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DETECTION OF PARASITES CONTAMINATING RAW CONSUMABLE VEGETABLES IN ASSIUT CITY, ASSIUT GOVERNORATE, EGYPT

By
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Abstract

Vegetables are a major way for the transmission of human parasites, especially those that are consumed raw and or not properly washed. The leafy vegetables act as a reservoir host for these parasites or act as a critical intermediate host for some parasites encysted to complete their life cycle. The study aimed to evaluate the extent of parasitic contamination of various raw consumed vegetables available commercially in Assiut City. A cross-sectional study was conducted in 2017 & 2018, where a total of 240 samples were collected (40 samples from each of Watercress, Radish, Parsley, Coriander, Green onion, and Lettuce). A direct simple washing technique available to households was used to recover parasites in each plant. Parasitic contamination rate was calculated.

The overall parasites were 202/240 (84.1%). Parsley was the highly contaminated vegetable whereas Lettuce was the least contaminated. The most encountered contaminating parasite was *Cryptosporidium* parvum oocysts 76.25% (183/240) most frequent in radish (38/40), while *Isospora* sp. was the least detected one (2.1%). Parasites were viable only in 50% of tested samples. The high percent of vegetable contamination detected by this study urge other alternative methods for farming, irrigation and washing in the local community.

Keywords: Assiut City, Raw green vegetables, Zoonotic parasites, Viability, Infectivity

Introduction

Raw vegetables are an essential element for human health due to their nutritional value, of vitamins, dietary fibers, minerals and associated with a reduction of risky diseases (Ezatpour *et al,* 2013).

The trend of eating raw green vegetables, as a salad or mild cooked vegetables to preserve their taste and content of heat-labile nutrients, increased the possibility of food-borne infections which could be contaminated with enteric bacterial, viral and parasitic pathogens (Said, 2012).

Unprocessed vegetables that have not been properly cleaned or peeled in particular have proven to be a medium for spreading large numbers of parasites. They become a potential source of human infection in their contamination (Slifko, *et al,* 2000).

Human infections associated with the consumption of contaminated raw green vegetables represent a public health concern mainly in developing countries, where that was still underestimated (Dorny *et al,* 2009). The soil transmitted vegetables parasites are due to manure, sewage sludge, irrigation water and wastewater from livestock operations or directly from wild and domestic animals, as well as bad hygienic practices during harvesting, production, transport, processing, preparation, distribution and marketing or even at home handlers and many other environmental factors (Punsawad *et al,* 2017).

Protozoa and helminths are the main parasites involved in vegetables, which were characterized by long survival time as cyst, oocyst or ova (Berger *et al,* 2012). More than 40 million persons worldwide were predisposed to zoonotic parasites (Shahnazi *et al,* 2010). Increased interest in organic farming might abate the parasitosis risk. Pickers, handlers, packers, and those involved in processing of vegetables have role in contamination (Robertson and Gjerde, 2001).

Generally speaking, *Taenia* spp., *Ascaris* spp., *Giardia* spp. and *Cryptosporidium* spp. were the most pathogenic parasites contaminated raw vegetables implicated in outbreaks. Others species were of *Entamoeba*, *Isospora, Enterobius, Trichuris, Toxocara, Hymenolepis,* and *Fasciola* contaminated the green vegetables (Bekele *et al,* 2017). Soil-parasites identification were critical for human health (Hunter and Thompson, 2005).

This study aimed to assess parasitic contamination of some raw consumed vegetables available commercially in Assiut City between 2017 and 2018. Usage direct simple washing technique available to households to recover parasites in each vegetable.

Material and Methods

Study type: This was a descriptive cross-sectional field and laboratory study.

Study area: Targeted area was Assiut City, the Capital of Assuit Governorate, with municipality of 27.252ºN 31.01ºE.

Sampling was monthly carried out between October 2017 and September 2018. A total of 240 samples as 40(~250gm) from each raw vegetable were collected, included radish (*Raphanus sativus*), watercress (*Eruca sativum*), parsley (*Petroselinum crispum*), green onion (*Allium fistolosum*), coriander (*Coriandrum sativum*) and lettuce (*Lactuca sativum*) were randomly purchased from different markets, and greengrocers in the City. Samples were transported in separate sterile nylon bags to theDepartment of Parasitology laboratory for examination.

Samples were prepared by separating them leaf by leaf and any damaged leaves were discarded, and about 50gm of each sample was rinsed in 500ml distilled water and removed immediately. The waste-water of the washes were collected in separate labeled conical flasks and each sediment was centrifuged (Garcia, 2007).

