# Results

The present study was carried out on 300 stool samples obtained from 300 diarrheic patients of different age groups for the detection of intestinal microsporidiosis. They included 149 males and 151 females.

The patients were divided into two groups: immunocompromised group including 109 cases and immunocompetent group including 191 cases.

Intestinal microsporidiosis was detected in forty nine cases (16.3%) by using Weber’s trichrome stain. PCR technique was performed to confirm intestinal microsporidiosis in microscopically microsporidia-positive samples and to identify species of microsporidia.

## 1) Detection of microsporidian spores by using Weber's trichrome staining technique:

Microsporidian spores appear pink against a green counterstained background and contain a central pink band and posterior vacuole which are useful indicators for differentiation these organisms from yeasts which also stain pink.

Spores of *E. bieneusi* are oval and small, measuring only 1.1 to 1.6 by 0.7 to 1.0 μm (Figure 4; plate 1).

Spores of *Encephalitozoon spp.* are broad and rod-like or kidney shaped, measuring 2.0 to 2.5 by 1.0 to 1.5 μm (Figure 4; plate 2).

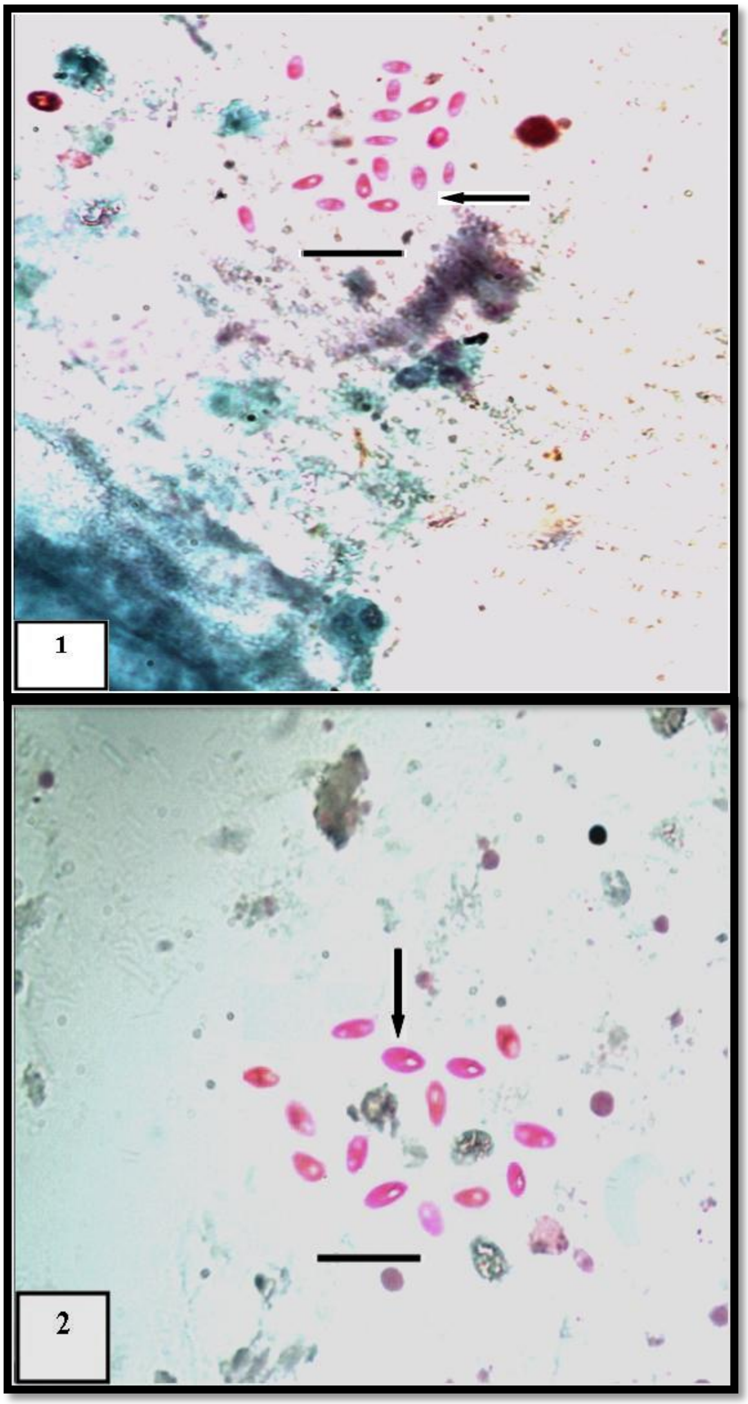


*Figure 4:*

***Plate (1):*** **Human stool specimen showing pink spores of *E. bieneusi* with vacuole stained with modified trichrome stain. Magnification, X 1000 (oil immersion).**

***Plate (2):*** **Human stool specimen showing pink spores of *Encephalitozoon SPP.* with vacuole stained with modified trichrome stain. Magnification, X 1000 (oil immersion), Bar=5 µm**

## 2) Clinical manifestations in microsporidia-positive patients:

Among microsporidia-positive patients, acute diarrhoea was found in 18/19 (94.7%) immunocompetent patients and 25/30 (83.3%) immunocompromised patients, while chronic diarrhoea was found in one (5.3%) immunocompetent patient and 5 (16.7%) immunocompromised patients. Microsporidia-positive patients suffered from nausea, abdominal colic, flatulence, fatigue and weight loss (Table 3 and Figure 4). Statistically ,there was no significant difference between clinical types of diarrhoea among immunocompetent and immunocompromised microsporidia-positive patients; as Pearson $Chi^2$ = 1.408 and P value (2-sided) of Fisher's exact test = 0.384 for D.F. = 1.

