



Evaluation of Efficiency of Silver Nanoparticles for the Diagnosis of Intestinal Parasites, Isolated from Displaced Children in Kirkuk Province-Iraq

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Abstract

Background: In Kirkuk province, the number of internally displaced people has reached 370,000 with more expected as government forces and affiliated militias are targeting the Islamic state of Iraq and Syria-controlled district of Hawija in south-west Kirkuk. Laboratory-intensive way like microscopy still remain the mainstay of some diagnostic laboratories and fast diagnosis has always been a priority to find out appropriate treatment and prevent fatalities also advances in diagnostics can assist prevent transmission and obtain active surveillance.

Objectives: This work is to evaluation of diagnostic efficiency of silver nanoparticles in staining technique for the diagnosis of intestinal parasites, because the first step in controlling and treatment of parasites is to isolate and identify them. Therefore we have decided to know the intestinal parasites which infect children aged 1-10 years among displaced population in Kirkuk province.

Methodology: The present study include the using of synthesized silver nanoparticles, with sizes going from 16 nm to 47 nm for the diagnosis of intestinal parasites with the exposure to ultraviolet (UV) or sun light. For this purpose a total of 411 random stool samples were collected by using disposable stool containers containing normal saline from children aged 1-10 years in Laylan 1 and Laylan 2 camps located 19 Km southeast of Kirkuk province northern of Iraq, twice in a week, during the period from April until the end of June 2018. **Results:** The study presented the occurrence of two large groups of parasites, protozoans and helminthes with high efficiency diagnosis due to use of silver nanoparticles during a staining technique, included five (5) protozoans belonging to following Classes: Sarcodina (*Entamoeba histolytica* 2.18% and *Entamoeba coli* 1.45%), Mastigophora (*Giardia lamblia* 2.67% and *Trichomonas hominis* 1.70%) and Ciliophora (*Balantidium coli* 0.72%); as well as four (4) parasitic Helminths belonging to class Cestoda (*Hymenolepis nana* 0.72%) , Trematoda (*Faciolopsis buski* 0.24%) and Nematoda (*Ascaris lumbricoides* 1.21% and *Enterobius vermicularis* 3.4%). Compression between stained with 2% Silver Nitrate and non stained samples were explained in the present study by presentation of a colored images.

Conclusion: prevalence of intestinal parasitic infection differ and make variation depending on the type of parasite groups and parasite species. The present estimation was within the range of 0.24% –

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3.40%. Also It can be concluded that the using of silver nanoparticles in staining technique can be consider as a first attempt in the intestinal parasitic diagnosis in Iraq with the high efficiency.

Keys; Intestinal parasite , Silver Nanoparticles, *Enterobius*, Displace area, Kirkuk.

1.Introduction

Building small structures for advanced materials design, high performance nano devices and miniaturized electronics is one of the central goals of nano sciencel and norganic nanoparticles are particularly attractive building blocks for such purposes (Alahmad, 2014).For over centuries, silver based compounds have been used as nontoxic, inorganic, and antibacterial agents owing to their biocidal properties in many applications such as wood preservatives or for water purification in hospitals (Rauwelet *al.*, 2015).Despite its wide prevalence, controversy still surrounds the best means of intestinal parasites diagnosis. Currently, the detection and diagnosis of parasitic infections rely on several laboratory methods in addition to clinical symptoms, clinical history, travel history, and geographic location of patient. Methods for the identification of parasitic infections have stagnated in the past three decades. Laboratory-intensive way like microscopy still remain the mainstay of some diagnostic laboratories and fast diagnosis has always been a priority to find out appropriate treatment and prevent fatalities also advances in diagnostics can assist prevent transmission and obtain active surveillance (Ndao, 2009; Kamel and Rahemo,2015).

In Kirkuk province, the number of internally displaced people has reached 370,000 with more expected as government forces and affiliated militias are targeting the Islamic state of Iraq and syria-controlled district of Hawija in south-west Kirkuk (Higel, 2016). Children below 18 years make up about 46 % of the world's refugees and these children spend their entire childhood far from home and may have experienced war and violence resulting in detrimental psychological consequences (Yaghi, 2014) . Intestinal parasitic infections are among the widest spread infections. About 3.5 billion people are affected resulting in 450 million cases of illness with the majority being children (Mahmood *et al.*, 2014). These infections lead to growth retardation in children and other physical and mental health problems so they represent as serious public

health problem (WHO, 1998). Now, intestinal parasites such as *Ascaris lumbricoides*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Giardia lamblia*, and *Cryptosporidium* spp. are among the major contributors to the global infection burden as they are prevalent in different parts of the world (Ricciardi, 2010).