Parasitological examination: All samples were subjected to wet mount microscopic exami-nation followed by floatation and sedimentation concentration technique (Zajac and Conboy, 2007), Lactophenol cotton blue stain (Khanna *et al*, 2014) and Modified Z-N stain (Garcia, 2007). Acridine orange technique was used for positive samples of four washes using a fluorescence microscope to detect parasite viability (Hassan *et al*, 2019).

Statistical analysis: Data were computerized, tabulated and analysis using SPSS version 20. Frequency and percent for qualitative data and mean ± SD were done. Analytic (inferential) statistics; Chi-squared test for comparing frequencies, and ANOVA test to compare means and results were considered significant when P-value ≤ 0.05.

Results

The overall positive parasites in the 240 examined vegetable samples were 202 samples (84.1%); 37 in Watercress, 37 in Radish, 38 in Parsley, 29 in Coriander, 34 in Green onion, and 27 in Lettuce (Fig. 1).

The different parasites were detected were helminthes eggs, filariform lavae and protozoal cysts and/or oocysts (Figs. 2-11).

Table 1: Distribution of parasite species and their count among each green vegetable.

Vegetables	Watercress	Radish	Parsley	Coriander	Green onion	Lettuce	Total	P-value
Cryptosporidium parvum	32	38	35	28	29	21	183	<0.0001
Giardia spp.	1	3	0	0	3	1	8	0.02
Entamoeba spp.	4	5	7	0	0	0	16	<0.0001
Eimeria	8	4	8	0	6	0	26	<0.0001
Isospora	0	1	0	0	4	0	5	<0.0001
Cyclospora	3	1	2	0	1	0	7	<0.0001
Cestodes egg	1	2	4	1	8	1	17	<0.0001
Nematodes larvae	3	7	2	3	13	3	31	<0.0001
Encysted metacercaria	5	5	11	0	2	0	23	<0.0001
Arthropods	9	9	8	3	0	2	31	<0.0001
Total	66	75	77	35	66	28	365	<0.0001

All vegetable types showed significant difference as to the recovered parasitic infective stages. Parsley was the highly contaminated vegetable and lettuce was the least contaminated. The highly detected parasite was *C. parvum* oocysts 76.25% (183/240), followed by radish (38/40), parsley (35/40), and watercress (32/40), they were on green onion (29/40), coriander (28/40), and lettuce (21/40). Nematode larvae were 12.9% (31/ 240), mainly on green onion (13/40), followed by radish (7/40), while coriander, lettuce and watercress each was (3/40), and parsley was lowest contaminated one (2/40).

The arthropods were mainly detected in watercress, radish, and parsley (9, 9, & 8, respectively) but coriander and lettuce showed lower contamination (3, & 2, respectively). None arthropods was on green onion.

Other parasites were detected with variable prevalence rates included: *Eimeria* spp. oocysts with a rate of 10.8% (26/240) distributed most frequent on parsley and watercress (8/40) followed by green onion (6/40) and then radish (4/40), but neither was detected on lettuce nor coriander.

Fasciola spp. encysted metacercariae were 9.6% (23/240) the most frequent on parsley (11/40), watercress and radish (5/40), and then green onion (2/40), but neither was detected on lettuce nor coriander.

Cestode eggs were 7% (17/240), with the high prevalence on green onion (8/40), parsley (4/40), radish (2/40), coriander, watercress and lettuce have the same rate (1/40) *Giardia* sp. cysts prevalence rate was 3.3% (8/240), high prevalence in green onion and radish (3/40), watercress and lettuce (1/40), parsley, but none on coriander.

Isospora spp. recorded a prevalence of 2.1% (5/240), on green onion (4/40), radish (1/40), watercress, lettuce, and parsley coriander, but none on coriander. *Cyclospora* spp. showed a prevalence rate of 2.9% (7/240), on watercress (3/40) and parsley (2/40), radish and green onion (1/40), but neither was detected on lettuce nor coriander. The details were given (Tabs. 2 & 3).

Table 2: Percent of viable parasites detected

Washing type	Viable Parasites in the samples (N= 240)		X^2	*P-value*
	Viable	Not viable		
Wash 1	120 (50%)	120 (50%)	131.4	<0.0001

Table 3: Total parasites no. and viability in different vegetables examined.