***Table (3): Types of diarrhea affecting microsporidia-positive patients.***

| Type of diarrhoea / Immune status | Total | Acute diarrhea | | Chronic diarrhea | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| Immunocompetent | 19 | 18/19 | 94.7 | 1/19 | 5.3 |
| Immunocompromised | 30 | 25/30 | 83.3 | 5/30 | 16.7 |
| **Total** | 49 | 43/49 | 87.8 | 6/49 | 12.2 |

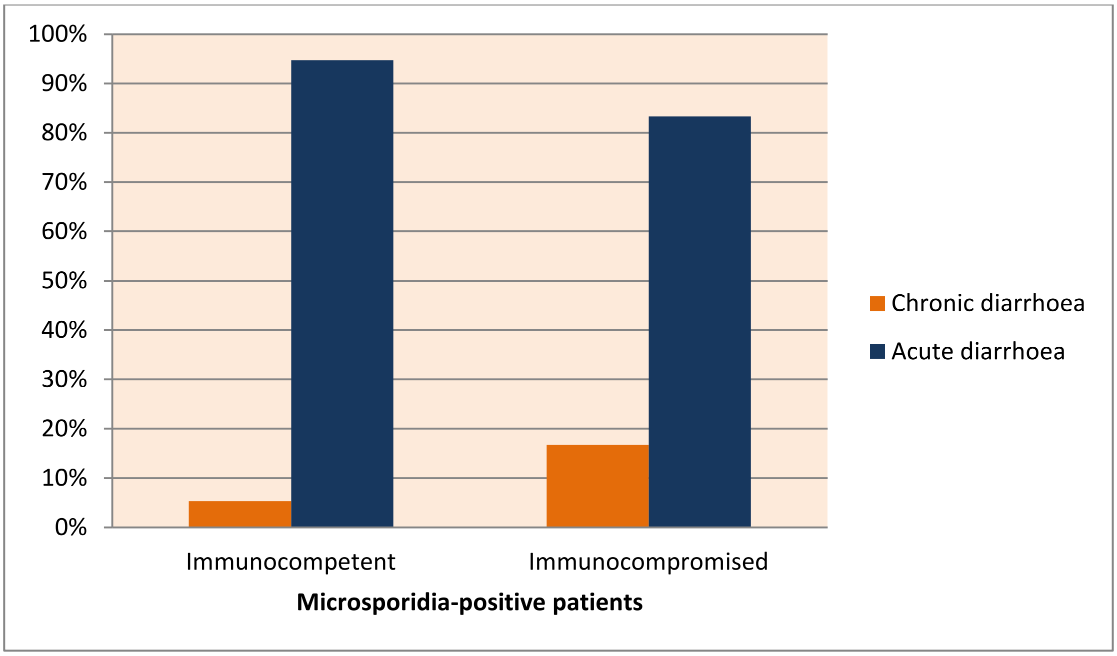


***Figure (5): Types of diarrhea affecting microsporidia-positive patients.***

## 3) The relation between immune status and rate of infection with intestinal microsporidiosis:

Regarding immune status of the examined cases, it was found that 19/191 (9.9%) immunocompetent cases and 30/109 (27.5%) immunocompromised cases were microsporidia-positive patients (Table 4 and Figure 6). Statistically, the presence of microsporidia was significantly more common among immunocompromised cases; as Pearson $Chi^2$ = 15.686 and P value (2-sided) < 0.001 for D.F. = 1) (Table 5).

***Table (4): The relation between immune status and rate of infection with intestinal microsporidiosis.***

| Cases / Immune status | Total cases | Microsporidia - positive cases | | Microsporidia-negative cases | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| **Immunocompetent** | 191 | 19 | 9.9 | 172 | 90.1 |
| **Immunocompromised** | 109 | 30 | 27.5 | 79 | 72.5 |
| Total | 300 | 49 | 16.3 | 251 | 83.7 |

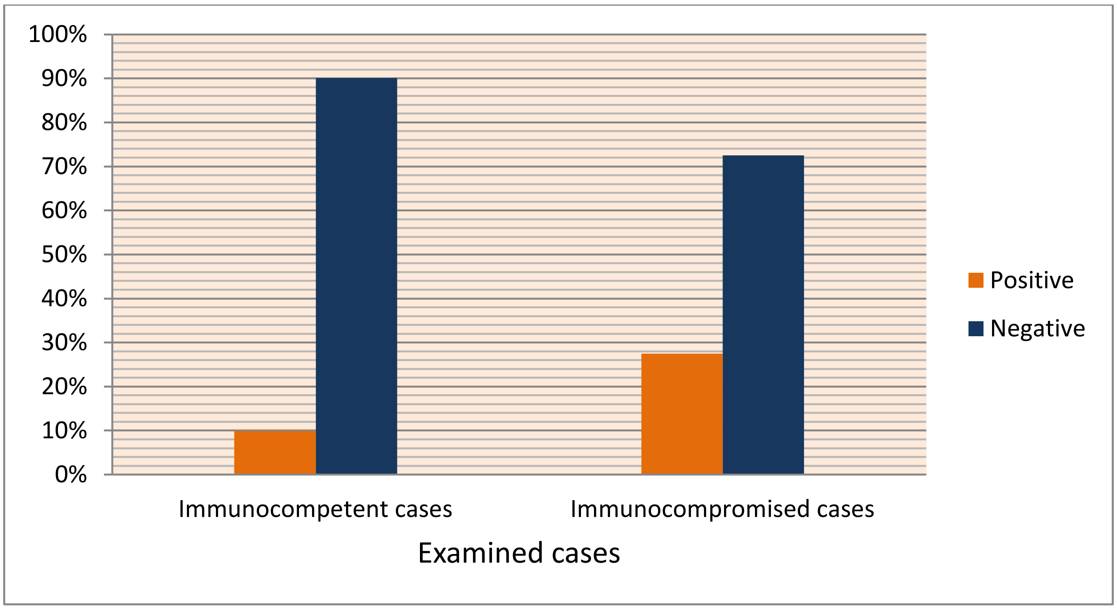


***Figure (6): The relation between immune status and rate of infection with intestinal microsporidiosis.***

***Table (5): Chi-Square Tests showing relation of sex, age, immune status, residency and source of water of patients with the rate of infection with intestinal microsporidiosis.***

