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Cryptosporidiosis in Human Immunodeficiency Virus patients in Tripoli Medical Center

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Abstract:

Cryptosporidiosis is a leading cause of protracted, life threatening diarrhea in Human Immunodeficiency Virus **(**HIV) infected patients. The aim of this study was to identify risk factors for transmission of cryptosporidiosis in HIV infected adults and to assess prevalence of Cryptosporidium in HIV patients in Tripoli Medical Center (TMC) Hospital. The study was a descriptive prospective cross sectional study carried out on 65 HIV patients in TMC hospital, only symptomatic (diarrheal) HIV infected patients were screened. All Cryptosporidium spp. positive cases were enrolled in the study and interviewed to record socio-demographic information, water supply and animal contact. The results showed the prevalence of infection with cryptosporidium in HIV infected patients was 5% (5 cases) out of 65 patients. The finding of this study indicates that *Cryptosporidium parvum* is an important cause of morbidity and mortality in immunocompromised patients in Tripoli, also pollution of water supply provides an important source of infection. And In addition to that the immune status of the patients play an important role in the infection. From the present study the following are recommended: Periodic examination of patients who are suspected to be in special risk, Improved environmental sanitation as water supply and sanitary sewage disposal system.

Keywords: Cryptosporidium parvum, HIV, Diarrhea

1. Introduction

CRYPTOSPORIDIOSIS is caused by Cryptosporidium, a parasite classified as an emerging pathogen by the Centers for Disease Control and Prevention (CDC) (Guerrant RL, 1997). The organism infects the gastrointestinal epithelium to produce a diarrhea that is self-limited in immunocompetent persons but potentially life-threatening in immunocompromised persons, especially those with the acquired immunodeficiency syndrome (AIDS). Infection by this parasite accounts for up to 6% of all diarrheal disease in immunocompetent persons. 24%of persons with both AIDS and diarrhea worldwide (Guerrant RL, 1997). The most of the HIV positive patients infected to opportunistic parasitic infections dies because of chronic diarrhea (MaKri A, 2004), (Paul RH, 2002). Those patients whose cellular immunity has beenweaken due to HIV, opportunistic infections such as toxoplasmosis, cryptosporidiosis, isosporiasis and micrisporidiasis, display a high prevalence rate (MaKri A, 2004), (Paul RH, 2002), (Ferreira MS, 2000), (Goodgame RW, 1993), (Ijzerman M, 1997), (Maniatis T, 1982). The mortality rate due to Cryptosporidium diarrhea in people infected with HIV, Particulaly AIDS, has been considerable in the last few years (Nahravanian H, 1991), (Shojaei S, 1991).

Cryptosporidium is an important waterborne pathogen that infect gastrointestinal or respiratory tract of mammals, including humans. The Cryptosporidium oocyst has enough resistant to drinking water disinfectants such as chlorine and chloramines to survive in many water treatmentand distribution systems (Korich D, 1990), (Listle J. T., 1995). Contamination of drinking water by infectious been the cause of large and serious outbreaks of cryptosporidiosis such as the Milwaukee outbreak in 1993. Where affected an estimated 403,000 persons (Mackenzie WR, 1994). Oral paromomycin is recommended for AIDS-infected patients as an effective and tolerable drugfor treatment and, it is efficiency depends on it is dose/age (Paul RH, 2002), (Oktedalen O, 1994), (Colford JM, 1996). The aim of this study is to assess the Incidence and Prevalence of *Cryptosporidium parvum* infection in HIV patientswith diarrhea as important cause of diarrhea, morbidity and mortality, and to explore the risk factors and mode of transmission of *Cryptosporidium parvum.*

2. Materials and methods

This research was a cross-sectional, descriptive, prospective study, in which stool sample were collected from 65 AIDS patients with diarrhea attending TMC hospital and the collected data was filled in the questionnaires together with their personal information, including their name,

sex and etc. where patients were interviewed record socio-demographic information, water supply and animal contact. Stool sample were examined using modified acid fast method.

