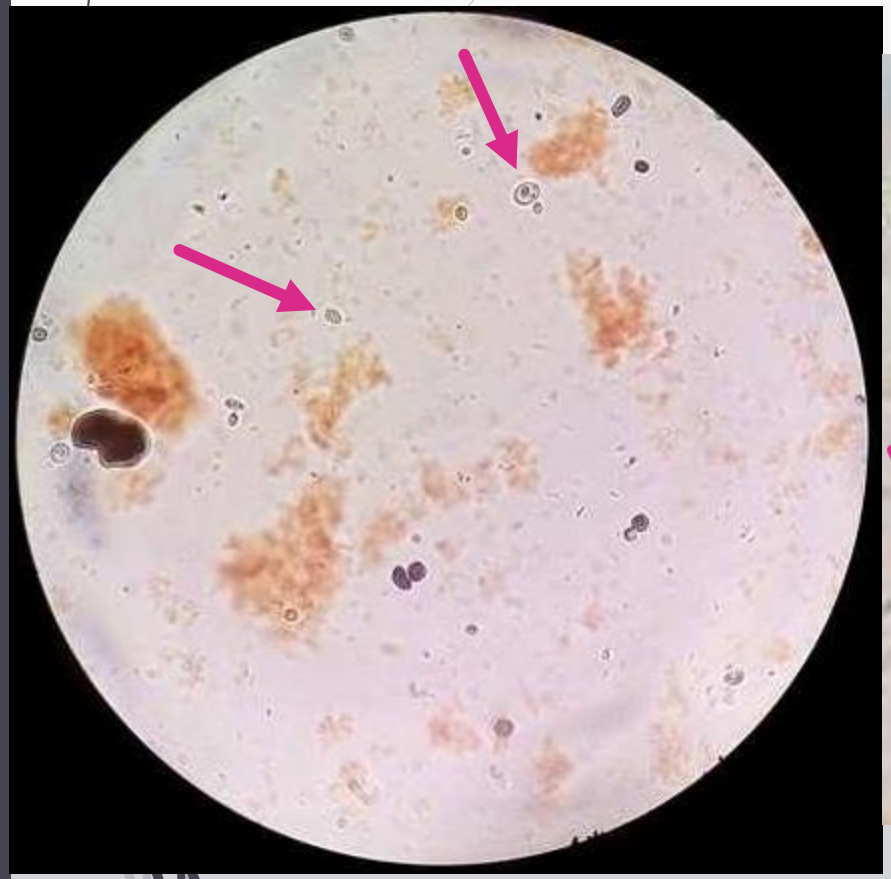
7. Perform the previous two steps until you finish the sample.