| | *N* | $\chi^2$ | *DF* | *P value* | *OR [95% CI]* |
|---|---|---|---|---|---|
| Age (all age groups) * Presence of microsporidia | **300** | **14.900** | **4** | **<0.01**<br>(= 0.005)** | **-** |
| Immune status * Presence of microsporidia | **300** | **15.686** | **1** | **<0.001***<br>(<0.001)** | **0.291 [0.154-0.548]<br>(competent/compromised)** |
| Sex * Presence of microsporidia | **300** | **1.086** | **1** | **>0.05<br>(= 0.297)** | **-** |
| Source of water supply * Presence of microsporidia | **300** | **7.441** | **1** | **<0.01**<br>(= 0.006)** | **0.428 [0.230-0.796]<br>(tape water/underground)** |
| Residence * Presence of microsporidia | **300** | **9.047** | **1** | **<0.01**<br>(= 0.003)** | **0.354 [0.176-0.711]<br>(urban/rural)** |

N = number of cases, DF = degree of freedom, OR = odds ratio, CI = confidence interval, * = significance, ** =high significance, *** =strong significance

### 4) The associations between medical history (risk factors) and presence of microsporidia:

with regards to medical history of immunocompromised cases, it was found that the highest rate of infection with microsporidia (38.5%) was recorded in patients with malignancies followed by 33.3%, 26.6% and 17.7% in patients with chronic liver diseases, patients used immunosuppressive drugs and patients suffered from chronic renal disease, respectively. While the lowest rate of infection 16.6 % was recorded in diabetic patients (Table 6 and Figure 4).

Statistically , Table (7) showed that:

1) The presence of intestinal microsporidiosis was significantly more common among cases with chronic liver diseases; as Pearson $Chi^2$ = 6.275 and P value (2-sided) = 0.012 for D.F. = 1.

2) The presence of microsporidia was significantly more common among cases with medical history of malignancy; as Pearson $Chi^2$ = 10.200 and P value (2-sided) = 0.001 for D.F. = 1.

3) There was no significant difference between microsporidia proportion among chronic renal cases and cases without chronic renal disorders; as Pearson $Chi^2$ = 0.023 and P value (2-sided) = 0.880 for D.F. = 1.

4) There was no significant difference between microsporidia proportion among diabetic and non diabetic cases; as Pearson $Chi^2$ = 0.002 and P value (2-sided) = 0.963 for D.F. = 1.

5) there was no significant difference between intestinal microsporidiosis proportion among cases whom had a history of taking immune-suppressive drugs and whom hadn't; as Pearson $Chi^2$ = 1.234 and P value (2-sided) = 0.267 for D.F. = 1

***Table (6): The relation between the medical history and rate of infection with intestinal microsporidiosis.***

| Disease \ Cases | Total examined cases | Microsporidia-positive cases | | Microsporidia-negative cases | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| Diabetes mellitus (D.M.) | 24 | 4 | 16.6 | 20 | 83.4 |
| Malignancy | 26 | 10 | 38.5 | 16 | 61.5 |
| Immunosuppressive treatment | 15 | 4 | 26.6 | 11 | 73.4 |
| Chronic renal diseases | 17 | 3 | 17.7 | 14 | 82.3 |
| Chronic liver diseases | 27 | 9 | 33.3 | 18 | 66.7 |
| Total | 109 | 30 | 27.5 | 79 | 72.5 |

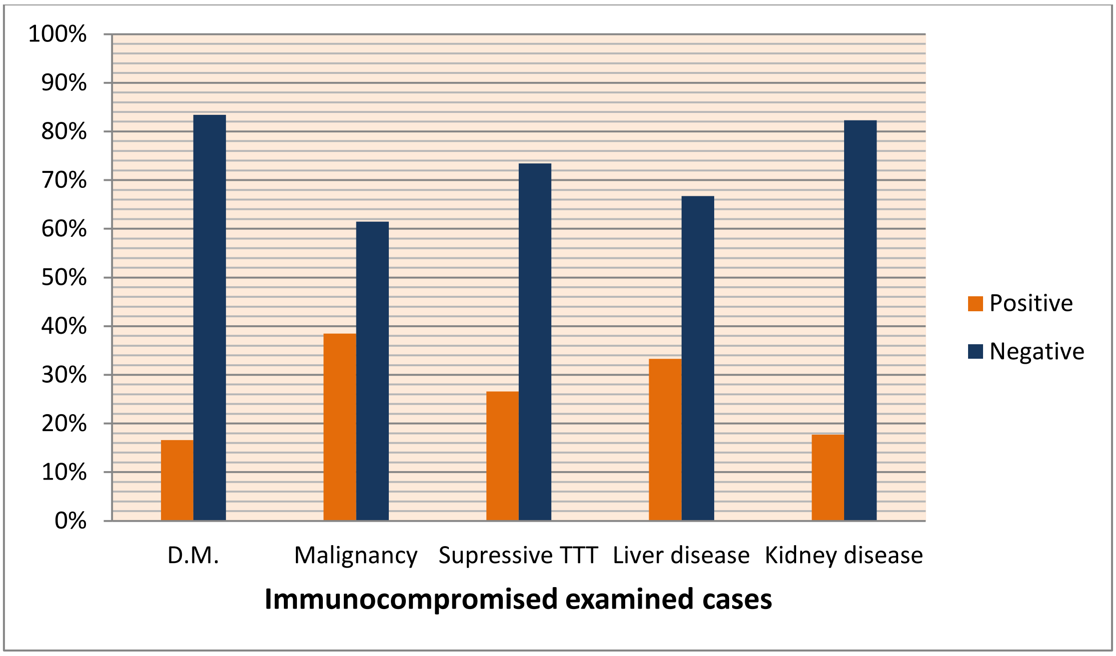


***Figure (7): The relation between the medical history and rate of infection.***

***Table (7): Chi-Square Tests showing relation between the medical history and the rate of infection with intestinal microsporidiosis.***