Vegetable	Parasites No.	*P-value*	Viable parasites	*P-value*
Watercress	3.25± 4.4	0.004	1.8 ±1.0	0.03
Radish	2.3 ± 1.7	<0.0001	1.8 ± 0.9	0.003
Green onions	2.1± 3.5	<0.0001	2.3 ± 1.5	0.003
Coriander	1.4 ±1.6	0.005	1.4 ±0.7	0.008
Lettuce	0.3 ± 0.5	<0.0001	1.0 ± 0.0	*NS*
Parsley	2.9±2.6	<0.0001	2.4 ±1.4	*0.03*

Discussion

The present study showed that 202 out of 240 vegetable samples were conta-minated with zoonotic parasitic (84.1%). Parsley showed the high contamination, followed by radish, green onion, watercress, coriander and the least contaminate was lettuce.

The inter-vegetables parasite contamination difference can be conveyed to the different height, density, and form of plant growth that affect the micro-environment of the free-living stages of parasites and thus play a role in plant-parasitic load. Leafy vegetables and/or vegetables with uneven surfaces make it easier for the parasite stage to attach to the surface on the farm or when washed with contaminated water; unlike those with smooth surfaces (Avcioglu *et al,* 2010; Alemu *et al,* 2019). Parsley and radish showed higher rates due to their irregular surfaces which make the parasitic stages attach more easily to the surface of these vegetables.

Several Egyptian studies showed different results from the present one. Said (2012) in Alexandria reported the highly contaminated samples was detected in the rocket (46.7%) and the least number of contaminated one was on green onion (13.3%) Eraky *et al*, (2014) in Qualyobia Governorate found that lettuce was the most contaminant vegetable (45.5%), followed by watercress (41.3%), parsley (34.3%), green onion (16.5%), and leek was the least one (10.7%). Etewa *et al.* (2017) in Sharkia Governorate reported that 55.7% of parasitic contamination in watercress; followed by lettuce (45.7%) and parsley (44.2%).

Abroad, Al-Binali *et al.* (2006) in Abha Province, Saudi Arabia reported that 28% of the parasitic contaminations were on green onion, 25% in radish, 17% in watercress, and 17% in lettuce. Damen *et al.* (2007) in Tripoli detected parasites contamination on 96% lettuce and 100% on cress samples. The discrepancy between the different results might be due to variation in geographical locations, agricultural method, climatic and environmental factors, vegetable types and sampling, diagnosis, and community sanitary status (Alemu *et al*, 2019).

Different parasites were detected in the ex amined vegetables included helminthic, protozoan and Arthropod parasites. *Cryptosporidium parvum* was the most frequently detected parasite (69.4%) on the green vegetables samples. *C. parvum* gave the highest density on radish samples 90% and on parsley 83.1%. No doubt, cryptosporidiosis is mainly one of veterinary parasites among livestock and stray dogs and cats as well as rodents (Youssef and Uga, 2014). Also, *C. parvum* remained active in water environments for over a year without loss of ability to multiply; that prevails as an extremely difficult problem in removal and inactivation of *Cryptosporidium* spp. from water supplies even by chlorination (Hassan *et al*, 2019). Oocytes from contaminated source(s) strongly adhered to aquatic plants, a habit common in developing countries (Said, 2012), a suggestion supported by a previous study conducted in the local community in Assiut city deal with waterborne *Cryptosporidium* contamination (Sayed *et al*, 2016).

The current study showed a higher contaminated rate of *Cryptosporidium parvum* than other Egyptian studies carried out as 7.6% in Sharkia (Etewa *et al,* 2017) and 29.3% in Alexandria (Said, 2012). Abroad, in Ethiopia it was 7.6% (Alemu *et al*, 2019), in Norway, it was 4% on lettuce (Robertson and Gjerde, 2001). Results difference could be explained by the previous studies conducted in Assiut city, which revealed a high *Cryptosporidium* contamination rate in water supplies (reach 79%) (Sayed *et al*, 2016; Hassan *et al*, 2019).

In the present investigation, beside *Cyclospora* spp.; *Giardia* spp. cysts were the least detected parasite (1.4%) of the total examined vegetable samples. This result disagreed with Eraky *et al,* (2014) reported *Giardia* as the most prevalent parasite (8.8%) in Benha, Egypt; while in Alexandria it was 6.7% in different vegetables (Said, 2012).