The aim of this work is to evaluation of diagnostic efficiency of silver nanoparticles in staining technique for the diagnosis of intestinal parasites, because the first step in controlling and treatment of parasites is to isolate and identify them. Therefore we have decided to know the intestinal parasites which infect children aged 1-10 years among displaced population in Kirkuk province.

2. Materials and Methods

2.1. Study Design and Sampling Area.

A prospective study had been done on Laylan 1 and Laylan 2 refugee camps (displacement area), located 19 Km Southeast of Kirkuk province northern of Iraq, at the latitude 35.478565 and longitude 44.401932 with the GPS coordinates of 35° 28' 42.8340" N and 44° 24' 6.552" E. Both camps constitute of 1744 houses. Number of displacement people equal to 8371. Displaced individuals are originated from different cities and villages like Hawija, Mosul, Salah- Eldin, Diyala and other areas which

severely affected from conflicts and crisis in Iraq.

2.2. Collection and Examination of Samples

A total of 411 random stool samples were collected from children aged 1-10 years in Laylan 1 and Laylan 2 camps using disposable stool containers containing normal saline twice in a week, during the period from April until the end of June of 2018. Samples were transported in a sterile containers containing normal saline to the medical laboratory.

2.3.Preparation of Nano-silver

The procedure is to place a plate of Silver of a very high purity (>99.99%) immersed in 10 ml of double distilled deionized water (DDDW) inside a glass vessel and then irradiated.

The laser used for ablation is Nd: YAG system with 20 ns pulse width with repetition rate of 5 Hz. The fundamental (1064 nm) and second harmonic (532 nm) output of Nd :YAG laser were used to irradiate Silver plate. The required exposure time of laser was 10 minutes and this Silver (Fouad H Kamel *et al.*2017).

2.4. Fixation, Preservation and Staining of Parasites.

In the laboratory, direct smears were prepared from samples. If parasites were present, slides were stained as follows: slides were fixed in absolute methyl alcohol for about 1-2 minutes then stained with synthesized silver nanoparticles, with sizes going from 16 nm to 47 nm for 8 minutes, (Silver nanoparticles can also be prepared in aqueous solution of silver nitrate of %0.02 after adding a reducing agent) followed by 10 minutes exposure to ultraviolet (200 - 800 nm.) or sunlight as described by Srikar *et al.*(2016),

Alahmad (2014) and Albaladejo and Arthur (1989).

3. Results and Discussion

The study presented the occurrence of two large groups of parasites, protozoans and helminthes with high efficiency diagnosis due to use of silver nanoparticles during a staining technique, included 5 protozoans belonging to following Classes: Sarcodina (*Entamoeba histolytica* 2.18% and *Entamoeba coli* 1.45%), Mastigophora (*Giardia lamblia* 2.67% and *Trichomonas hominis* 1.70%) and Ciliophora (*Balantidium coli* 0.72%); as well as 4 parasitic Helminths belonging to class Cestoda (*Hymenolepis nana* 0.72%), Trematoda (*Faciolopsis buski* 0.24%) and Nematoda (*Ascaris lumbricoides* 1.21% and *Enterobius vermicularis* 3.4%). Comparison between stained with 2% Silver Nitrate and non stained samples were explained in the present study by presentation of a colored images. types of parasites and the prevalence of each parasites are summarized in Table 1 and Fig.1.

Table (1) Shows isolated parasite ,stage and prevalence of infection among children aged 1-10 years in laylan camps (n = 411).

