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ORIGINAL ARTICLE

Prevalence of Intestinal Parasitic Infections among Egyptian Children with Chronic Kidney Diseases in Zagazig University Children's Hospital.

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ABSTRACT

Background: Chronic kidney disease (CKD) in children is associated with reduced immune responses that predispose them to frequent parasitic infections. This present work aimed to determine the prevalence rates of intestinal parasitic infections in children with CKD.

Methods: This case-control study was carried out on 136 cases aged 2-18 years, divided into three groups; CKD on hemodialysis, CKD on conservative treatment, and healthy control. This work was carried out from March 2018 to April 2019. Three fecal samples were obtained from each case and samples were subjected to direct wet mount and iodine stained smear, formol ether sedimentation concentration technique, modified Ziehl-Neelsen stain, and stool culture on an agar plate for nematode larvae. Blood samples were examined for anemia and kidney function tests.

Results: The prevalence rate of parasitic infections was 53% for the hemodialysis group, 51% for CKD on conservative treatment group, and 36% for the control group. The infecting parasites were as follows, *Cryptosporidium* (13.2%), *G. lamblia* (2.9%), *E. histolytica* (5.1%), *Entameba coli* (5.1%), *Blastocystis* (9.6%), *Cyclospora cayetanensis* (1.5%), *Strongyloides stercoralis* (1.5%), *Ascaris lumbercoides* (2.2%) and *H. nana* (3.7%). Mixed infections were reported in 2.9% of the studied groups. 42% of the hemodialysis cases were anemic as well as 63% of the other CKD cases and 23% of the control group. In the infected group, 57% were anemic as well as 28% in the non-infected group.

Conclusions: The elevated prevalence rate of parasitic infections among the CKD cases was related to their reduced immune responses.

Keywords: Dialysis; Intestinal parasites; Anemia; Diarrhea

INTRODUCTION

Chronic kidney disease is a clinical condition of irreversible kidney damage and insufficiency that can progress to end-stage renal failure. This CKD condition is a worldwide health problem, especially in the pediatrics sector [1].

The accumulation of metabolites that are not excreted by the kidney as urea which progresses to uremia, is a major complication of chronic renal insufficiency [2]. Uremia induces polymorphonuclear leucocyte dysfunction and immune system suppression [3].

Immunocompromized patients have quantitative and/or qualitative impairment of their humoral and cellular immune responses that interfere with their effective action against infections. So, these patients are easily infected by enteric parasites with a high degree of severity [4].

Since their immune systems are not fully developed, children are vulnerable to acquire microbial infections, including parasites [5]. Immunocompromized children with chronic renal diseases may show a higher prevalence of protozoan infections than immunocompetent children [6].

Infection by intestinal parasites represents a major public health problem, mainly in developing countries. However, in developed countries, these parasites may become a severe problem due to either intentional immune suppression or other causes of immunodeficiency diseases [7]. So, the acquisition of parasitic infections and their outcome depend mainly on the host immune response [8].

Intestinal parasitism is a significant cause of illness and even death all over the world. They can cause serious clinical symptoms in

immunocompromised patients [3]. They can cause severe diarrhoea and dehydration, threatening kidney function. Also, it is commonly presented by anorexia, bloating, abdominal pain, malabsorption, loss of weight, and occasionally fever [2].

Patients with CKD undergoing hemodialysis have a broad spectrum of gastrointestinal symptoms, similar to those caused by some intestinal parasites, and this may act as a confounding factor. Therefore, it is recommended to evaluate these patients for parasitic infections before therapeutic interventions [9].

Cryptosporidium species, *Blastocyst* species, *Entameba histolytica*, *Giardia lambalia*, and *Cyclospora caytanensis* represent the most common causes of parasitic diarrhea in humans [10].

Helminthic infections such as *Ascaris lumbricoides*, *Enterobius vermicularis*, *Trichuris trichiura*, *H. nana* and *Strongyloides stercoralis* are among the most common intestinal parasitic infections worldwide. These organisms produce serious public health problems in children, especially the immunocompromised such as HIV-positive and hemodialysis patients [11].

This current study aimed to assess the prevalence rates of intestinal parasitic infections in children patients suffering from chronic kidney diseases

METHODS

This study is a case-control study performed on 136 cases (76 males and 60 females) aged from 2 years up to 18 years old. They were divided into three groups: the CKD on hemodialysis (HD) group, the CKD on conservative therapy group and the control group.