2.1. Apparatuses and Materials

A Microscopic slides, Marker and pencil, Pipettes, stool containers, wooden sticks, Rack for slides, surgical gauze, light microscope, Forceps.

2.2. Stains and reagents

Normal saline, Carbolfuchsin, Methylene blue 0,1% solution, Decolorizer (acid alcohol).

2.3. Study time and sitting

The present study was carried out from January 2017 to July 2017 in Tripoli medical center.

2.4. Study design

A cross- sectional descriptive study was done.

2.5. Study sample

A total of 65 AIDS patients with different sex and age groups were participated in this study.

The study was designed to investigate HIV patients presented with diarrhea. The size of the sample was calculated according to this equation as advised by statistic group:

$$n = \frac{(p-n)}{\frac{(p-n)}{N} + \frac{E_2}{z(1-\frac{\alpha}{2})}}$$

n=size of required sample. P= p value, N= population size from which sample was taken, E= constant=0.05.

2.6. Data Collection

History was obtained from patients or their relative in a pre-designed questionnaire specially prepared to fulfill the required information for this study (Annex 1).

2.7. Laboratory samples

Each patient was provided with one fecal collection vial 5 ml and the fresh sample was immediately worked out.

2.8. Procedures

The screening method used was modified Ziehl-Neelsen (acid-fast) staining technique. After gross examination of the sample characteristics, we label a microscopic slide with number which is the same number on the stool container of the patient, and then with an applicator stick, we pick up a small amount of the stool and spread it in a thin smear by rubbing the material back and forth with the wooden stick over the middle part of the slide.

We try to make the layer uniform and even as possible and of the correct density. If the stool was not watery weadd a drop of a normal saline and mix with a feces, some samples contains mucus so we pick up mucus also with the stool. In attempt to minimize the number of false negative three slides were prepared from each sample.

Fecal smear were air-dried, then stained as follow:

1. Smears were fixed in absolute methyl alcohol for three minutes.
2. The smear were stained in cold carbol fuchsin (Annex 2).
3. Decolarization was done using 3% Acid-Alcohol (Annex 3) until color ceased to flood out(from 30-60 second).
4. This was followed by rinsing in tap water and draining.
5. Counter staining was done using 1% methylene-blue (Annex 4) for 1 minute, then slides wererinsed in tap water.
6. Smears were left to dry by standing slides upright and then examined using high power and100x-oil immersion objective.

   Oocyst in a modified Ziehl-Neelsen stained smear appears as acid fast densely stained redspherical structure measuring 4-6µm in diameter.

2.9. Statistical analysis:

The Statistical Package for Social Science (SPSS) version 15 which is a software for biostatic analysis was used to achieve valid and reliable results in this study.

3. Results:

Cryptosporidium oocyst were detected in 5 stool samples of HIV diarrheic patients.

Cryptosporidium oocyst were detected more frequently among diarrheic HIV patients between (30-40 years) of age (80%, 4/5; p <0.08). (Table 1) shows the distribution of Cryptosporidium isolated from HIV patients with diarrhea according to the age. Although Cryptosporidium were detected at a higher rate in female (60%, 3/5) than in male (40%, 2/5) diarrheic HIV patients, the difference was statistically significant (P < 0.05). (Table1) shows the distribution of Cryptosporidium isolated from HIV patients with diarrhea according to the gender, there was no significance difference between males and females.

Out of the five HIV Cryptosporidium diarrheic patients were civilized in their living (60%, 3/5) while the others were rural in their living (40%, 2/5), (Table1) shows the distribution of Cryptosporidium isolated from HIV patients with diarrhea according to their living.