Parasite groups	Parasites	Stages of parasites	No. of positive samples	prevalence of infection (%)
Sarcodina	<i>Entamoeba histolytica</i>	Trophozoite and cyst	9	2.18
Sarcodina	<i>Entamoeba coli</i>	Trophozoite and cyst	6	1.45
Mastigophora	<i>Trichomonas hominis</i>	Trophozoite	7	1.70
Mastigophora	<i>Giardia lamblia</i>	Trophozoite and cyst	11	2.67
Ciliophora	<i>Balantidium coli</i>	Trophozoite and cyst	3	0.72
Cestoda	<i>Hymenolepis nana</i>	Egg	3	0.72
Trematoda	<i>Faciolopsis buski</i>	Egg	1	0.24
Nematoda	<i>Ascaris lumbricoides</i>	Egg	5	1.21
Nematoda	<i>Enterobius vermicularis</i>	Adult and egg	14	3.40

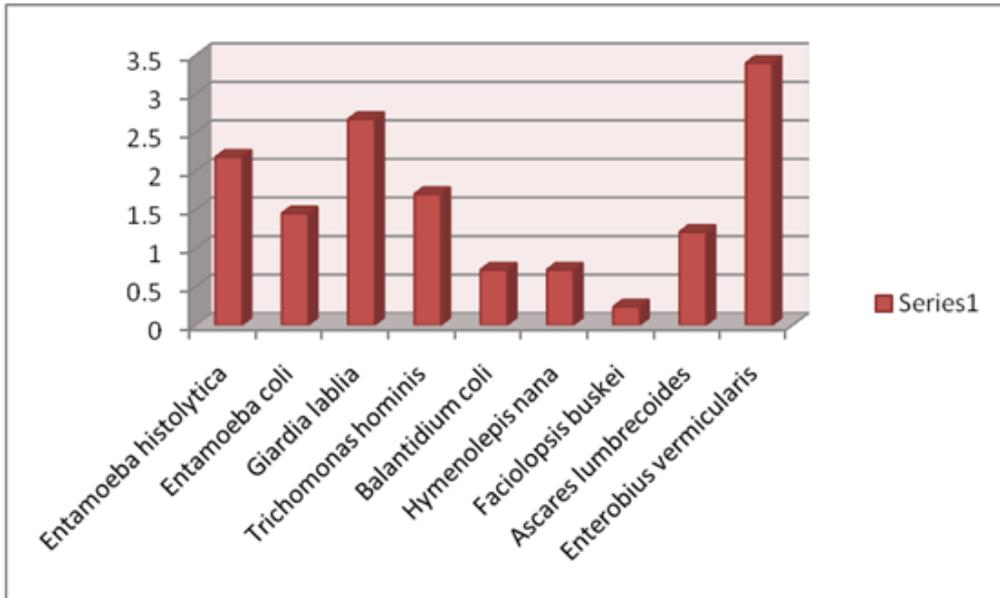


Fig.(1): Prevalence of infection among children aged 1-10 years in laylan camps

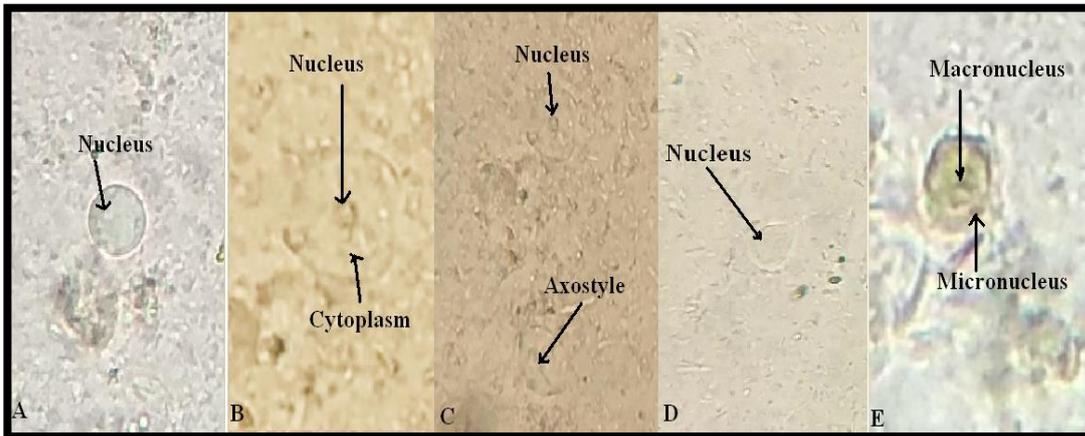


Fig. (2): Isolated intestinal protozoans, without staining, A-Immature *T. hominis*, D- Trophozoite of *G. lamblia* and E-Cyst of *B. coli*. (400X).