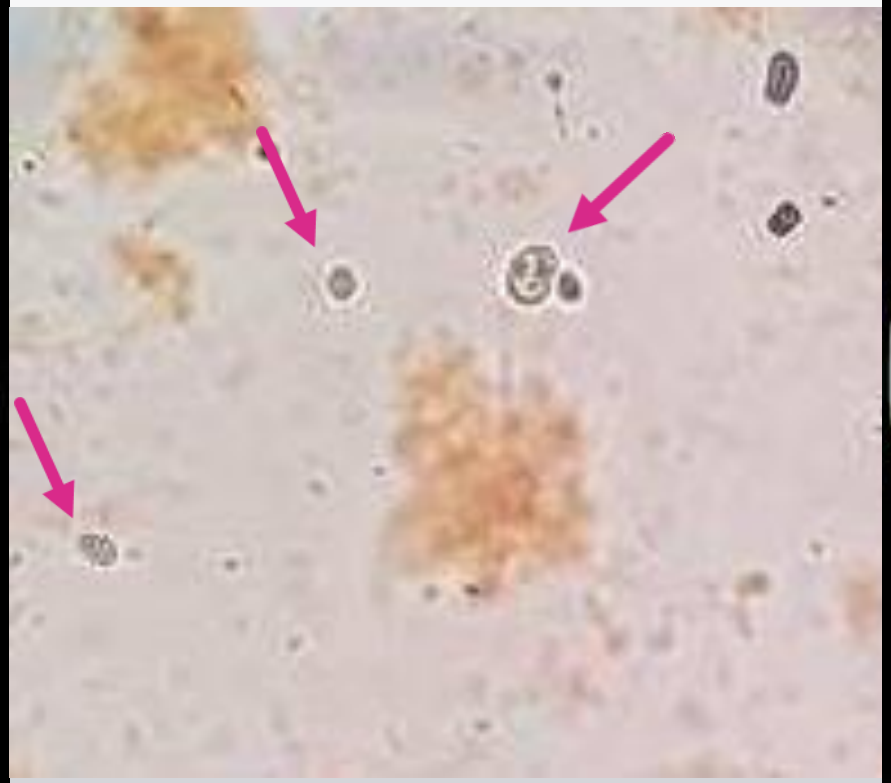


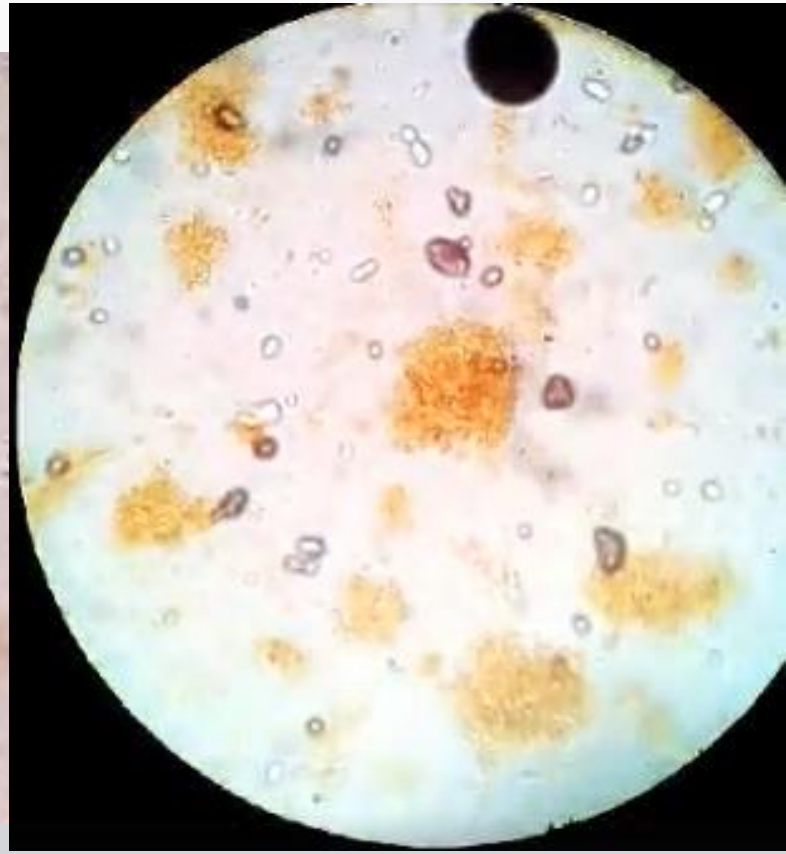
8. Using a pipette take a quantity of the sediment and place on a slide with a drop of lugol.

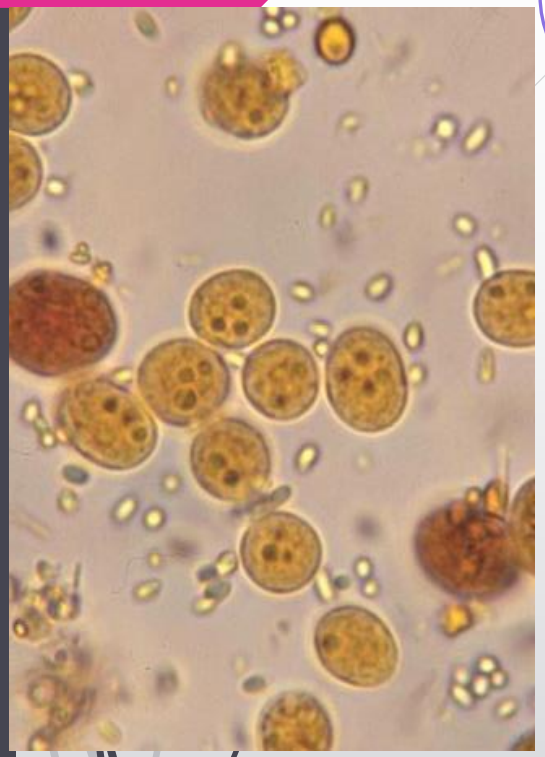
9. Place the coverslip and observe under the microscope at 10X and 40X.