Table (1). The distribution of Cryptosporidium isolated from HIVpatients with diarrhea according to the age, sex and living

	Positive (n=5)	Negative (n=60)
	No. (% of positive)	No. (% of negative)
Age in years		
=< 29	1 (20%)	16 (26.7%)
30 - 44	4(80%)	23 (38.3%)
+ 45	0 (0%)	21 (35%)
Chi-square= 3.75	*P*=0.08	
Gender		
Male	2 (40%)	32 (53.3%
Female	3 (60%)	28 (46.7%)
Chi-square= 0.33	*P*=0.5	
Living		
Rural	2 (40%)	18 (30%)
Civilized	3 (60%)	42 (70%)
Chi-square= 0.22	*P*=0.6	

In addition to diarrhea the main clinical presentation were fever in 4 of 5 HIV diarrheic patients (80%), 3 (60%) had nausea, 2 (40%) had abdominal pain, and 3(60%) had vomiting, Table (2) shows clinical symptoms associated with Cryptosporidium isolated from HIV diarrheic patients in Tripoli.

Table (2): Distribution patients who had positive of cryptosporidiumaccording to symptoms

	Positive (n=5)	Negative (n=60)
	No. (% of positive)	No. (% of negative)
Fever		
Yes	4 (80 5)	50 (83.3%)
No	1 (20%)	10 (16.7%)

Nausea		
Yes	3 (60%)	34 (56.7%)
No	2 (40%)	26 (43.3%)
Abdominal pain		
Yes	2 (40%)	33 (55%)
No	3 (60%)	27 (45%)
Vomiting		
Yes	3 (60%)	34 (56.7%)
No	2 (40%)	26 (43.3%)
Diarrhea		
Yes	5 (100%)	35 (58.3%)
No	0 (0%)	25 (41.7%)

(2/5) they have other medical condition which affect their immunity: fever, nausea, and vomiting were significantly associated with HIV diarrheic patients associated with other medical condition affecting their immunity (Table 3-a) distribution patients who had a medical condition that is known to affect their immunity according to symptoms.

Table (3-a) Distribution patients who had a medical condition that is known to affect their immunity according to symptoms

	Yes (n=21)	**No (n=30)**	**Don't know(n=14)**
	No. (% of yes)	**No. (% of no)**	**No. (% of don't know)**
Fever			
Yes	19 (90.5%)	23 (76.7%)	12(85.7%)
No	2 (9.5%)	7 (23.3%)	2 (14.3%)
Nausea			
Yes	15 (71.4%)	16 (53.3%)	6 (42.9%)
No	6 (28.6%)	14 (46.7%)	8 (57.1%)
Abdominal pain			
Yes	12 (57.1%)	15 (50%)	8 (57.1%)

No	9 (42.9%)	15 (50%)	6 (42.9%)
Vomiting			
Yes	15 (71.4%)	15 (50%)	7 (50%)
No	6 (28.6%)	15 (50%)	7 (50%)
Diarrhea			
Yes	14 (66.7%)	18 (60%)	8 (57.1%)
No	7 (33.3%)	12 (40%)	6 (42.9%)

In Table (3-b) shows the distribution 5 patients who had a medical condition that is knownto affect their immunity according to symptoms.

Table (3-b) Distribution 5 patients who had a medical condition that is known to affect their immunity according to symptoms

	Yes (n=2)	**No (n=3)**
	No. (% of yes)	**No. (% of no)**
Fever		
Yes	2 (100%)	2 (66.7%)
No	0	1 (33.3%)
Nausea		
Yes	2 (100%)	1 (33.3%)
No	0	2 (66.7%)
Abdominal pain		
Yes	1 (50%)	1 (33.3%)
No	1 (50%)	2 (66.7%)
Vomiting		
Yes	0	3 (100%)
No	2 (100%)	0
Diarrhea		
Yes	2 (100%)	3 (100%)
No	0 (0%)	(0%)

In Table (4-a) Shows the Distribution patients have history with contact with other family member had diarrhea according to taking a medical condition which affect immunity.