| | *N* | $\chi^2$ | *DF* | *P value* | *OR [95% CI]* |
|---|---|---|---|---|---|
| History of DM * Presence of microsporidia | **300** | **0.002** | **1** | **>0.05 (= 0.963)** | **-** |
| History of malignancy * Presence of microsporidia | **300** | **10.200** | **1** | **<0.01** (= 0.001)** | **3.766 [1.594-8.897] (present/absent)** |
| History of immune-sup. * Presence of microsporidia | **300** | **1.234** | **1** | **>0.05 (= 0.267)** | **-** |
| History of CRD * Presence of microsporidia | **300** | **0.023** | **1** | **>0.05 (= 0.880)** | **-** |
| History of CLD * Presence of microsporidia | **300** | **6.275** | **1** | **<0.05* (= 0.012)** | **2.913 [1.223-6.935] (present/absent)** |

N = number of cases, DF = degree of freedom, OR = odds ratio, CI = confidence interval, DM = diabetes mellitus, CRD = chronic renal diseases, CLF = chronic liver diseases, * = significance, ** =high significance, *** =strong significance

## 5) Associations between age and the presence of microsporidia:

With respect to age of the examined cases, it was found that the highest rate of infection with intestinal microsporidiosis (25.7%) was recorded in age group (0-5 years), followed by 23.3%, 16.6% and 7.8% in age groups (≥ 51) , (6 - 12) and ( 20-50),respectively. While the lowest rate of infection with microsporidia 4.5% was recorded in age group (13-19 years) (Table 8 and Figure 8).

Statistically, Tables 5 and 9 showed that:

1) The presence of microsporidia was significantly common among cases aged between 0-5 years old than whom aged between 12-55 years old; as Pearson $Chi^2 = 13.393$ and P value (2-sided) $< 0.001$ for D.F. = 1.
2) The presence of microsporidia was significantly common among cases aged ≥ 51 years old than whom aged between 12-51 years old; as Pearson $Chi^2 = 10.326$ and P value (2-sided) $= 0.001$ for D.F. = 1.
3) The presence of microsporidia was significantly common among cases aged between 6-12 years old than whom aged between 12-55 years old; as Pearson $Chi^2 = 4.579$ and P value (2-sided) $= 0.032$ for D.F. = 1.

***Table (8): The relation between the age and rate of infection with intestinal microsporidiosis.***

| Cases / Age | Total | positive cases | | Negative cases | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| **0-5** | 70 | 18 | 25.7 | 52 | 74.3 |
| **6-12** | 60 | 10 | 16.6 | 50 | 83.4 |
| **13-19** | 46 | 2 | 4.5 | 44 | 95.5 |
| **20-50** | 64 | 5 | 7.8 | 59 | 92.2 |
| **≥51** | 60 | 14 | 23.3 | 44 | 76.7 |

***Table (9): Chi-Square Tests showing relation between the age of examined patients and the rate of infection with intestinal microsporidiosis.***

| | ***N*** | $\chi^2$ | ***DF*** | ***P value*** | ***OR [95% CI]*** |
|---|---|---|---|---|---|
| Age (all age groups) * Presence of microsporidia | **300** | **14.900** | **4** | **<0.01**<br>(= 0.005)** | **-** |
| Age (0-5 ver. 12-51 years old) * Presence of microsporidia | **180** | **13.393** | **1** | **<0.001***<br>(<0.001)** | **5.093 [2.001-12.968] (6-11/12-55 years old)** |
| Age (6-11 ver. 12-51 years old) * Presence of microsporidia | **170** | **4.579** | **1** | **<0.05*<br>(= 0.03)** | **2.943 [1.058-8.188] (0-5/12-55 years old)** |
| Age (12-55 ver. ≥51 years old) * Presence of microsporidia | **170** | **10.326** | **1** | **<0.01**<br>(= 0.001)** | **4.484 [1.693-11.765] (>55/12-55 years old)** |

N = number of cases, DF = degree of freedom, OR = odds ratio, CI = confidence interval, * = significance, ** =high significance, *** =strong significanc.