The study was performed on patients attending the nephrology unit and nephrology outpatient clinic in Zagazig University Children's Hospital. This work was carried out from March 2018 to April 2019. Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

The inclusion criteria of patients were children whose ages ranged from 2 to 18 years old and children suffering from CKD. While exclusion criteria were children below 2 years old and more than 18 years old, children with chronic debilitating diseases as bronchial asthma, diabetes mellitus, and children having malignancies…..etc., and children with a recent history of taking anti-parasitic treatment. All cases involved in the study were subjected to full history taking, including age, gender, consanguinity, family history of renal diseases, initial presentation, and duration of dialysis. Sanitation habits such as washing hands after bathing and before eating; washing fresh fruits and vegetables before eating; food and drinking water source. Patient complaints include prolonged diarrhea, abdominal pain, distension and bloating, renal complaints like oliguria, hematuria, urine retention, or edema. Full general and local examinations were done on each child with special emphasis on signs of anemia, and growth assessment via anthropometric measures (weight, height, and body mass index (BMI) percentile curves) [12].

Blood samples were collected from all patients for the diagnosis of anemia and kidney function tests (KFTs). Anemia is diagnosed in CKD children patients if Hb level is <11.0 g/dl in cases aged 0.5–5 years, <11.5 g/dl in cases 5–12 years, and < 12.0 g/dl in cases 12–15 years. and <13.0 g/dl in adult male patients and <12.0 g/dl in adult female patients [13]. KFTs include glomerular filtration rate (GFR). The normal value for GFR is 90 mL/min/1.73 m2 or above. Chronic kidney dysfunction is defined as GFR<60 mL/min/1.73 m2. When GFR decreases below 15 mL/min/1.73 m2 this indicates kidney failure that needs treatment either by dialysis or kidney transplant, serum creatinine (normal values are 0.5-1.0 mg/dL for children ages 3-18 years and 0.3-0.7 mg/dL for children <3 years old; greater values mean renal impairment) and blood urea nitrogen (BUN), normal value is from 7 to 20. BUN value elevates when renal function deteriorates [14].

After completing the patients' questionnaire, 3 fecal samples were collected from every case in clean wide-mouthed disposable cups labeled with the date of sample collection and the patients' name and age on 3 consecutive days with 1-day intervals. They were carefully collected in order not to be contaminated with urine and then transported to the laboratory for examination.

The stool samples were examined by the naked eye for determination of the consistency of the stool, its color, its odor, and the presence of mucous, blood, or fat.

Microscopic examination of stool samples was done to detect parasitic infections through direct wet mount smear and iodine stained smear [15], formol ether sedimentation concentration technique [16], staining with modified Ziehl-Neelsen stain [17], and stool culture on an agar plate to detect nematode larvae [18]. Each fresh stool sample was cultured on an agar plate to

detect hookworm or *S.stercoralis* larvae. In the center of the plate, 2 gm of stool were placed and the plate was incubated for 2 days in a humid chamber at ambient temperature. Then the plates were rinsed with 10 ml of 10 % acetyl-formalin solution followed by centrifugation at 500 g for 1 min and the sediments were examined microscopically at x400 magnification.

Statistical analysis

The positive findings were presented as Mean±SD, percentage, and the statistical analysis was carried out by SPSS software (version 16, Chicago, USA) using Student's *t*-test, ANOVA (*F*-test) and Chi-square test (X^2). Probability, the p-value was considered statistically significant if <0.05.

RESULTS

Demographic data and anthropometric measures of the studied groups showed that females (53%) represented the majority of CKD in the HD group, while males (63%, 64%) represented the majority of CKD in the conservative therapy group and control group, respectively, with insignificant statistical differences. They were mostly rural dwellers, with insignificant statistical differences. There were statistically significant differences regarding age, height, and BMI, but there was no statistical difference regarding weight between the studied groups (Table 1).

The most frequent gastrointestinal manifestations detected in the studied groups were diarrhea, flatulence, abdominal pain, and vomiting. All manifestations were significantly different between all groups except for abdominal pain (Table 2).