Table (4-a): Distribution patients have history with contact with other familymember had diarrhea according to taking a medical condition which affect immunity

taking a medical condition which affect immunity	***family member had diarrhea***	
	Yes (n=18)	**No (n=47)**
	No. (% of yes)	**No. (% of no)**
Yes	10 (55.6%)	11 (23.4%)
No	7 (38.9%)	23 (48.9%)
Don't know	1 (5.6%)	13 (27.7%)

It was detected that among 5 HIV diarrheic patients who are Cryptosporidium positive having history of contact with other family member with diarrhea (66.7%, 2/5), (Table 4-b) shows the distribution of 5 patients have history with contact with other family member had diarrhea.

Table (4-b): distribution 5 patients have history with contact with other family member had diarrhea according to taking a medical condition which affect immunity

taking a medical condition which affect immunity	***family member had diarrhea***	
	Yes (n=3)	**No (n=2)**
	No. (% of yes)	**No. (% of no)**
Yes	2 (66.7%)	0
No	1 (33.3%)	2 (100%)

In regard to water supply as seen from this work water from home filter represent an important source of infection, (56.9%, 3/5) of positive cases were using water processed from home filter, (38%) were using well water, (35.4%) using water from tab, Figure (1): water supply.

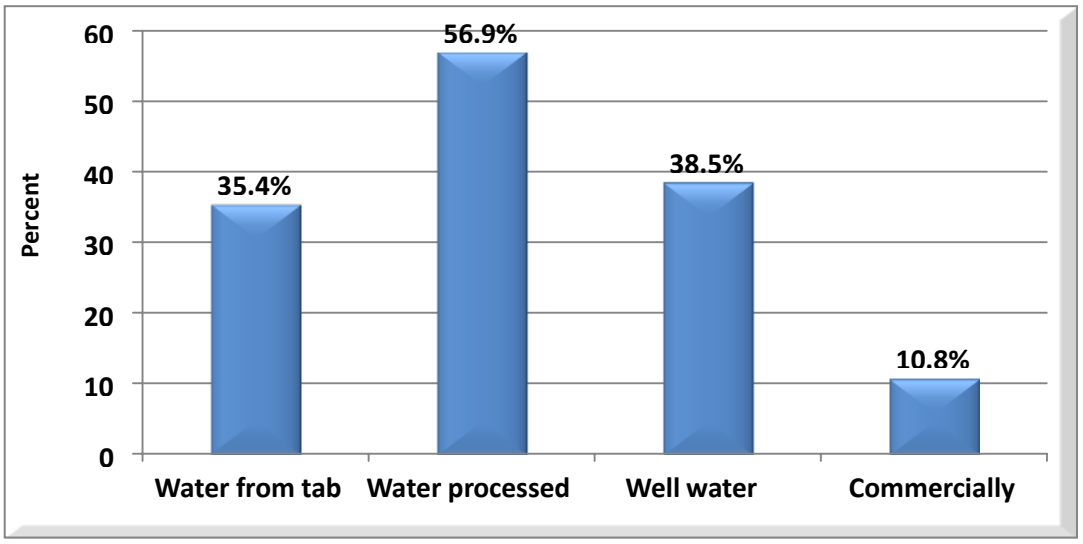

Figure (1): water supply

4. Discussion:

Cryptosporidiosis has been increasingly recognized as a cause of diarrheal illness both in immunocompotent as well as immunocompromised patients, which is why should be taken and weighting up the risk factor that might be induced from it is spread, as diarrhea can result in dehydration with significant morbidity and mortality.

Although to my knowledge, no previous study of Cryptosporidiosis in HIV patients in Libya done before, But the finding of this study confirms the importance of Cryptosporidium as a cause of diarrhea especially in immunocompromised patients.