Results







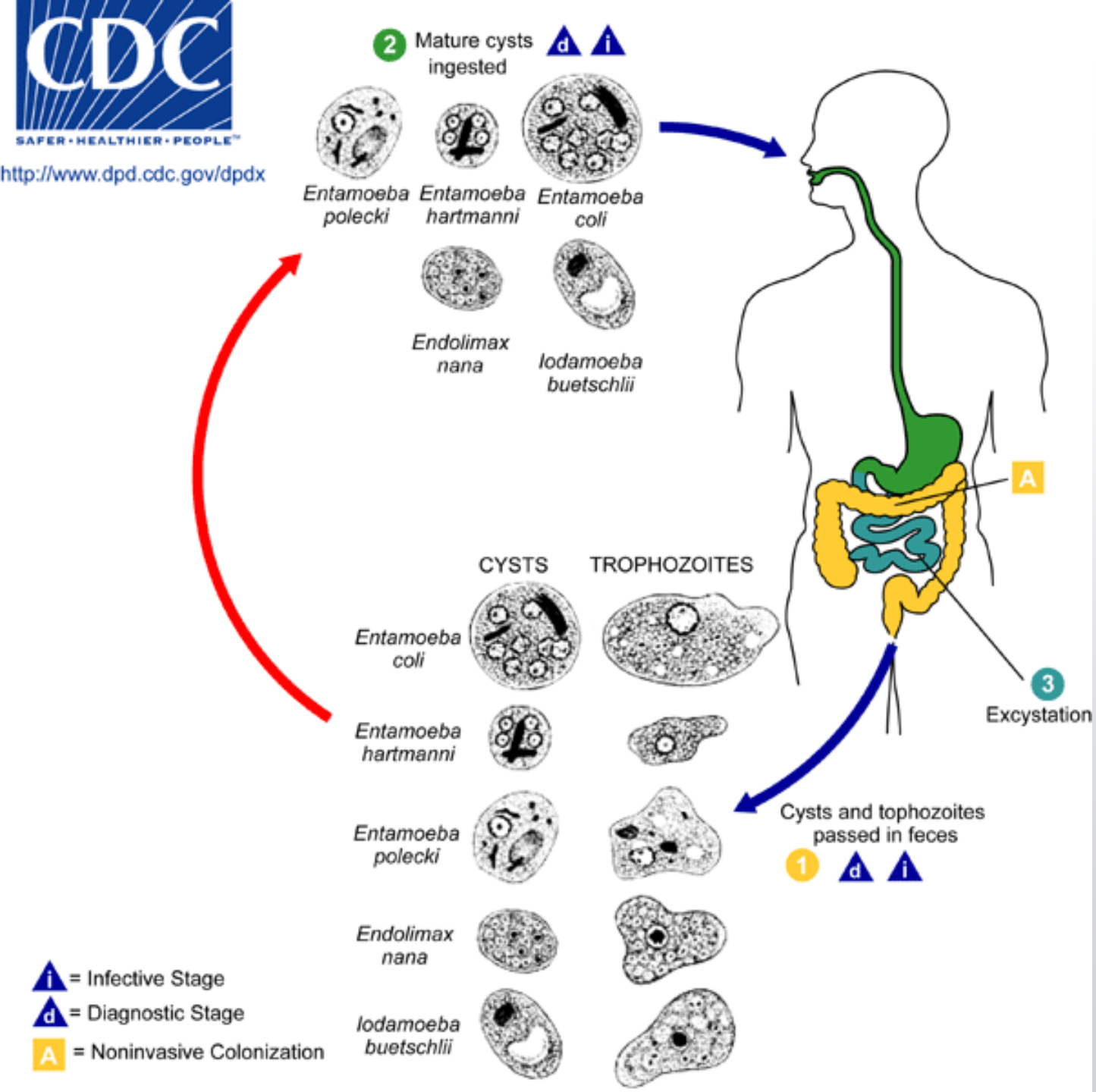


- Three samples of 40 g each were collected; Carrots, potatoes and cilantro obtained from markets.
- The corresponding procedure for parasite search was carried out.
- No parasites were observed in potato and coriander. Only bacteria
- In carrots were found bacteria and parasites, which, by their morphology, was deduced that it was Endolimax nana.