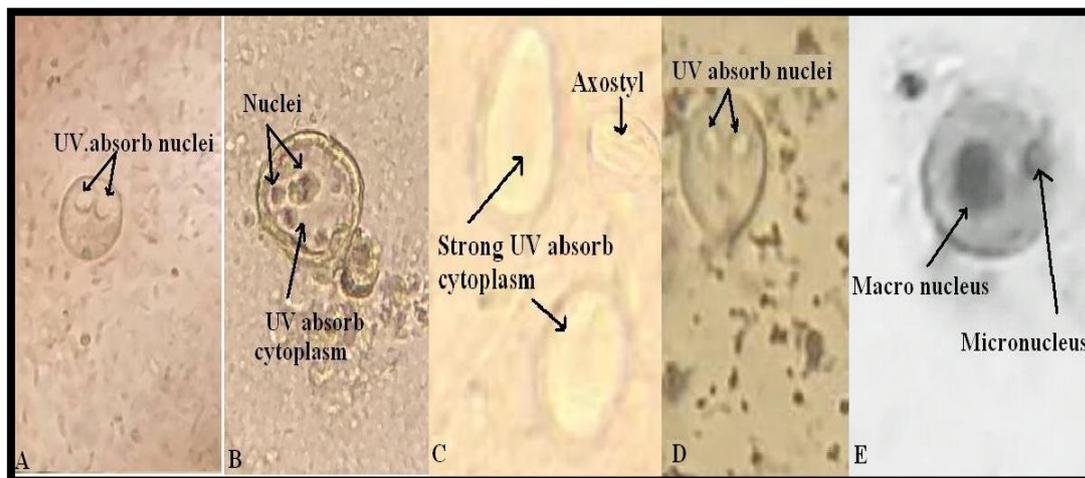


Fig. (3): Isolated intestinal protozoans, staining with the Silver nanoparticles after exposure to Ultra violet ray. A-Immature cyst of *E. histolytica*, B-Mature cyst of *E. coli*, C- Trophozoite of *T. hominis*, D- Trophozoite of *G. lamblia* and E-Cyst of *B. coli*. (400X).

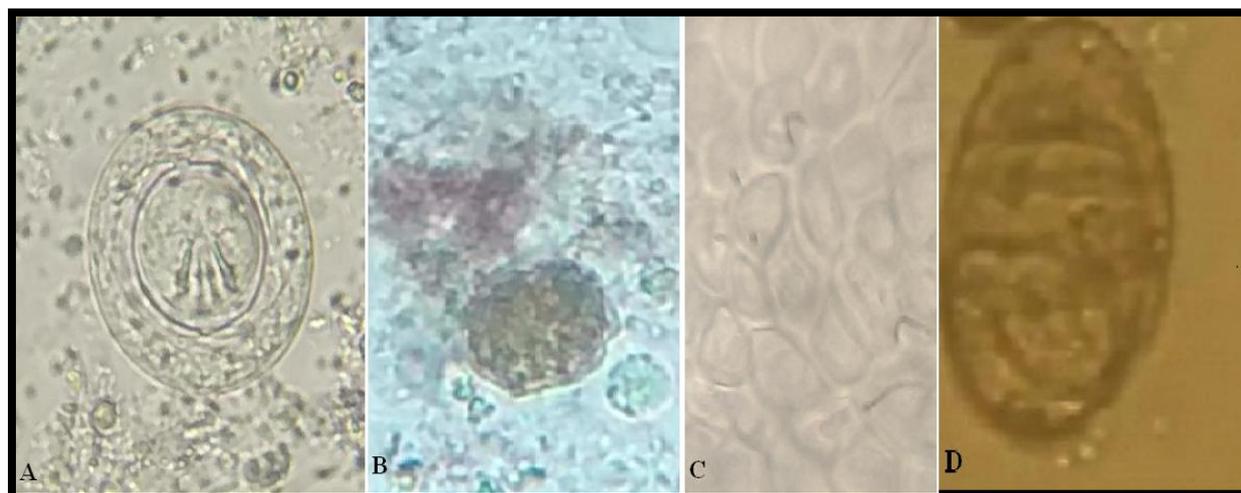


Fig.(4): Isolated intestinal helminthes, without staining. A-Egg of *H. nana*, B-Egg of *A. lumbricoides*, C-Egg of *E. vermicularis*, D-Egg of *F. buski*. (400X).