The prevalence of 5% in HIV-infected patients with cryptosporidial diarrhea observed in this study is similar to that in a study from the UK (Cotte L, 1999), lower than those from Haiti (Malebranche R,1983), Italy (Brandonisio O, 1993) and higher than in a North American study (Sorvillo FJ 1994). This diverge may be a reflection of different periods studies, environmental conditions or therapeutic fashion. Since Cryptosporidium is one of the diseases capable of even causing death, a lot of researches have been done on it. In most of cases high prevalence is reported.

Cryptosporidium prevalence in HIV + patients was reported by Pape, otler, Bran-danezeu and sauda as 30%, 23%, 33.3% and 19.5% respectively (Brandonisio O, 1993), (Hazrati-Tappeh KH, 2004). In other study conducted on HIV patients living in southwest of Iran during 2014-2015 the prevalence rate was (10.8%) (S. Tzipori, 2002).

Srisuphanunt M et al. conducted a study in urban areas of Thailand to detect the prevalence and genotyping of Cryptosporidium isolated from HIV/AIDS patients using nested-PCR-RFLP technique. They showed that the prevalence of cryptosporidiosis in hospitalized HIV/AIDS patients during their study was 18.4% (S. Tzipori, 2002). Moreover, high rates of infection among HIV/AIDS patients have been reported in Indonesia (11.9%), India (56.5%) and Malaysia (11.5) (Tumwine JK, 2005), (Taherkhani H., 2007).

Data regarding the role of Cryptosporidiosis in HIV patient's diarrhea in North Africa and the Middle East was scarce. Currently, 18 reports on cryptosporidiosis among HIV-infected individuals are available from 12 country in Africa, with prevalence ranging from 3.8% to 73.6% (Meamar AR, 2007). Studies in Ethiopia, which contributes 2.1% of the African HIV/AIDS patients, have reported high prevalence of cryptosporidiosis (11% to 25%) (Meamar AR, 2005), (Sarfati C, 2006). A study in Imam Khomeini Hospital (Tehran) in 1995 on 35 patients, has reported 11.4% infection (Meamar AR, 2007). Hazrati-Tappeh et al reported 11.5% and 30, 48% infection rate in renal transplants and hemodialysis patients, respectively (Hazrati-Tappeh KH, 2006). The distribution of cryptosporidiosis does not differ significantly between sexes; this finding disagrees with that recorded in KirKuk (Al-Moula, 1999) in Baghdad, (Al-Gelany, 1998) and in china (Chen et al., 1998). These studies reported that the distribution of infection in males was higher than females. On the other hand (Al-Moula, (1999), found that the rate of infection in females were higher than males in Kirkuk. Concerning the age distribution, there were no statistically significant differences between the age group, most age affected. In this study all HIV patient were affected between 30-40 years of age (80%, 4/5; $p<0.08$), indicating no significant association was observed between infection rate and gender, nor infection rate and age group. Although they detected Cryptosporidium at higher rates from patients with history of contact with other family member with diarrhea compared with other negative patients the difference was statistically significant and ($p<0.05$). So, person to person transmission seems to play a significant role. Prior to eating and food preparation after touching clothing, bedding, toilets, or bed pans from anyone with diarrhea or coming in contact direct or indirect with anything contaminated with human stool in the environment and the hospital setting.

In this work we evaluate the role of immunity on the presence of infection the result was not statistically significant compared with other studies, in one study on 34 Children hospitalized in Oncology Pediatric Department of Tripoli Medical Center, with various forms of malignancies,

suspected immunodeficient from chemotherapy, nine of them had diarrhea and the rest were asymptomatic. Infection were detected in this group. Same study performed in Naples city (Sarfati C, 2006), situated in the temperate Mediterranean region of southern Italy. The parasite in this study was found in 11(22%) of asymptomatic immunodeficient patients and2 (4.8%) of 42 symptomatic patients. This result consistent with our result in this work.

As regards the residency of the patients under study, it was shown that patients in rural areashave lower rates of infection than those in urban areas. Similar finding were reported in Kirkuk province (Al-Moula, 1999), but these finding disagrees with the finding in orea (Chai et al., 1996) where higher rate of infection was reported in rural (14%) than urban areas (3.7%).