Parasitic infections were detected in 53% of the CKD in the HD group, 51% of the CKD in the conservative treatment group, and 36% of the control group. The single intestinal parasites recovered from stool examination of the studied groups were as follows: *Cryptosporidium spp.* (13.2%), *Giardia lamblia* (2.9%), *E. histolytica* (5.1%), *Entameba coli (*5.1%), *Blastocystis hominis* (9.6%), *Cyclospora cayetanensis* (1.5%), *Strongyloides stercoralis* (1.5%), *Ascaris lumbercoides* (2.2%) and *H. nana* (3.7%). Mixed infections were reported in (2.9%) of the studied groups (Table 3)(Fig 1S-9S).

Table (4) showed that males represented the majority of the infected group, while females represented the majority of the noninfected group with significant statistical differences. The majority of both groups inhabited rural areas, with significant statistical differences. Significant statistical differences were observed between the two groups concerning age, weight and height, but there was no statistical difference regarding the BMI.

The majority of gastrointestinal manifestations detected in studied cases were diarrhea, flatulence, abdominal pain and vomiting. Only diarrhea and abdominal pain were found to be significantly different between the two groups (Table 5).

In the HD group, (42%) were anemic, as well as (63%) in the CKD on conservative treatment group and (23%) among the control group with a significant statistical difference between them. In the infected group, (57%) were anemic, as well as (28%) in the noninfected group with a significant statistical difference between them (Table 6).

Table (1): Demographic data and anthropometric measures of the studied groups.

	CKD on hemodialysis (n=62)	**CKD on conservative treatment** (n=35)	**Control** (n=39)	**Statistical analysis**	
	No. (%)			**Chi-squares (*X2*)**	***P*-value**
Gender Male Female	 29 (47) 33 (53)	 22 (63) 13 (37)	 25 (64) 14 (36)	3.8	0.14
Residene Rural Urban	 38 (61) 24 (39)	 23 (66) 12 (34)	 27 (69) 12 (31)	0.68	0.71
	Mean±SD			***F*- test**	***P*-value**
Age (Y)	13.1±3.6	9.7±2.3	11.7±2.9	3.65	0.01*
Weight (Kg)	27.8±4.1	24.4±3.4	29.6±3.7	1.97	0.14
Hight (Cm)	135±12.5	126±10.4	129±11.6	11.82	0.00*

	CKD on hemodialysis (n=62)	CKD on conservative treatment (n=35)	Control (n=39)	Statistical analysis	
BMI (kg/m2) Z-score Percentile	15.25±1.6 -1.8 3.4	15.37±2.3 - 0.7 25.8	17.8±4.7 0.08 53	13.24	0.00*

*Significant. BMI (Body mass index). Percentile ≥5th and<85th means healthy weight.

Table (2): Distribution of gastrointestinal manifestations among the studied groups.

	CKD on hemodialysis (n=62)	CKD on conservative treatment (n=35)	Control (n=39)	Statistical analysis	
	No. (%)			Chi sequare *(X2)*	P-value
Diarrhea (n=64)	**40 (65)**	**20 (57)**	**4 (10)**	**30.2**	**0.0000***
Abdominal pain(n=36)	**19 (31)**	**9 (26)**	**8 (21)**	**1.28**	**0.53**
Flatulence (n=56)	**24 (39)**	**21 (60)**	**11 (28)**	**7.98**	**0.02***
Vomiting (n=25)	**13 (21)**	**10 (29)**	**2 (5)**	**7.26**	**0.03***

*Significant.

Table (3): Prevalence of parasitic infections among the studied groups.

Intestinal parasites	CKD on hemodialysis (n=62)	CKD on conservative treatment (n=35)	Control (n=39)	Total (n=136)
	No. (%)			
I-Protozoa				
Cryptosporidium spp. *Giardia lamblia* *E.histolytica.* *Entameba coli* *Blastocystis hominis* *Cyclospora cayetanensis*	12 (19.4) 3 (4.8) 2 (3.2) 3 (4.8) 5 (8.1) 1 (1.6)	6 (17.1) 0 (0.0) 1 (2.9) 1 (2.9) 5 (14.3) 1 (2.9)	0 (0.0) 1 (2.6) 4 (10.3) 3 (7.7) 3 (7.7) 0 (0.0)	18(13.2) 4(2.9) 7(5.1) 7(5.1) 13(9.6) 2(1.5)
II-Helminthes				
Strongyloides stercoralis *Ascaris lumbercoides* *H.nana*	2 (3.2) 1 (1.6) 2 (3.2)	0 (0.0) 0 (0.0) 2 (5.7)	0 (0.0) 2 (5.1) 1(2.6)	2(1.5) 3(2.2) 5(3.7)
Mixed infections	2 (3.2)	2 (5.7)	0 (0.0)	4 (2.9)
Total	33 (53)	18(51)	14(36)	65(47.8)