Fig. (5): Isolated intestinal helminthes, staining with the Silver nanoparticles after exposure to Ultra violet ray. A-Egg of *H. nana*, B-Egg of *A. lumbricoides*, C-Egg of *E. vermicularis* , D-Egg of *F. buski*. (400X)

In this study silver nanoparticles had been used for the intestinal parasite identification. Good results appear after exposure to ultra violet light (Fig. 3 and Fig .5). Several taxonomic organelles or structures of parasite appear clearly and easy to be diagnosis like number of nuclei, axostyle, cytoplasm granules, and embryo shape and structures because UV light can change silver nanoparticles to metallic silver nanoparticles as mentioned by Aziz *et al.* (2017). Among all none organic nanoparticles, silver nanoparticles having several special cheaters, such as catalytic effect, microbiocidal activity, and high

electrical and thermal conductivities (Liu *et al.*, 2009)

On the other hand, direct photos of parasites were taken for diagnosis. It's not clear and independent for the accurate diagnosis because most taxonomic properties of the parasites were not appears (Fig. 2 and Fig.4).

In Iraq, several study had been done for the evaluation and determination of the best technique for the parasitic diagnosis. Mahmood *et al.*, (2014) reported several diagnostic methods for stool specimens by examine all received stool samples by direct wet mount

microscopic exam. Of these, any sample give negative result then must be submit to floatation method and acid-fast staining so as to give more accurate results in suitable time, cost, and efforts.

In Kurdistan region, a comparison between zinc sulfate floatation and formalin: ether sedimentation with two natural extract, pomegranate molasses and honey was studied by Koyee and Faraj (2011). They reported that the pomegranate molasses and Honey sedimentation can be consider as a first attempt in the intestinal parasitic diagnosis. Also several studies had been done for the using of AgNO₃ with Ultra violet light as an alternative stain for fish parasitic protozoans diagnosis in Iraq by Al-Marjan and Abdullah (2008); Shwani and Abdullah, (2010).

In the present study, Depending on the use of silver nanoparticles and UV-light in their diagnosis, results revealed that the prevalence of intestinal parasitic infection among displaced children in Kirkuk province differ and make variation depending on the type of parasite groups and parasite species among children . The present estimation is within the range of 0.24% – 3.40%. The difference in estimations could be attributed to some factors include parasite ecology, personal health care, and nutrition.

The high rates of infection by protozoans (8.75%) were recorded in compression with that of helminthes (5.6 %) at the same study area (Table 1). High distribution of protozoans related to its simple life cycle, because most intestinal parasitic protozoans not required to intermediate host (having simple life cycle) and it reproduce by simple cell division (binary fission) and this fact leads to produce huge number of generation in a minimum time and rapid spread out of the parasite take place among people especially those with low personal hygiene , children, mental person...ect.

In Iraq and Kurdistan region, Most protozoans are endemic, means occur in low rate continuously throughout the time in a special area, while when an crisis came, it leads to rapid elevation and become epidemic in such area. Several crises came like internal ware, ISIS ware

at the beginning of 2014 and this leads a new wave of violence and human rights abuses has left large numbers of people dead and caused mass population displacement at an unprecedented scale. Sectarian and generalized violence has been acute in mixed areas, particularly in Kirkuk. High density population in a small area is among a strong factors in the distribution and spread out of the protozoans because whose population has inadequate access to shelter, food, clean water and employment opportunities. Vector-transmitted is also a main factor in camps that leads to increase the rate of infection. Cyst of most protozoans is resistant outside the body of the host and it may reach to human via vector host (biological and mechanical vectors). House fly, cockroaches, mosquito among the most important vectors that have ability to transmit parasite cysts from one person to another at the study area.

The spread of parasitic diseases of the digestive tract are facilitated by unsatisfactory sanitary conditions which result from the damage of plumbing and sewage systems (mostly as the effect of the warfare). Every day 0.5 million tons of sewage is dumped into Iraqi rivers which contaminated the major source of drinking water in the country. This situation leads to outbreak of various diseases such as giardiasis and amoebiasis as mentioned by Ibrahim (2012).