Robertson and Gjerde (2001) in Norway found *Giardia* cysts in only 5.5% of lettuce samples. It was 6.5% in Tehran (Gharavi, *et al,* 2002); 9% in Iran (Daryani *et al*, 2008); 31.6% in Saudi Arabia (Al-Megrin, 2010); 4% in Qazvin, Iran (Shahnazi *et al,* 2010); 8.2% in Shahrekord, Iran (Fallah *et al*, 2012); 22.5% in North of Iran (Siyadatpanah *et al*, 2013); in Jordan, *Giardia spp*. was found in lettuce (23%) (Ismail, 2016).

The relative difference in results can be conveyed to different geographic and atmospheric\ ambient conditions like high humidity and using different studied vegetables. *Cyclospora* oocysts in the present study were detected in (1.4%) of the examined samples. Higher contamination rates were recorded from previous studies conducted in different parts of Egypt and the world; in Alexandria, Egypt Said (2012) reported a contamination rate of 21.3%. In Nepal was 29.8% of green leafy vegetables (Sherchand *et al,* 2004). Ortega *et al.* (1997) in Peru reported 1.8 % *Cyclospora* spp., which agreed with current results.

Eimeria oocysts were 9% in all examined samples. Although *Eimeria* oocysts resist standard wastewater treatment and can be found in sewage sludge end products commonly used for fertilization of ready-to-eat crops or in runoff-impacted surface water used for irrigation. Haniloo *et al.* (2016) in Iran reported that *Eimeria* oocysts were scanty as 0.6% in fresh lettuce.

In the present study *Entamoeba* spp. was 4.1% that more or less agreed with 6.2% in Sharkia (Etewa *et al,* 2017) and 6.8% in Qualyobia respectively (Eraky *et al,* 2014).

Al-Sanabani *et al.* (2016) in Yemen found *E. histolytica* was 26.9% and *E. coli* was 18.2%, Shahnazi *et al.* (2010) in Iran found *Entamoeba* coli cysts were 2.8%, while *E. histolytica* was 1.4% and Guimarães-Luz *et al.* (2010) in Brazil found *E. coli* was 26.0%. *Isospora spp*. recorded (3.3%), it is agreed with the study of (Bekele *et al*, 2017) conveyed *Isospora belli* contamination rate was 3.06%.

In this study, nematode larvae were 8.9% in all samples. In Alexandria, nematode larvae were 1.6% in all samples (Said, 2012), and in Benha were 3.6% (Eraky *et al,* 2014). This disagreed with 36.5% in Brazil (Guimarães-Luz *et al,* 2017) and 22% to 25.8% in Iran (Shahnazi *et al,* 2010; Siyadatpanah *et al,* 2013). The cultivation fields were irrigated from the River Nile and local freshwater canal, with subsequent silting and deposition of sandy loam top-soils and increased soil moisture promoting the emergence of these larvae, as well as the prevalence of nematode larvae in wastewater, within the domestic animal and human population (Etewa *et al,* 2017).

Fascioliasis encysted metacercariae were found in 7.6% of all raw vegetable samples. This high rate denoted the re-emergence of fascioliasis among 67 patients (Ramadan *et al,* 2019). In the past few decades, the incidence of human fascioliasis increased worldwide, particularly in South America, Asia, and Africa including Egypt (Mas-Coma, *et al,* 2005). Animal manure or sewage from livestock pens or abattoirs as fertilizers for plants introduced fascioliasis to aquatic vegetables, and non-aquatic ones such as lettuce eaten raw (WHO, 1995).

In Sharkia, Benha, and Alexandria, cestode eggs rates were 2.8, 4.9%, 2.6% & 4.7% & 5.7% respectively (Etewa *et al,* 2017, Eraky *et al,* 2014; Said, 2012). Shahnazi *et al.* (2010) in Iran found trematodes and cestodes parasites were (1.4% & 2.3, respectively).

Collectively, agricultural use of sewage, soils, vegetables, and grass contaminated by helminths ova and protozoa cysts, due to the discharge of untreated sewage, surface water and seawater. By consumption of raw vegetables, drinking or swimming in contaminated water, man and animals were exposed to pathogenic microorganisms. In order to stop this health hazard the vegetables and products should be treated mainly at home level, and at industrial level (Ayed *et al,* 2009).

Conclusion

The study showed a high rate of vegetables parasitic contamination in a variable pattern, the issue requires modulation or even finding other safe methods of farming, irrigation and washing to diminish or eliminate such contamination in the local community.