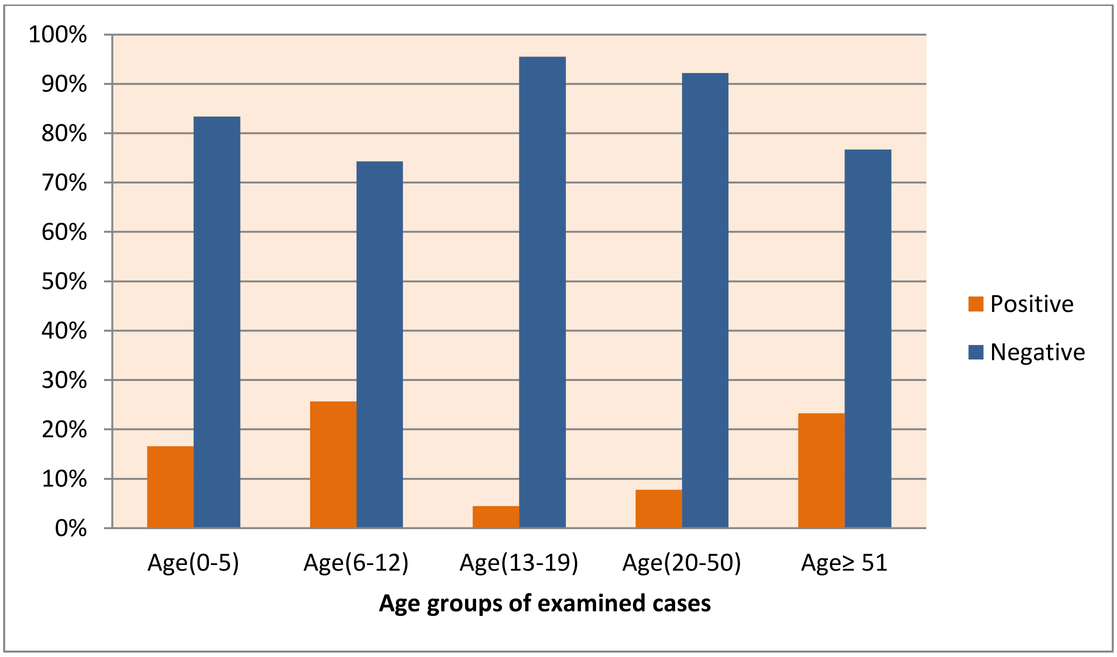


***Figure (8): The relation between the age and rate of infection with intestinal microsporidiosis.***

## 6) Associations between sex and the presence of microsporidia:

Concerning sex of examined patients, 21/149 (14%) male cases and 28/151 (18.5%) female cases were microsporidia-positive patients. The highest rate of infection with microsporidia (32%) was recorded in immunocompromised females, followed by 23.2%, 11.2% and 8.6% in immunocompromised males; immunocompetent females and immunocompetent males, respectively (Table 10 and Figure 9). Statistically, there was no significant difference between proportion of microsporidia-positive patients among males and females; as Pearson $Chi^2$ = 1.086 and P value (2-sided) = 0.297 for D.F. = 1 (Table 5).

***Table (10): The relation between sex and the rate of infection with intestinal microsporidiosis.***

| Cases / Result | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Total | Immuno-competent cases | Immuno-compromised cases | Total | Immuno-competent cases | Immuno-compromised cases |
| Positive | 21/149 (14%) | 8/93 (8.6%) | 13/56 (23.2%) | 28/151 (18.5%) | 11/98 (11.2%) | 17/53 (32%) |
| Negative | 128/149 (86%) | 85/93 (91.4%) | 43/56 (76.8%) | 123/151 (81.5%) | 87/98 (88.8%) | 36/53 (68%) |

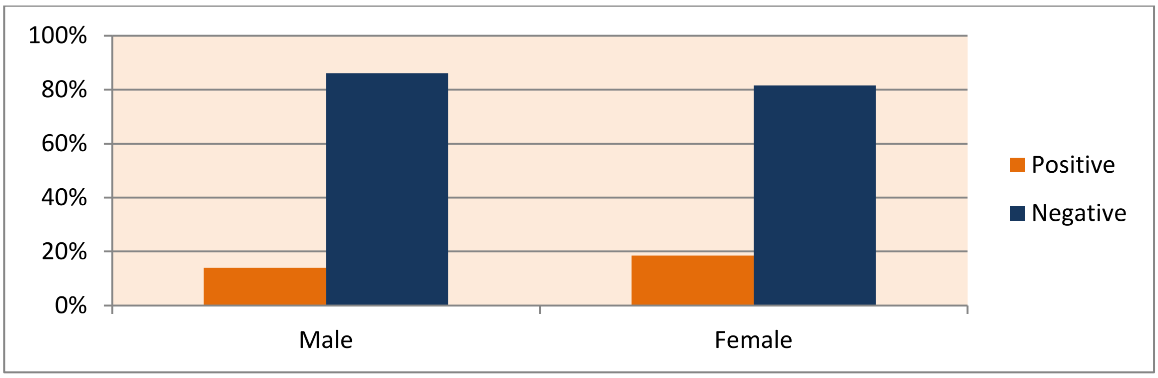


***Figure (9): The relation between sex and the rate of infection with intestinal microsporidiosis.***

## 7) The relation between time and the rate of infection with intestinal microsporidiosis:

### A) The monthly rate of infection with intestinal microsporidiosis:

Statistically, as shown in Table (13), the rate of infection varied markedly among the months of the year of study. It was recorded that the highest rates of infection with microsporidia reached 11/34 (32.4%), 9/32 (28.2%) and 7/26(26.9%) were recorded in August, July and September, respectively. On the contrary, the lowest rate of infection with microsporidia 1/25(4%), 1/22(4.5%) and 1/22(4.5%) were recorded in January, February and November, respectively (Table 11 and Figure 10).

### B) The relation between seasonal variation and the rate of infection with intestinal microsporidiosis:

Regarding the effect of seasonal variation, the highest rate of infection with microsporidia (27/82, 32.9%) was recorded in summer, followed by (10/72, 13.9%), (7/70, 10%) and (5/71, 6.6 %) in spring, autumn and winter, respectively (Table 12 and Figure 11).

statistically, there was a significant relation between the months of summer (July, August and September) and the prevalence of intestinal microsporidiosis; as Pearson $Chi^2 = 24.183$ and P value (2-sided) $< 0.001$ for D.F. = 3 (Table 13).