Children walk on the soil without shoes and play on soil and sewage water that contains parasite cysts and mature stage. The infective stages of parasites have the ability to easily enter their host, usually through the mouth as a contaminant of diet, any liquid, or fingers, while the next generation leaves the body in feces through the anus in the form of spores, cysts, eggs, or larvae to affect new host. The effect of poverty on the intestinal parasitic infection was complex and could be attributed to many factors, such as an unhygienic environment, lack of safer potable water, safer clothes, and poor nutrition. Studies conducted in different countries showed that parasitic infections were higher in those with a low socioeconomic status and was more common among immigrants as mentioned

by Alver and Tore (2006). Small aged patient, who had at least one stool sample in which an enteric parasite was isolated were classified as positive for that parasite. Significantly, children more infected with intestinal parasites, similar to that reported in other studies because, children aged two and below mostly remain in house and are milk fed and this is closely agree with that previously reported by Doni *et al.* (2015) .

The low rate of infection by helminthes (5.6%) were recorded in comparison with that of protozoans (8.75 %) at the same study area because worms need to special ecology parameters like intermediate host, suitable humidity and temperature for embryonation also need to opposite sex (male and female) in sex separated parasites. It means that the worm has a complex life cycle, so its spreading is little when compare with the distribution rate of the protozoans. In addition to that the number of parasite generation low and time consumer during their reproduction process. Worms are in endemic sites for children. Infections that are of major public health importance are mostly spread in tropical and subtropical site, with the largest numbers occurring in sub-Saharan Africa, the Americas, China, and East Asia. Infected children are physically, nutritionally, and cognitively impaired because of the malabsorption of nutrients.

Conclusion:

1-The study presented the occurrence of two large groups of parasites, protozoans and helminthes and these include five (5) protozoans belonging to following Classes: Sarcodina (*Entamoeba histolytica* 2.18% and *Entamoeba coli* 1.45%), Mastigophora (*Giardia lamblia* 2.67% and *Trichomonas hominis* 1.70%) and Ciliophora (*Balantidium coli* 0.72%); four (4) helminthes belonging to class Cestoda (*Hymenolepis nana* 0.72%) , Trematoda (*Faciolopsis buskei* 0.24%) and Nematoda (*Ascares lumbricoides* 1.21% and *Enterobius vermicularis* 3.4%). Results revealed that the prevalence of intestinal parasitic infection differ and make variation depending on

In addition, some helminthes also cause loss of appetite, and therefore a reduction in nutritional intake and physical fitness (Doni *et al.*, 2015).

Displacement area are often dependent on external help because countries of asylum are not able to provide relief on the needs scale. Rapid public health needs assessments evaluate the extent and magnitude of the emergency, the current and potential public health impact, the availability of local human and material resources, the need for external resources, and ongoing information needs. Systematic notification should follow a predetermined checklist of characteristics such as population density and composition, family size, environmental conditions (e.g., water supply, sanitation, shelter, drainage, and possible vector breeding sites), food availability, and morbidity (e.g., malnutrition, dehydration, febrile illnesses, and injuries). The source of infection is mainly human (carrier or patient). Principal factors in maintaining endemicity of intestinal parasitic infections are favorable qualities of the soil and frequent contamination of the environment by human feces. Water cycle (WC) system not fit with number of camps population each eight (8) house having only four (4) WC. It can be consider as one of the other factors affected the health of camps population.

the type of parasite groups and parasite species. The present estimation is within the range of 0.24% – 3.40%.

2- The high rate of infection by protozoans (8.75%) were recorded in comparison with that of helminthes (5.6 %) at the same study area.

3- Among protozoans *G.lamblia* was recorded in a high prevalence (2.67 %) and *E. coli* was recorded with the lowest rate (1.45%) of infection.

4- The result of this study showed that 3.40 % were affected with the *E. vermicularis* and this is a highest rate of infection which was recorded in this study followed by *A. lumbricoides* (% 1.21).

5-The second part of the present study deals with the using of new staining technique during parasite diagnosis known as Silver impregnation

technique (using AgNO₃ and Ultraviolet). It can be considered as a first

Recommendation

1- Control and prevent spread and transmission of parasitic infestations within the community in general and among families from displaced camps through the following recommendation: 5- Health education at community level about personal hygiene by explaining how to protect food and drinks from flies and dust, storage and handling water by filtration for drinking water.

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attempt in the intestinal parasitic diagnosis in Iraq.

2- Diagnosis and treatment of all cases infected individual.

3- Courses awareness health about the transmission of intestinal parasite monthly in camps.

4- Improve water safe in camps.

Reduce the number of the population or families in the camps.

6-Higher education level to health hygiene among children and improve toilet use

7-Proper control of insects and rodents

development of new Nano-silver printing ink. *RJPBCS Vol 8(4), 100pp.*

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