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Explanation of figures

Fig. 1: No. of +ve samples in each vegetable

Fig. 2: *Cryptosporidium* spp. oocyst on raw vegetables examined stained with Ziehl Nelsen stain at 400& 1000x in A, B & E respectively; C & D parasite oocysts stained with acridine orange at 400 and 1000x respectively

Fig. 3: *Cyclospora* spp. oocyst on raw vegetables examined stained with Ziehl Nelsen at 1000x.

Fig. 4: Sporulated oocyst of *Eimeria species* on raw vegetables examined at 1000x

Fig. 5: *Isospora oocyst* on raw vegetables A) stained with wet method B) parasite oocysts stained with acridine orange and C) stained with lactophenol cotton blue stain at 1000x

Fig. 6: *Blastocyst hominis* stained with lactophenol cotton blue stain at 1000x.

Fig. 7: *Entamoeba* sp. stained with lactophenol cotton blue stain at 400x in A & B respectively; **C**, parasite stained with acridine orange at 400x.

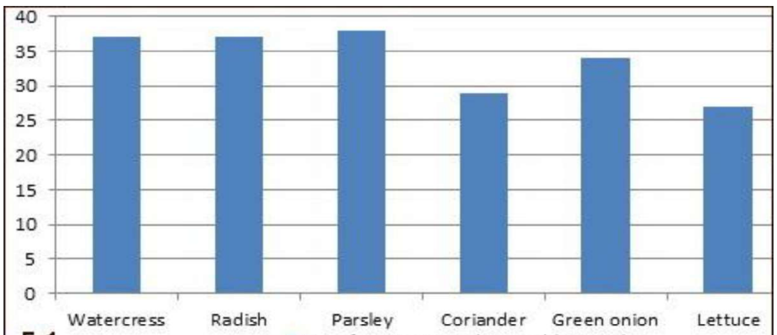
Fig. 8: G*iardia lamblia* cyst stained with lactophenol cotton blue at 400x.

Fig. 9: Encysted *metacercariae* on raw vegetables A) with wet method at 200x; B) stained with lactophenol cotton blue stain 400x. C) stained with acridine orange at 400x

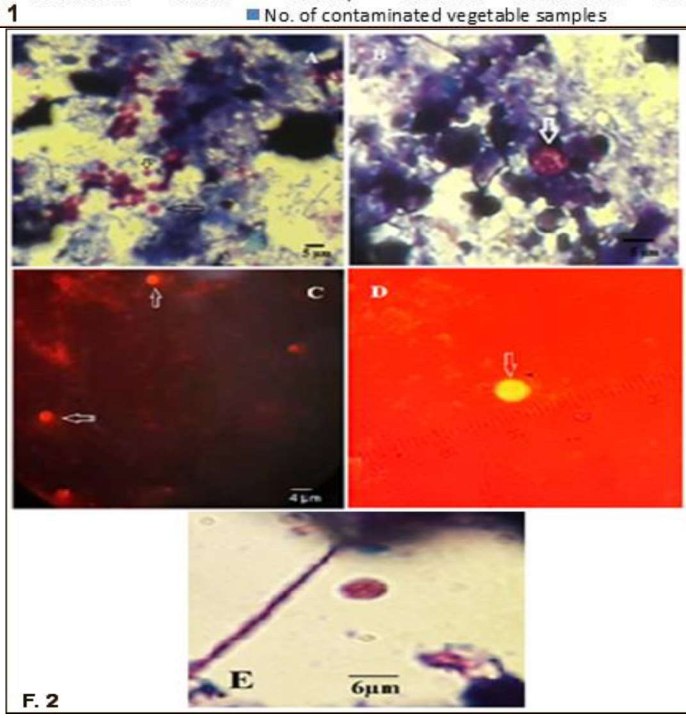
Fig. 10: Eggs: A & B) *Toxocara* spp. at 200x & 400x respectively. C) *Dipylidium caninum*, *Hymenolepis nana* viewed in D) at 400x (with lactophenol cotton blue) and F) at 200x with acridine orange stain, E) *Anclystoma* spp. stained by lactophenol cotton blue at 200x.