***Table (11): The monthly rate of infection with intestinal microsporidiosis.***

| Cases / Month | Total (N=300) | Positive cases (N=49) | | Negative cases (N=251) | |
|---|---|---|---|---|---|
| | No | No. | % | No | % |
| **January** | 25 | 1 | 4.0 | 24 | 96.0 |
| **February** | 22 | 1 | 4.5 | 21 | 95.5 |
| **March** | 21 | 3 | 14.3 | 18 | 85.7 |
| **April** | 23 | 2 | 8.7 | 21 | 92.3 |
| **May** | 25 | 4 | 16.0 | 21 | 84.0 |
| **June** | 25 | 6 | 24.0 | 19 | 76.0 |
| **July** | 32 | 9 | 28.1 | 23 | 71.9 |
| **August** | 34 | 11 | 32.4 | 23 | 67.6 |
| **September** | 26 | 7 | 26.9 | 19 | 73.1 |
| **October** | 24 | 2 | 8.3 | 22 | 91.7 |
| **November** | 22 | 1 | 4.5 | 21 | 95.5 |
| **December** | 21 | 2 | 9.5 | 19 | 90.5 |
| **Total** | 300 | 49 | 16.3 | 251 | 86.7 |

***Table (12): The effect of seasonal variation in the rate of infection with intestinal microsporidiosis.***

| Season \ Cases | total | Positive | | Negative | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| **Winter (21/12-21/3)** | 76 | 5 | 6.6% | 71 | 93.4% |
| **Spring (21/3-21/6)** | 72 | 10 | 13.9% | 62 | 86.1% |
| **Summer (21/6-21/9)** | 82 | 27 | 32.9% | 55 | 67.1% |
| **Autumn (21/9-21/12)** | 70 | 7 | 10% | 63 | 90% |

***Table (13): Chi-Square Tests showing relation between the seasonal variation and the rate of infection with intestinal microsporidiosis.***

| | ***N*** | $\chi^2$ | ***DF*** | ***P value*** | ***OR [95% CI]*** |
|---|---|---|---|---|---|
| Seasonal variations (all) * Presence of microsporidia | **300** | **24.183** | **3** | **<0.001*** (<0.001)** | **-** |
| Seasonal variations (Summer vs. Others) * Presence of microsporidia | **300** | **22.737** | **1** | **<0.001*** (0.001)** | **4.374 [2.312-8.273] (summer/other seasons)** |
| Seasonal variations (Summer vs. winter) * Presence of microsporidia | **158** | **16.953** | **1** | **<0.001*** (<0.001)** | **6.971 [2.521-19.276] (summer/winter)** |

N = number of cases, DF = degree of freedom, OR = odds ratio, CI = confidence interval, * = significance, ** =high significance, *** =strong significance