Table (4): Demographic data and anthropometric measures of parasitic infected and noninfected groups:

	Infected (n=65)	**Non-infected** (n=71)	**Statistical analysis**	
Gender male female	**No.(%)**		**Chi-squares (*X2*)**	***P*-value**
	43 (66) 22 (34)	33 (46) 38 (54)	5.3	0.02*
Residence Rural Urban	 49 (75) 16 (25)	 39 (55) 32 (45)	6.2	0.01*
	Mean±SD		***t*-test**	***P*-value**
Age (Y)	12.6±2.8	8.3±1.9	4.93	0.001*
Weight (Kg)	28.6±5.3	25.8±4.7	1.79	0.024*
Height (Cm)	136±9.8	128±7.5	3.12	0.013*
BMI (kg/m2) Z-score Percentile	15.46±1.3 -1.5 6.8	15.75±1.9 - 0.1 47.2	0.216	0.513

*Significant. BMI (Body mass index). Percentile ≥5th and<85th means healthy weight.

Table (5): Distribution of gastrointestinal manifestations among the infected groups.

	Infected(n=65)	**Non infected**(n=71)	**Statistical analysis**	
	No. (%)		**Chi-squares (*X2*)**	**P-value**
Diarrhea (n=64)	**45 (69)**	**19 (27)**	**24.6**	**0.0000***
Abdominal pain (n=36)	**24 (37)**	**12 (17)**	**6.9**	**0.01***
Flatulane (n=56)	**31 (48)**	**25 (35)**	**2.18**	**0.14**
Vomiting (n=25)	**14 (22)**	**11 (15)**	**0.83**	**0.36**

*Significant.

Table (6): Distribution of anemia among the studied groups.

	Anemia		**Total**	**Statistical analysis**	
	+ve	**-ve**		**Chi-squares (*X2*)**	***P*-value**
CKD on hemodialysis CKD on conservative treatment Control group	26 (42) 22 (63) 9 (23)	36 (58) 13 (37) 30 (77)	62 35 39	12	0.002*
Parasitic infected Non-parasitic infected	37 (57) 20 (28)	28 (43) 51 (72)	65 71	11.52	0.000*
Total	**57 (42)**	**79(58)**	**136**		

*Significant.

DISCUSSION

CKD patients have a significantly compromised immune system that makes them more vulnerable to infections, which may be responsible for significant morbidity in these patients [19]. Immunodepression detected in these patients is mainly attributed to accumulated uremic toxins which impair both lymphocytic and granulocytic functions [20]. So, these patients have a decreased ability to clear infections that may be severe enough to cause death in these patients [21].

Parasitic infections are among the most widespread causes of chronic human infections, especially in developing countries. However, even in countries with adequate sanitary conditions and education, some of these parasites may play an important role in worsening diseases in specific groups, such as immunocompromised individuals and young children [22]. These parasites particularly infect individuals with altered cellular immunity as patients with malignancies, organ transplanted patients, CKD and dialysis patients, and patients taking high doses of corticosteroids [8].

Chronic kidney diseases and intestinal parasitic infections are higher in rural areas than in urban areas due to many factors such as lower socioeconomic status of the families, lower levels of education, household crowding, nonuse of footwear, lack of toilets, and inaccessibility of clean water [23], [24]. However, El-Kady *et al.* [25] reported that gender and residence cannot be considered as risk factors for acquiring intestinal parasitic infections in hemodialysis cases. We found that the majority of both infected and noninfected groups inhabited the rural areas (72% and 61%), respectively with insignificant statistical differences between them.

Chronic kidney disease can slow a child's growth through many factors, such as causing vitamin D and phosphorus impairment, which results in bone weakness; a decreasing appetite, which may lead to poor nutrition and slower growth; anaemia, which in turn can cause growth to slow or stop; also, when the kidneys are damaged, wastes accumulate in the blood and the body does not properly process the growth hormone [26]. In the present study, there is a decrease in BMI among children with CKD on dialysis from the standards for the same age and sex, with a percentile 3.4th however normal BMI should range from percentile ≥5th to <85th. This agreed with Lotfy *et al.* [27] who assessed the nutritional status of Egyptian children on regular HD and reported that most of the patients' BMI was below the 3rd percentile.