The high rate of infection in patients using filtered water for their drinking (56.9%). Could be attributed mainly to small size of oocyst round 4-6 µm, and it is resistant to routine chlorination. Each mature oocyst containing sporozoites, May to avoid unboiled tap water, filter with appropriate filters, home distiller can be used. However, water that comes from protected well or spring water sources is less likely to be contaminated. Which indicate statistical significance between.

Diarrhea and water supply. The first documented waterborne outbreak in San Antonio, Texas was linked to swage contamination of a well-water supply that was chlorinated but not filtered (Antonio RG, 1985). In 1987, an outbreak in Georgia resulted in an estimated 13,000 cases of cryptosporidial enteritis despite the filtered and chlorinated public supply (Hayes EB, 1989).

5. Conclusion:

To my knowledge, this is the first study to identify Cryptosporidium oocysts as pathogen causing diarrhea in HIV patients in Libya. The finding of this study indicates that Cryptosporidium. Parvum is an important cause of morbidity and mortality in immunocompromised patients in Tripoli, also pollution of water supply provides an important source of infection. In addition to that the immune status of the patients play an important role in the infection. More studies are necessary in other major cities of Libya to identify various risk factors which play major role in transmission and spread of infection.

From the present study the following are recommended

1). Periodic examination of patients who are suspected to be in special risk.

2). Applying more sensitive diagnostic techniques like monoclonal antibody immunofluorescence.

3). Training of parasitologists in diagnosis of cryptosporidium sp. As this parasite represents a problem especially among immune-suppressed patients, and it might emerge in the community at any time.

4). Improved environmental sanitation as water supply and sanitary sewage disposalsystem.

5). Cryptosporidium sp. As considered an important opportunistic infection which may be life threatening in immunosuppressed, special attention and awareness to the immune status of positive cases should be taken in account.

6. Ethical Considerations:

The samples from human and with approved from the Research Ethics Committee of Libyan Board of Health Specialties (Research Approval Letter) REC protocol Number: REC 01.10/2017 Date: Monday, October 9, 2017.

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Annex1

Cryptosporidiosis Project: Case Sheet

Questionnaire for Cryptosporidiosis IN HIV Patients IN TMCAdult Cases

Please answer as many of the questions as you can. There are noor wrong answers. It is OK to answer "don't know"

Your personal details

First Name …… Last Name …….

Sex (please tick) Male ☐ ☐Female

Age..... years

Date of Birth ……/……/…….……

Address:…… a house …… a farm ……...

Main Occupation…… …

Address of Workplace……

Country of Birth ……..

Your medical details

9- Do you take regular medication that is known to affect your immunity? (that is your body's ability to fight infections) Please tick

Yes ☐ ☐ NO ☐ Don’t know

If YES, please give the name and dose of medication ……

10- Do you have a medical condition that is known to affect your immunity (that is your body's ability to fight infection) Please tick

☐ Yes ☐ NO ☐ Don't know

Recent Illness

11- Did you have diarrhea (3 or more loose stools in 24 hrs) in the 2 weeks before you provided stool sample? Please tick

Yes ☐ ☐ NO ☐ Don't know

If NO, continue to answer the following question

If YES, on what date did your diarrhea start? …../…../…..

If you are better now, how many days did it last in total?.....

If you still have diarrhea, for how many days have you had it now?.....

12- In the two weeks before you provided a stool sample did you have any of the following symptoms? Please tick

Yes ☐ ☐ NO ☐ Don't know

Loss of weight

Fever Yes ☐ No ☐ Don't know

Nausea Yes ☐ No ☐ Don't know

Abdominal pain Yes ☐ No ☐ Don't know

Vomiting Yes ☐ No Don't know ☐

Diarrhea Yes ☐ No Don't know ☐

Other……

If Other please say what ……..……..