Endolimax nana

- Exclusive commensal parasite of the human
- Infection: ingestion of viable cysts
- Contamination of food and drink or poor personal hygiene
- High distribution in warm climates, and populations with hygienic efficiency
- Two stages of development: cyst and trophozoite

Reino	Protista
Phylum	Amoebozoa
Class	Archamoebae
Order	Mastigamoebida
Family	Mastigamoebidae
Genus	Endolimax
Specie	Endolimax nana



Diagnosis:
By demonstrating E. histolytica in the feces, colon, hepatic abscess wall or any other location.

Treatment:
Metronidazole (500 mg / 6 hours) for 10 days

Prevention:
Adequate sanitary control of water used for consumption purposes.

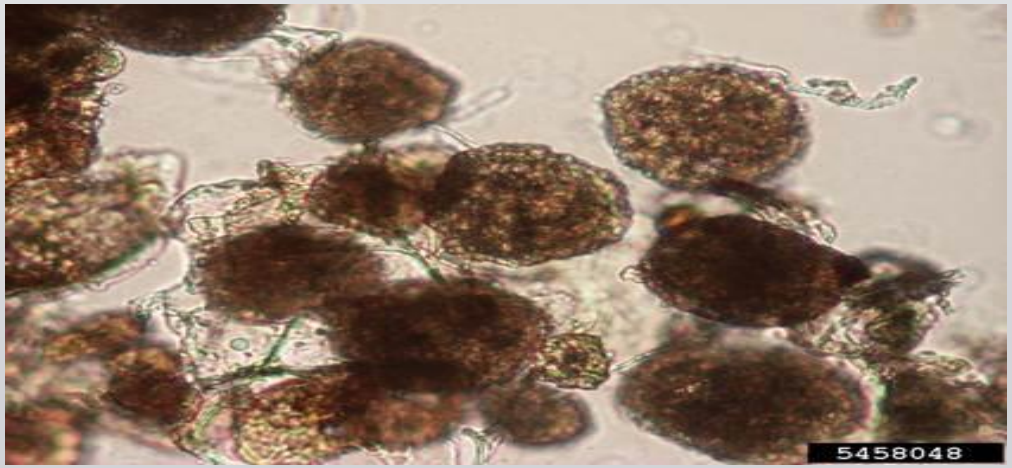
Paper
Spongospora subterranea

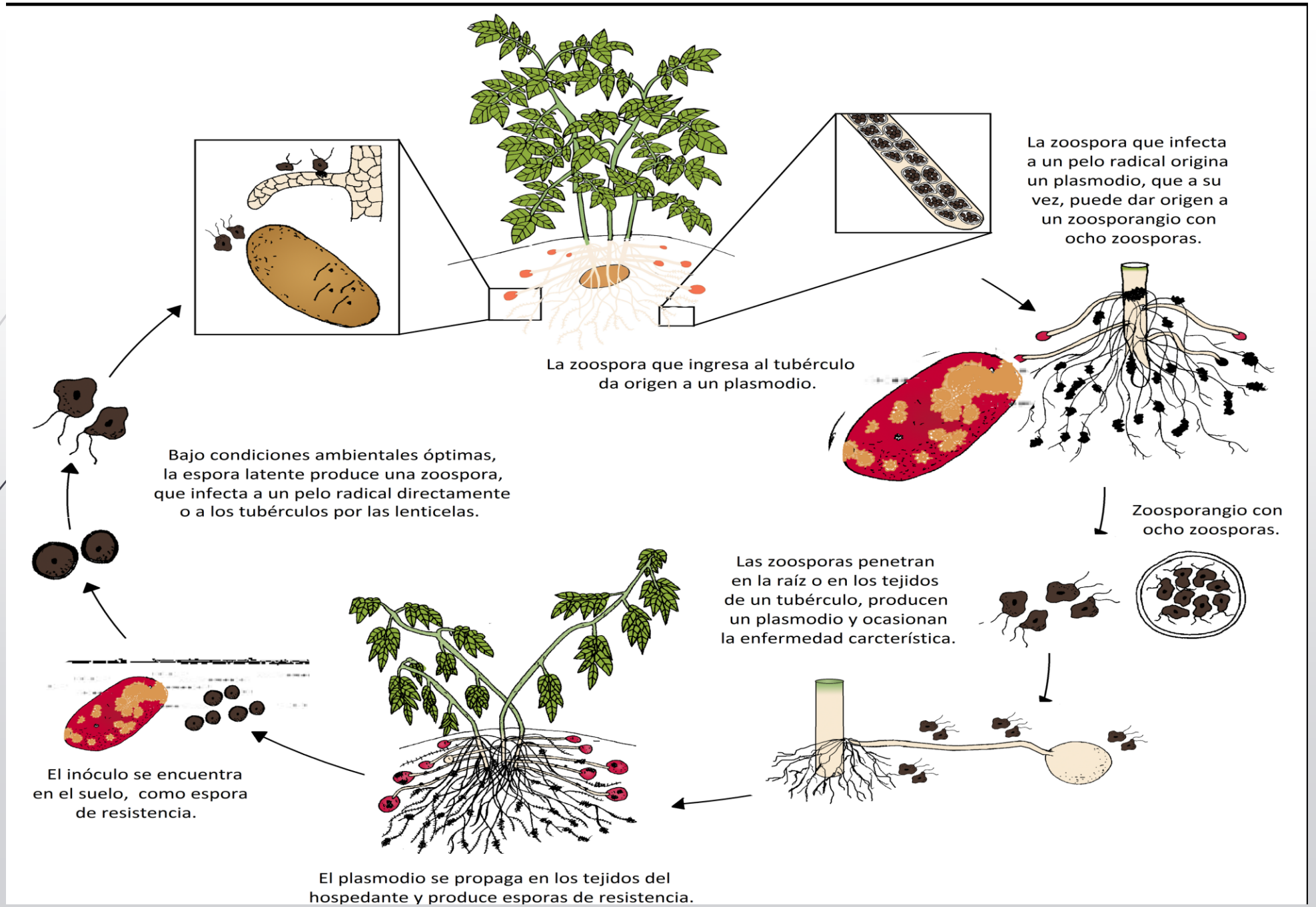
- Spongospora subterranea (Wallroth) Lagerheim f. Sp. Subterranea Tomlinson is the causal agent of the poplar mange. This pathogen is a protozoan, its infection occurs through zoospores which are released from cysts and are the main mode of dispersion of the disease.
- MATERIALS AND METHODS Obtention of cysts. To evaluate the best inoculum three sources of cysts were established from root galls, tuber pustules and soil collected