The most frequent gastrointestinal manifestations detected in the studied groups were diarrhea, flatulence, abdominal pain, and vomiting. Faidah *et al.* [4] concluded that there were no associations relating the gastrointestinal symptoms to the presence of any intestinal parasite, even when comparing the symptomatic and nonsymptomatic patients in the studied groups. Also, chronic uremia may mask some gastrointestinal symptoms associated with intestinal parasitism [28]. Similarly, Ferreira-Filho *et al.* [29] did not observe any differential signs or clinical symptoms in the hemodialysis patients with and without the intestinal parasites.

The overall detection rate of parasitic infections among the total examined cases was 47.8. The rate was 53% among the CKD on HD group, 51% among the CKD on conservative treatment group, and 36% among the control group. In a study performed on children with CKD at Menoufyia governorate, Rady *et al.* [5] reported a prevalence of parasitic infections of 66.7% in HD patients and 42.9% in non-dialysis patients. Similar results in Qena governorate were obtained by El-Kady *et al.* [25] who detected a prevalence of 66% among HD patients. A higher prevalence (94%) was detected in HD cases in Sohag governorate [30]. On the other hand, lower prevalences (33.3% and 28.8%) were reported by Abaza *et al.* [31] and Ali *et al.* [32] respectively. The prevalence rates reported by many researchers all over the world range from 25% to 51.6% [33], [34], [3].

In the current study, the most prevalent intestinal parasite was *Cryptosporidium* spp., with a prevalence of 19.4% among the HD group and 17.1% among the other CKD group. In Egypt, higher results were reported by El Nadi and Taha [30]. They reported that the prevalence rate of Cryptosporidial infection among HD cases was 48%. Similarly, El-Kady *et al.* [25] detected a 40% prevalence of Cryptosporidial infection among HD patients. Rady *et al.*[5] reported *Cryptosporidium* oocyst infection in 29.2% of HD patients and 14.3% of non-dialysis cases. In Iran, Omrani *et al.* [3] reported *Cryptosporidium* spp. infection in 11.5% of end stage renal disease (ESRD) patients. In Saudi Arabia, Hawash *et al.* [34] reported a prevalence of *Cryptosporidium* infection of 8%. In Brazil, Kulik *et al.*[2] detected Cryptosporidial infection in 4.6% of the HD cases.

Blastocystis hominis was the second most common parasite infecting children, 8.1% in HD group, and 14.3% in the other CKD group. These results are lower than those reported by Gama *et al.* [35] who detected a prevalence rate of 41.2%

of *Blastocystis* spp. among HD patients. Also, higher results were reported in previous Brazilian studies, such as those described by Gil *et al.* [28], (24.5%) and Kulik *et al.* [2], (20.9%). In Iran, for instance, studies by Omrani *et al.* [3] and Barazesh *et al.* [9], on HD patients, reported the parasite in 14.1% and 13.6%, respectively.

Concerning infection by *Giardia lamblia* in our study, it infected 4.8% of the HD patients. These results are higher than those reported by Gil *et al.* [28] who detected *G. lamblia* in only 0.9% of HD patients. Similarly, Omrani *et al.* [3] detected *G. lamblia* infection in 1.3% of the HD patients. Our findings are lower than those reported by El-Kady *et al.*, [25] who reported a prevalence of 12% among CKD patients.

In the current study, *Entamoeba histolytica* was detected in 3.2% of the HD group and 2.9% of the non-dialysis group. Higher results were observed by Ferreira-Filho *et al.* [29] its prevalence has been reported to be about 8% in HD patients. Rady *et al.* [5] reported a prevalence of *E.histolytica* infection of 4.2% in HD patients and not detected in non-dialysis patients. Our results disagreed with that reported by El-Kady *et al.* [25] who reported *E. histolytica* infection in 14% of CKD group and 16% of the control group.

The prevalence rate of *Entamoeba coli* in our study was 4.8% and 2.9% among the HD and non-dialysis CKD groups, respectively. Our results agreed with that reported by Kulik *et al.* [2] who detected *Entamoeba coli* infection in 4% of HD patients. These results are lower than those reported by Gil *et al.* [28] who detected *Entamoeba coli* in 6.4% of HD patients.