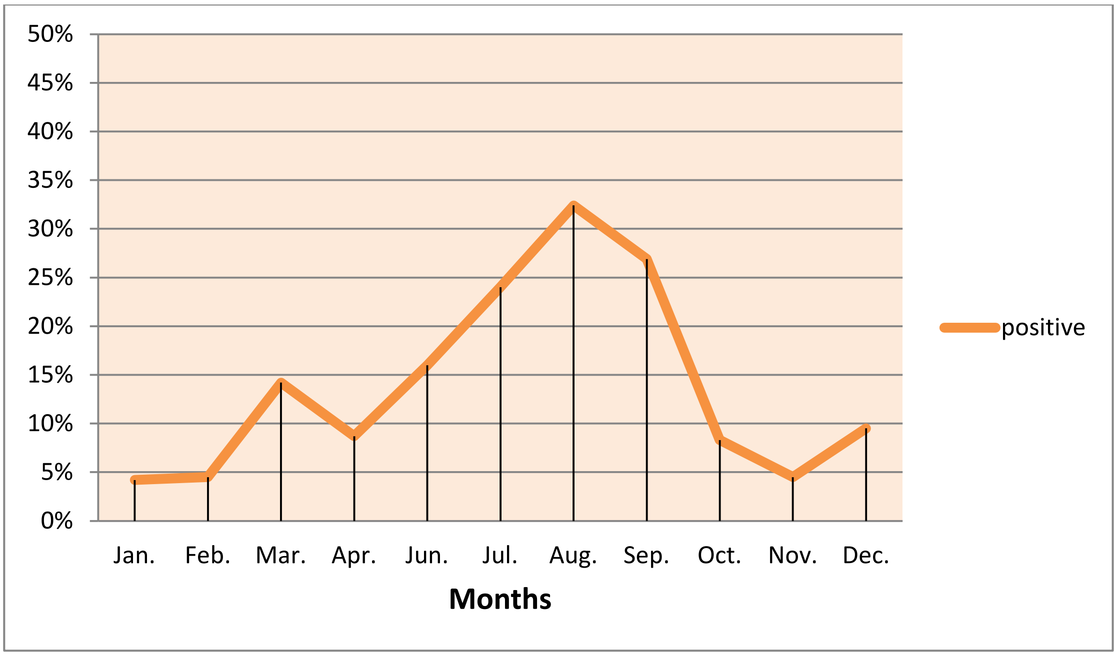


***Figure (10): The monthly rate of infection with intestinal microsporidiosis.***

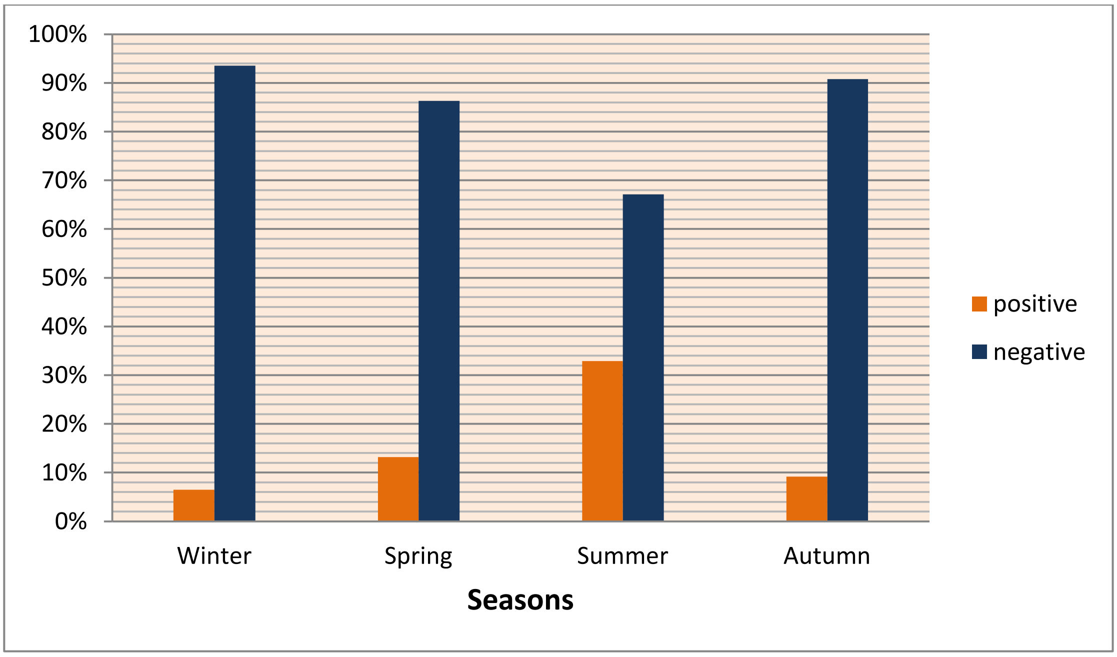


***Figure (11): The effect of seasonal variation in the rate of infection with intestinal microsporidiosis.***

## 8) Molecular identification of microsporidia species in microscopically microsporidia-positive samples:

Molecular examination of 49 microscopically microsporidia-positive samples revealed that all samples proved to be PCR positive by using generic primers**.** *E. bieneusi* and *E. intestinalis* were identified by species specific primers in microsporidia- positive samples. The mixed infection with both *E. bieneusi* and *E. intestinalis* was 24.5%, whereas the single infection with either *E. bieneusi* or *E. intestinalis* by PCR reached 65.3% and 10.2%, respectively. The mixed infection with both *E. bieneusi* and *E. intestinalis* was higher (28.6%) among females than males (19%). Generally, the rate of infection with *E. bieneusi* (89.8%) was higher than that with *E. intestinalis* (34.7%). Infection with *E. bieneusi* was higher (39.3%) among females than males (28.6%). Infection with *E. intestinalis* was higher (90.5%) among males than females (89.3%) (Table 14 and Figures 12, 13, 14 and 15).

***Table (14): Detection of microsporidia species detected in positive samples.***

| **SEX** | **Microsporidia-positive samples** | | | | | |
|---|---|---|---|---|---|---|
| | Total +ve samples by weber trichrome | **Mixed infection** | ***Enterocytozoon bieneusi*** | | ***Encephalitozoon intestinalis*** | |
| | | | **Single infection** | **Total** | **Single infection** | **Total** |
| microsporidia-positive **Male** | 21 | 4 (19%) | 15 (71.4%) | 19 (90.5%) | 2 (9.5%) | 6 (28.6%) |
| microsporidia-positive **Female** | 28 | 8 (28.6%) | 17 (60.7%) | 25 (89.3%) | 3 (10.7%) | 11 (39.3%) |
| **Total** microsporidia positive | 49 | 12 (24.5%) | 32 (65.3%) | 44 (89.8%) | 5 (10.2%) | 17 (34.7%) |

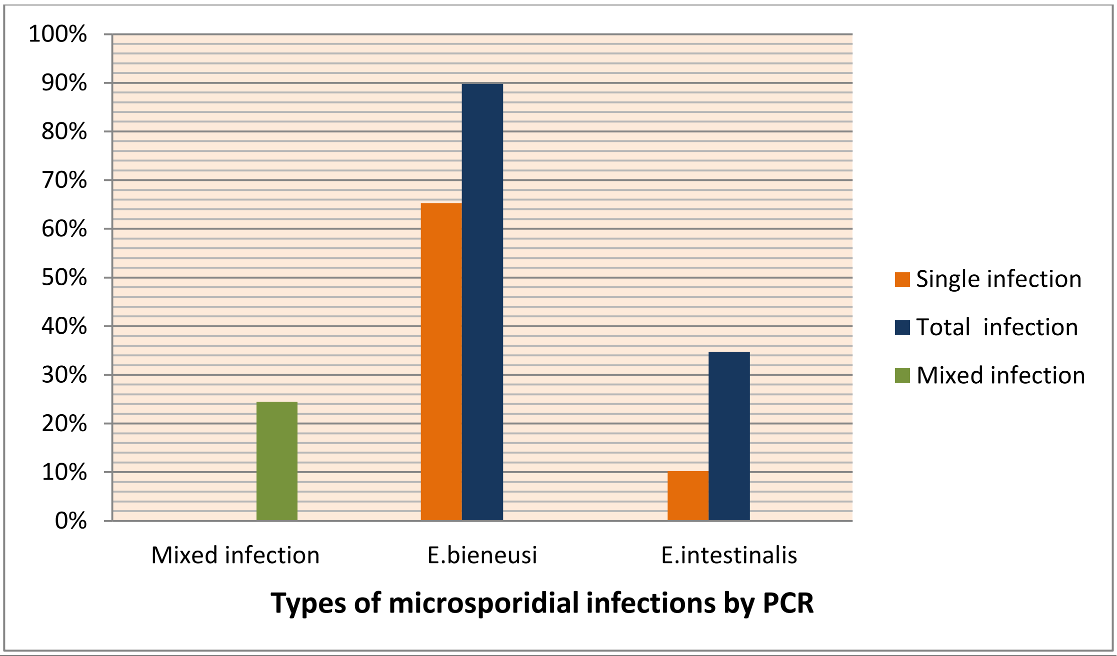


*Figure (12): Detection of microsporidia species in microsporidia- positive samples.*