13- Were you admitted to hospital because of this illness? Please tick

Yes ☐ No ☐

If YES, please give Name of hospital ……

Date of admission ...../……/……

Date of discharge …../…./….. name of drug you receive……..

14- In the 2 weeks before your symptoms started was anyone else who lives in your house ill with diarrhea? Please tick

Yes ☐ ☐ NO ☐ Don't know

Background InformationHousehold details

15- Please tick which best describes where you live:

Private house/flat ☐ apartment ☐

Other (please state) …….

16- How many people live with you

1 2 3 4 5 6 7 8 9 10

17- How many bath room you have?

1 2 3

18- How many other people use the same bathroom as you? Please tick

1 2 3 4 5 6 7 8 9 10

19- Which of the following best describes your water supply? Please tickWater from the tap

Water from the tap ☐

Water processed with a home filter ☐

☐

Well water ☐

Commercially bottled water ☐

Others "please specify"……

The following questions are about your activities in the 2 weeks before yoursymptoms started.

Travel

20- In the 2 weeks before your symptoms started did you travel outside the country? Please tick

Yes ☐ ☐ NO ☐ Don't know

If YES, please give the countries that you visited…….

The date you left the country……/……/……

The date you returned to the country ……/…../…...

21- Did you travel within the country? Please tick

Yes Nowhere…. Contact with Pets

22- In the 2 weeks before your symptoms started were there any domestic pets living in your home? Please tick

Yes NO Don't know

23- Did you touch or handle any of these pets? Please tick

Yes ☐ ☐ NO ☐ Don't know

Please tick the type of pets

Cat: , dog: , bird: , others ……

Contact with farm animals:

24- What type of Animal care were you involved in? pleas tick.

Work in a pet store ☐
Work in a farm store ☐
Work on a dairy farm ☐
Veterinarian ☐
Refused ☐
Unknown ☐

25- During the 2 weeks before you become ill. Were you involved in any of the following types of Activities?

food handling ☐
Child care ☐
Animal care ☐
Patients care ☐
Refused ☐
Unknown ☐

Number of meals:

26- In the 2 weeks before your symptoms started how often did you eat the following? (Please tick:

Food	Not at all	1-2 times	3-7 times	Mostdays	Not sure
green salad					
Tomatoes					
Raw vegetables					
Fresh fruit					

27- If you eat raw fruit and vegetables, do you wash them before eating? Please tick

Always Usually Sometimes Never

28- Do you wash your hands before eating or handling food? Please tick:

Always Usually Sometimes Never

29- Do you regularly bite your nails or chew fingers? Please tick:

Yes NO Don't know

This is the end of the questions. Many thanks for your help in completing this questionnaire.

Annex 2

Carbolfuchsin

Basic fuchsin	10 gm
Absolute methanol	100 ml
Phenol	50 gm
Distilled water	1 liter

1.-The basic fuchsin was weighted on a piece of clean paper (pre-weighted), the powder was then transferred to a bottle of 1.5 capacity.

2.-The ethanol (ethyl alcohol) was measured, added to the bottle with mixing until basic fuchsin was fully dissolved. The mixture was then filtered.

Annex 3

Decolorizer (Acid alcohol)

Concentrated hydrochloric acid (HcL)	3ml
Ethanol (95%)	97ml

1.- Ethanol was measured and filled in a cylinder2.-Slowly the (HcL) was added to the ethanol

3.-The reagent was transferred to a labeled, clean and leak-proof bottle.

Annex 4

Methylene blue-saline 0.1%

Physiological saline (0.85%NaCl Solution) 100 ml Methylene blue 0.1 gm

1.-Methylene blue was weighted, and transferred to a clean leak-proof bottle.

2. Saline was measured, added to the dye, and mixed until the Methylene blue is completely dissolved.

The bottle was labeled and stored at room temperature for use, small amount of stain was filtered into a dropper bottle.

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