Cyclospora cayetanensis was detected in 1.6% of children with CKD on HD. These results were lower than those reported by Ali *et al.* [32] who detected *Cyclospora cayetanensis* infection in 7.5% of the dialysis group.

As regards the helminthic infections prevalence rates, they were much lower than protozoal infections, and the detected helminths were *Strongyloides stercoralis* 1.5%, *Ascaris lumbricoides* 2.2% and *H. nana* 3.7%. These results were near to that of Gil *et al.* [28] who reported a *Strongyloides stercoralis* prevalence of 1.2% among HD patients. Among Iranian renal transplant recipients, Azami *et al.* [8] reported *Ascaris lumbricoides* infection among (0.7%) of the examined cases. Health education of CKD patients to eat properly cooked meat, green vegetables, and fruits could explain the lower prevalence of parasites such as *Oxyuris*, *Ascaris*, *Trichuris trichiura*, *Hymenolypis nana*, and *Taenia* spp. among them when compared to the control group [2].

Anemia is a common feature of CKD associated with poor outcomes [36]. In our study, 42% of the CKD patients in the HD group were anemic, as well as 63% in the CKD on conservative treatment and 23% among the control group. These results were near to that reported by Rady *et al.* [5] who detected anemia in 41.7% of HD patients and 47.6% of non-dialysis patients. Also, Shaheen *et al.* [37] detected a prevalence of anemia in children ranging from 42% to 82% in CKD stages from 1 to 5.

There is a close relationship between intestinal parasitosis and the nutritional status of the patient. It is difficult to determine the effect of each one on the other. Also, iron deficiency anemia is a major complication of intestinal parasitic infections [38]. In our study, 57% of the infected group were anemic as well as 28% in the non-infected group. These findings agree with Rady *et al.* [5] who reported a prevalence of anemia of 60% in infected children and 25% in non-infected children. Parasitic infections may worsen anemia that commonly occurs in dialysis patients and aggravate immunosuppression. So, this necessitates early diagnosis and treatment of parasitic infections to minimize their impact on patients [39].

CONCLUSIONS

The high prevalence of parasitic infections among the CKD patients was related to their reduced immune response. Anemia in the studied groups was mostly attributed to the CKD and parasitic infection is a cofactor that may aggravate renal disease and promote its deterioration.

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Supplementary Files

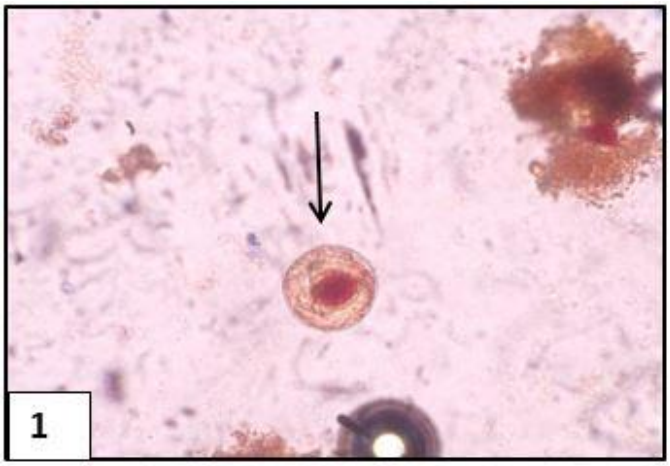

Fig.(1S): *H.nana* egg. Iodine stained smear (X400).

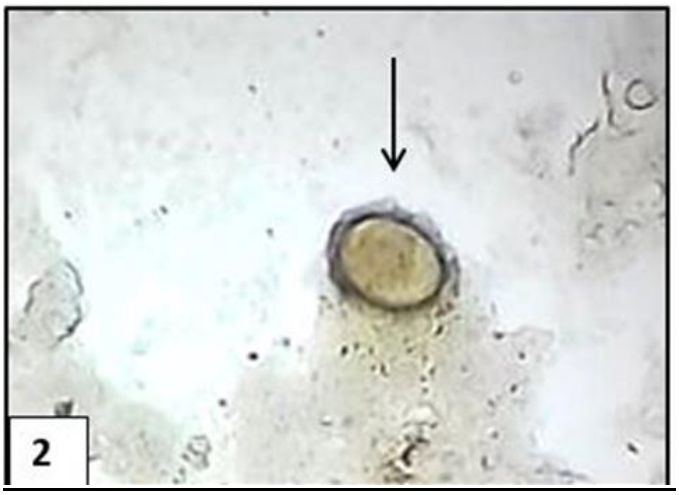

Fig.(2S): *Ascaris lumbericoides* egg. Wet mount smear (X400).