- Standardization of the concentration of cysts used in the experiment. A suspension of purified cysts in a 0.1% Tween 80 solution of 10 mg • ml of isolated kieselguers from the three sources was prepared and chamber counts of Neubauer, following the methodology of Castaño (1994), were prepared. To obtain a standard concentration of 2.4 x 104 cysts / ml of inoculum for all sources to be used

- Obtaining extracts of potatoes. The extracts were obtained from healthy potato roots (Solanum tuberosum), Diacol Capiro variety, for which 75 g of fresh root weight were taken and liquefied in 750 ml of distilled water.

- In this work, the effect of exudates of potato root, temperature and source of inoculum with isolated soil, root and tuber kerate was evaluated in order to verify the conditions in which the release of zoospores occurs.
- For this purpose two experiments were carried out, the first one evaluated the effect of water and extract of roots and in the second the extract of roots and the different sources of inoculum.
- Evaluations were done by counting mobile zoospores for 120 hours every 24 hours. It was observed that the root exudate has a high influence on the release of zoospores, which occurs between 15 and 23 ° C from 48 and up to 96 hours, no differences were detected between the sources of inoculum, which Indicates that regardless of their origin, if there are adequate environmental conditions, the kistars have the capacity to release a sufficient number of zoospores that can potentially initiate the infection process in the host.