Fig. 11: *Nematode larvae* on raw vegetables stained with lactophenol cotton blue stain at 200x in A, B, C &D respectively; E&F parasite stained with acridine orange stain at 200x& 100x

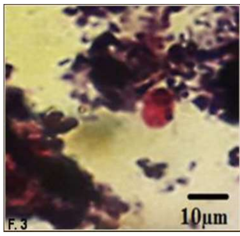
Fig. 12: Arthropod species detected on raw vegetables included A) sucking lice, C&D) mites and B) Crustacean (*Cyclops*) at100x & 200x



F. 1



F. 2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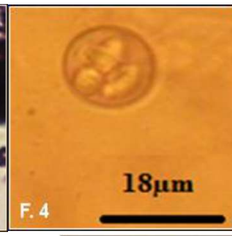
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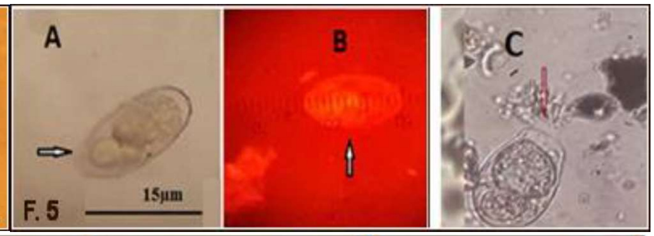
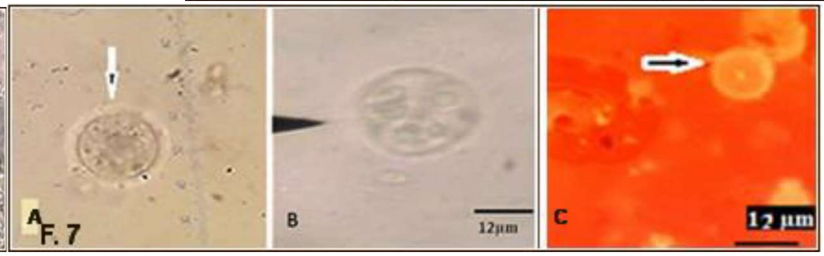
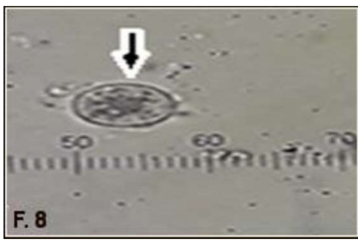
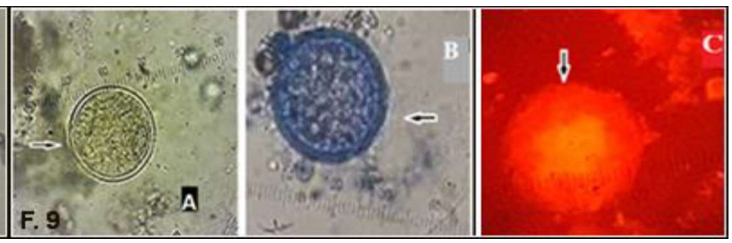
F. 4

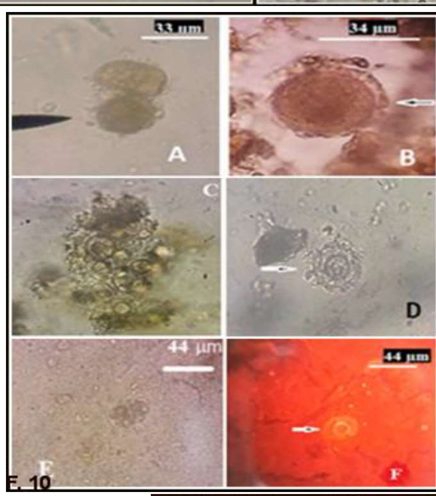

A
B
C
15µm
F. 5

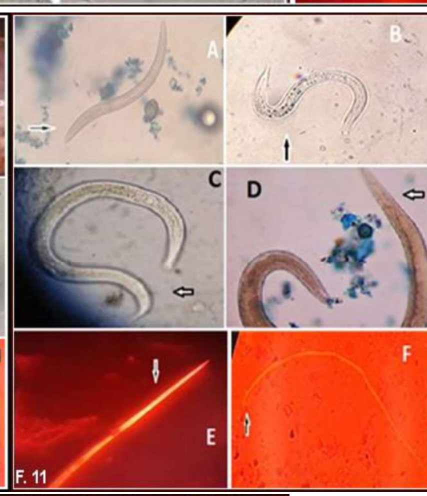

F. 6


A
F. 7
B
12µm
C
12 µm


50
60
70
F. 8


F. 9
A
B
C


33 µm
A
34 µm
B
C
D
44 µm
E
44 µm
F
F. 10


A
B
C
D
E
F
F. 11


A
B
C
D
F. 12