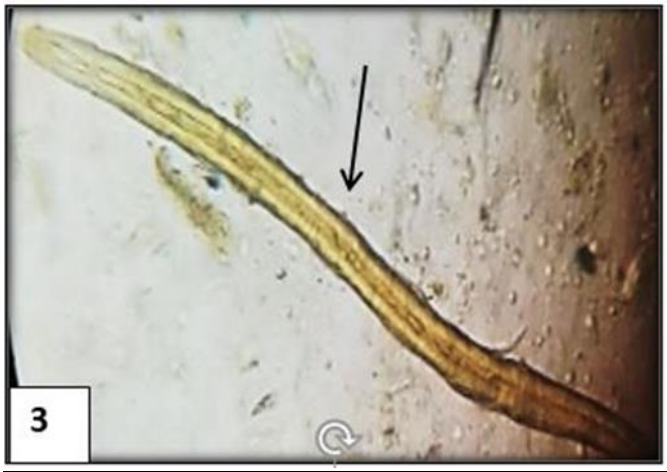

Fig.(3S): *Strongyloides stercoralis* larva from stool culture on an agar plate (X100).

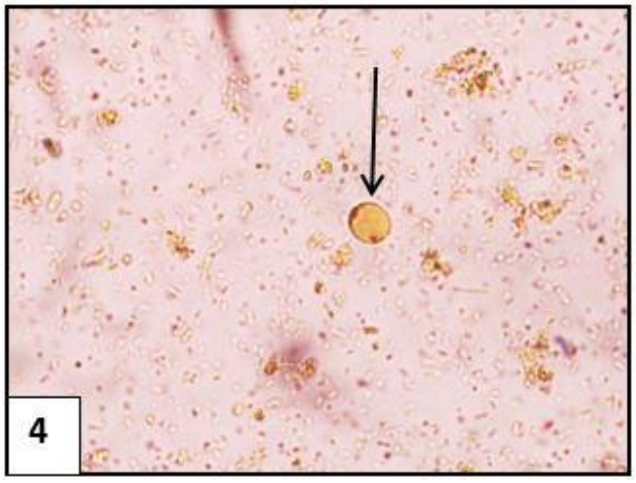

Fig.(4S): *Blastocystis* spp. Iodine stained smear (X1000).

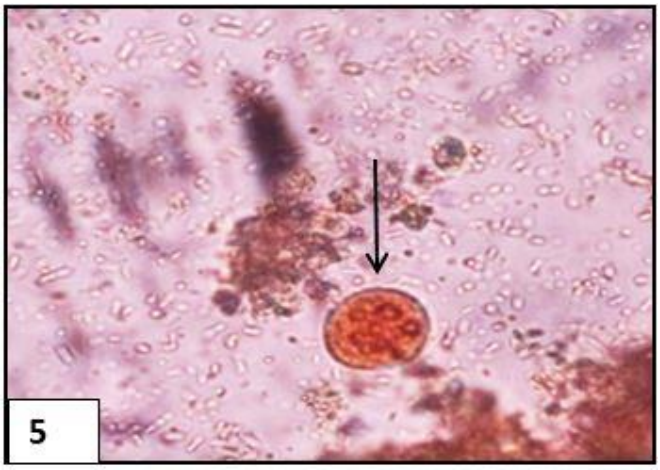

Fig.(5S): *Entameba coli* cyst. Iodine stained smear (X1000).

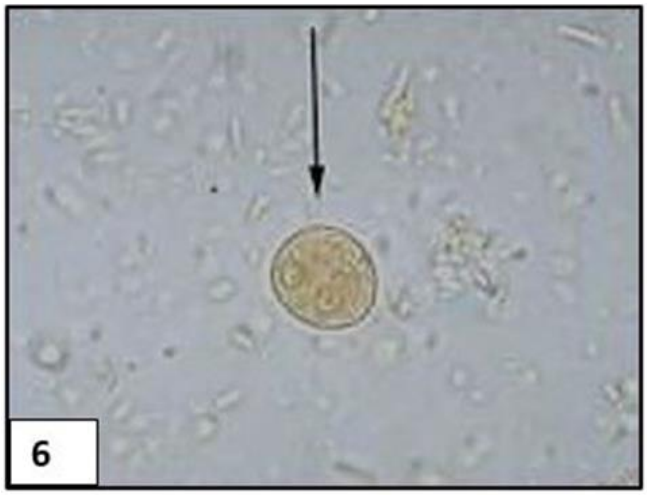

Fig.(6S): *Entameba histolytica* cyst.Wet mount smear (X1000).