La zoospora que infecta a un pelo radical origina un plasmodio, que a su vez, puede dar origen a un zoosporangio con ocho zoosporas.
La zoospora que ingresa al tubérculo da origen a un plasmodio.
Zoosporangio con ocho zoosporas.
Las zoosporas penetran en la raíz o en los tejidos de un tubérculo, producen un plasmodio y ocasionan la enfermedad carcterística.
El plasmodio se propaga en los tejidos del hospedante y produce esporas de resistencia.
El inóculo se encuentra en el suelo, como espora de resistencia.
Bajo condiciones ambientales óptimas, la espora latente produce una zoospora, que infecta a un pelo radical directamente o a los tubérculos por las lenticelas.

Paper
Comparative analysis of methods for the detection of parasites in vegetables for human consumption

- METHODS

Ten samples of lettuce, 10 watercress and 10 arugula were collected in free markets and supermarkets in the western region of the city of Sao Paulo. The vegetables had been packed in individual plastic bags. They were sent to the Clinical Analysis Laboratory. They were determined as unit of sample for the vegetables, whole leaves, that in the laboratory separated in 2 lots, intact and fragmented.

- PROCEDURE

Partial fragmentation of the 10 samples of each of the vegetables in duplicates was terminated with 30 samples. They were then immersed in distilled water and left to stand for sedimentation for a period of 24 H

- 10 mL of the separated from each of the samples was collected, the material was passed into 13 x 100 mm tubes and centrifuged at 2500 rpm for 1 min for 4 times; The top was discarded. In the last wash, 3 mL of zinc sulfate solution (33%) was added. 2.16
- Shortly thereafter, a film formed on the surface of the tube was collected with a platinum spatula, then passed to glass plates and then stained with lugol.2
- After the separation and centrifugal-flotation processes, the samples were numbered and identified according to the type of plant and technique used. All samples were analyzed in triplicate for 3 min in an optical microscope, both the fragmented and the intact of the 2 techniques reached a total of 360 readings. The positive samples were counted in numbers of cysts, eggs and larvae, verified by field for the statistical analyzes.

- ANALYSIS OF THE DATA

Data were pooled and analyzed by percentages. A graphical analysis methodology proposed by Bland and Altman (of Bland-Altman graph analysis) 17 was used to evaluate the concordance between the 2 methods of analysis, which marked the differences between the values obtained with the tests against the means of both values.

- RESULTS

Of the samples analyzed in triplicate (n = 120), 46.6% presented positive results for some type of parasite. Lettuce samples were the most contaminated with 52.5% (Table 1), followed by the arugula with 45.0% (Table 2) and, finally, the watercress with 42.5% (Table 3). Among the pollutants observed, there was a predominance of Balantidium coli, positive in 24 samples in total, representing 20.0% of the contamination of the lots in general and when evaluated separately plant species, the percentage was 45.0 % Of lettuce contamination, 15.0% of rocket pollution and 2.5% of watercress contamination.

The presence of Entamoeba coli cysts was found in 21.6% of the total lots (most common structure), with 12.5% in lettuce, 17.5% in watercress and 27.5% in arugula. Entamoeba histolityca was detected in 5% of the lots, with a frequency of 7.5% in lettuce, 7.5% in arugula and in watercress. As for helminths, the analysis revealed the presence of Trichuris trichiura eggs in 4 watercress samples (3.3% of the lots and 10.0% of the total contamination of the plant), and Strongyloides stercoralis larvae (Identified according to the dichotomous key of Valada18) was also verified in the same watercress samples (2.5% of the total).

Paper

- Parasicium strains can be obtained from banana peels, potato peeling, cattle manure, goats, small amounts of the organic horizon of any soil

Obtaining the Population of Microorganisms

The production of the Paramecium Sp. Strain was achieved by harvesting the grass that is subjected to a dehydration process to the environment. The preparation of paramecia can be made directly from non-dehydrated grass by making an infusion, but the breeding cup is very low with respect to the first procedure.

Preparation of culture medium

Fill the bottle with normal water from the aqueduct and there are two ways to eliminate the traces of chlorine contained in this: One is to let the water rest for 48 hours to later pour the dehydrated fodder and the second is to add to the water of the bottle a gram of Sodium hyosulfite and immediately introduce the grass

Sowing

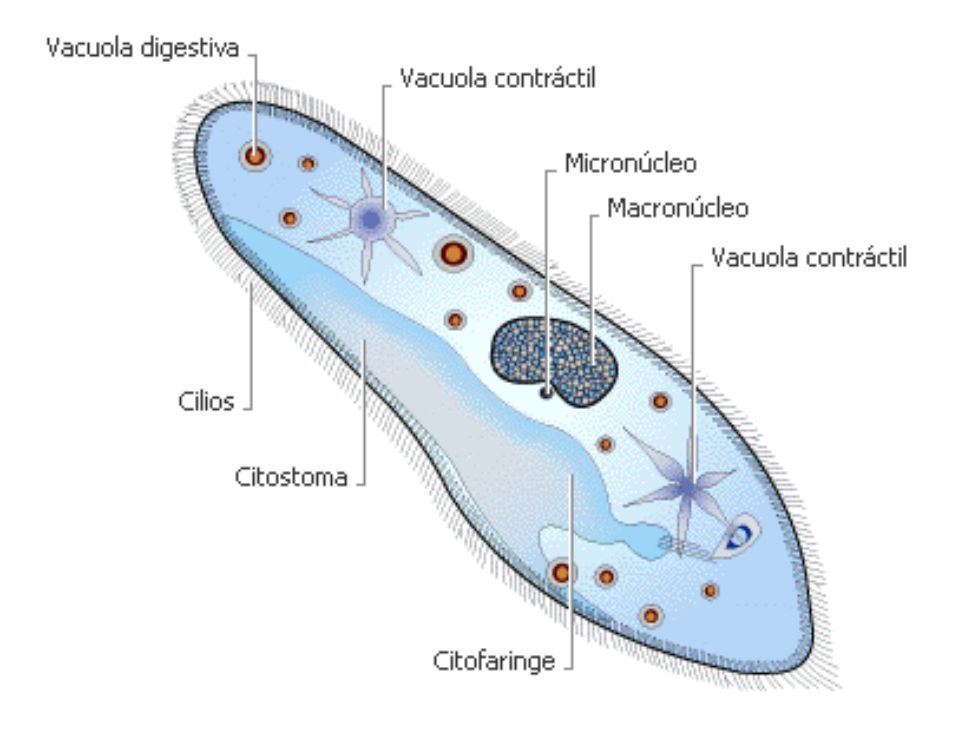
Once the water has been stored and conditioned, the completely dehydrated grass (after exposure of the forage to the sun for 15 days) is introduced into the containers. The amount of grass to be planted is 7gr for every 4 liters of water. Higher concentrations of organic matter inside the flasks leads to poor fermentation and subsequent rotting of the culture medium.

Nutrition and Cultivation Feeding

During the first two days a bloom of bacteria occurs which feed on the detritus that are suspended in the liquid, on the third or fourth day there are eclosion of paramecios and these will feed on the bacteria since they are holozoic; When the crop takes a transparent brown color and does not release any odor, a phenomenon that happens around the fifth or sixth day is the moment to begin to nourish and to feed the paramecia colony.