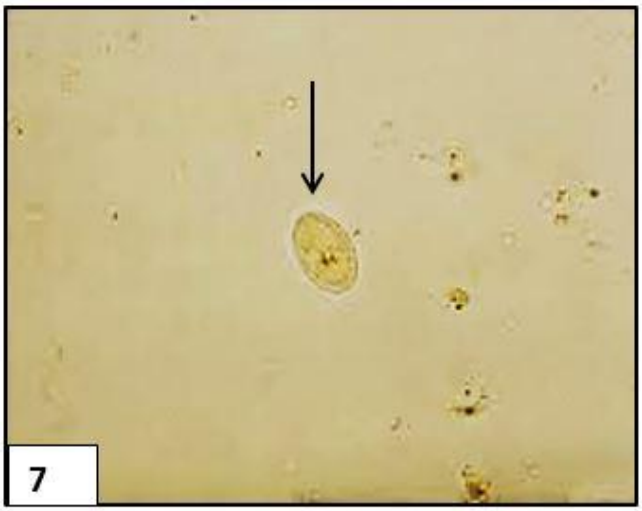

Fig.(7S): *Giardia lamblia* cyst. Iodine stained smear (X1000).

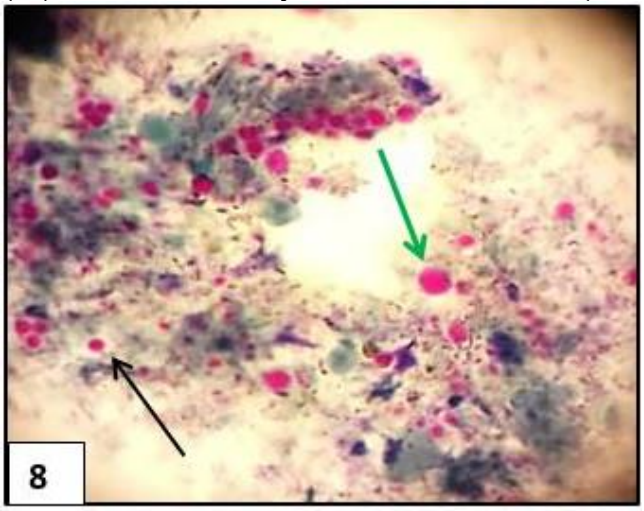

Fig.(8S): *Cyclospora* (green arrow) and *Cryptosporidium oo*cyst (black arrow). Modified Ziehl Neelsen stain (X1000).

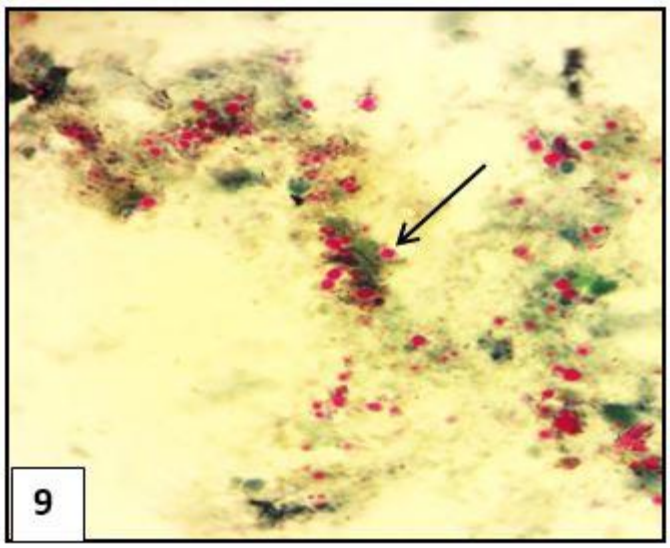

Fig.(9S): *Cryptosporidium oo*cyst. Modified Ziehl Neelsen stain (X1000).