Extraction of Paramecios

Most of the protozoa, among them paramecia, have a positive phototropism. This particularity is useful for directing a light beam (a flashlight), these microorganisms are grouped in large clouds from which it is very easy to extract them with a syringe or a dropper


Vacuola digestiva
Vacuola contráctil
Micronúcleo
Macronúcleo
Vacuola contráctil
Cilios
Citostoma
Citofaringe

Paper. Search for cysts and eggs of human intestinal parasites in vegetables and fruits

Sampling

- We investigated two markets in the metropolitan area (A and B)
- In each market 25 samples of 25 grams of each fruit were taken

- Samples of celery, watercress, coriander, cabbage, strawberries, lettuce, paprika, cucumber, radish and carrot were studied.
- Most contaminated vegetable: A: cilantro. B: celery

Findings

- Diners: Entamoeba coli and Endolimax nana
- Pathogens: Entamoeba histolytica, Giardia lambllia and Trichuris trichura

I = Fruta o verdura sin lavar
II = Fruta o verdura lavada al chorro del agua
III = Fruta o verdura escaldada
IVa = Fruta o verdura tratada con Hidroclonazone
IVb = Fruta o verdura tratada con Microdyn
V = Fruta o verdura tratada con detergente

Eh = *Entamoeba histolytica*
G1 = *Giardia lamblia*
Tt = *Trichuris trichiura*
En = *Endolimax nana*
Ec = *Entamoeba coli*

Porcentaje de quistes y huevos de parásitos intestinales humanos, contaminantes de los vegetales estudiados

	Mercados	
PARÁSITOS	**"A"**	**"B"**
Entamoeba coli	50.0	50.0
Entamoeba histolytica	31.8	35.0
Endolimax nana	9.0	0.0
Giardia lamblia	4.5	15.0
Trichuris trichiura	4.5	0.0

Resultados obtenidos según la variante aplicada Mercado "A"

VEGETALES	I	II	III	IVa	V	TOTAL
Apio	0	0	0	Eh	Eh	2
Berro	2 Ec, Eh	0	0	Ec, Eh	0	5
Cilantro	Ec, Tt, Eh	0	Eh, Ec	0	2 Ec	7
Col	0	0	0	0	0	0
Fresa	0	0	0	0	0	0
Jícama	0	0	0	0	0	0
Lechuga	0	3Ec, Eh	0	0	Ec	5
Pápalo	0	0	0	0	0	0
Pepino	0	0	0	0	0	0
Rábano	Ec	Ec	G1	0	0	3
Zanahoria	0	0	0	0	0	0
TOTAL	7	5	3	3	4	22

Resultados obtenidos según la variante aplicada Mercado "B"

VEGETALES	I	II	III	IVb	V	TOTAL
Apio	0	G1, Ec	Ec	2 Eh	G1	6
Berro	0	Eh	0	0	Eh	2
Cilantro	0	0	0	Ec	0	1
Col	Ec, G1	0	Ec	Ec	0	4
Fresa	0	0	Ec	0	Eh, Ec	3
Jícama	Ec	0	0	0	0	1
Lechuga	0	Eh	0	Ec	0	2
Pápalo	0	0	0	0	0	0
Pepino	0	0	0	0	0	0
Rábano	Ec	0	0	0	0	0
Zanahoria	0	Eh	0	0	0	1
TOTAL	3	5	3	5	4	20

Interpretation of results

- *Endolimax nana is* a parasitic parasite that spreads through dirty water that is used improperly to wash fruits and vegetables.
- In other studies, as in the General Microbiology laboratory, evidence has been found of the presence of Endolimax nana, in vegetables such as; Carrot, celery, cilantro, among others. The search for parasites in different samples is carried out.
- Later it is verified when studying its morphology.
- The parasite that we found experimentally, coincides with the parasite (among others) that has been found in various samples of vegetables.
- The articles were reduced only to our country (Mexico), so that there were no variations due to climate, soil, etc.
- The bacteria found in vegetables can cause damage to the health of people, especially as it is a very large number of bacteria

Conclusions

- We searched for parasitic forms of protozoa and intestinal helminths in samples of vegetables purchased from markets or grocery stores. We observe how some protozoa may be present in vegetables and cause intestinal diseases, which can be avoided by washing and disinfecting the vegetables.

- The obvious presence of intestinal parasites in the analyzed foods reinforces the issue under discussion, such as the failure of agricultural processes and the mismanagement of products at distribution points, since this type of products is easily accessible and Are products of mass consumption because of their nutritional properties, the vulnerability of the population is higher and the propensity to spread diseases of this type is high, and thus becomes a public health problem. The detected parasites are responsible for intestinal disorders mainly in the child population, the elderly and immunocompromised patients

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