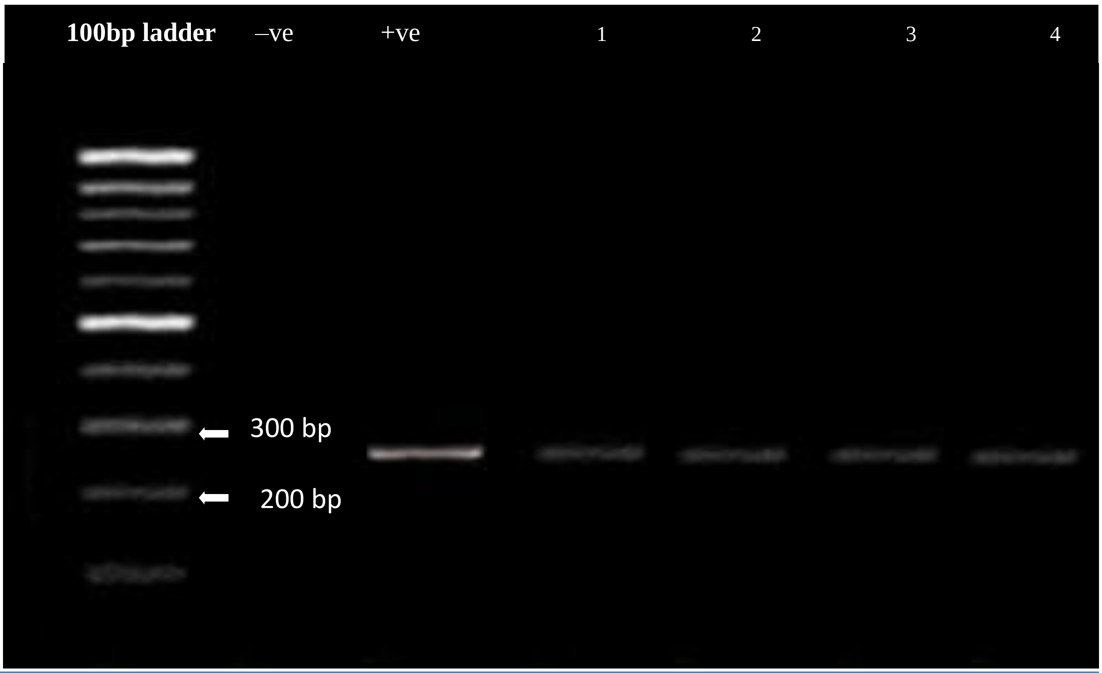


*Figure (13): Ethidium bromide stained 2% agrose showing PCR amplified product of intestinal microsporidia. M: marker; -ve: negative control; +ve: positive control; lanes 1-4: positive samples.*

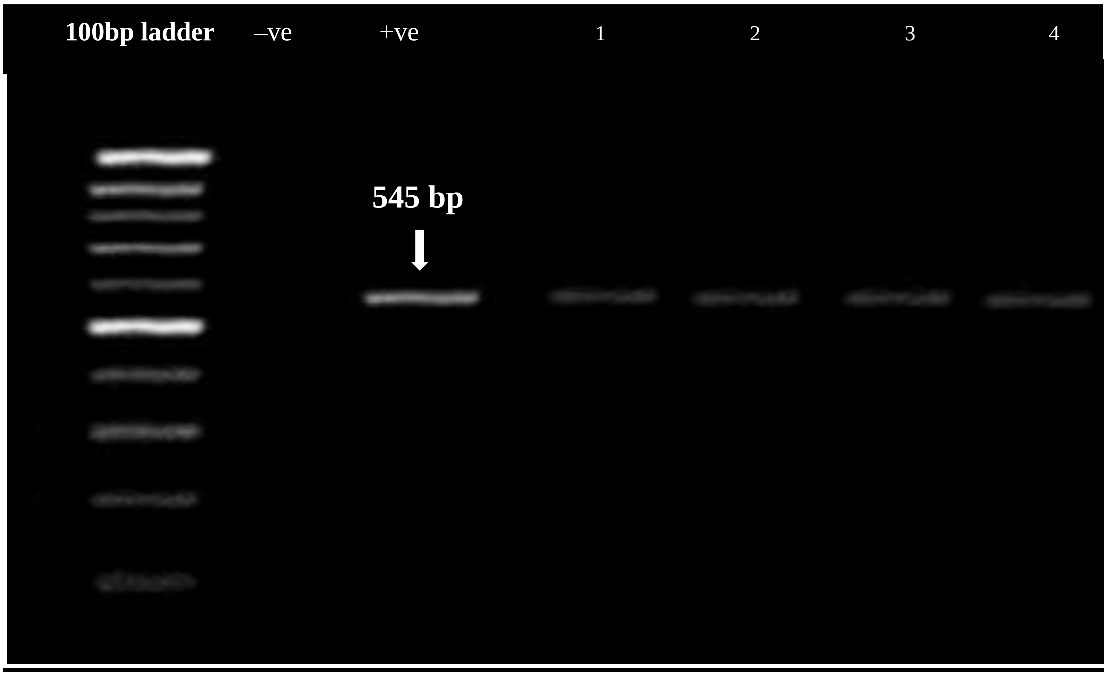


*Figure (14): Ethidium bromide stained 2% agrose showing PCR amplified product of Encephalitozoon intestinals. M: marker; -ve: negative control; + ve: positive control; lanes 1-5: positive samples.*

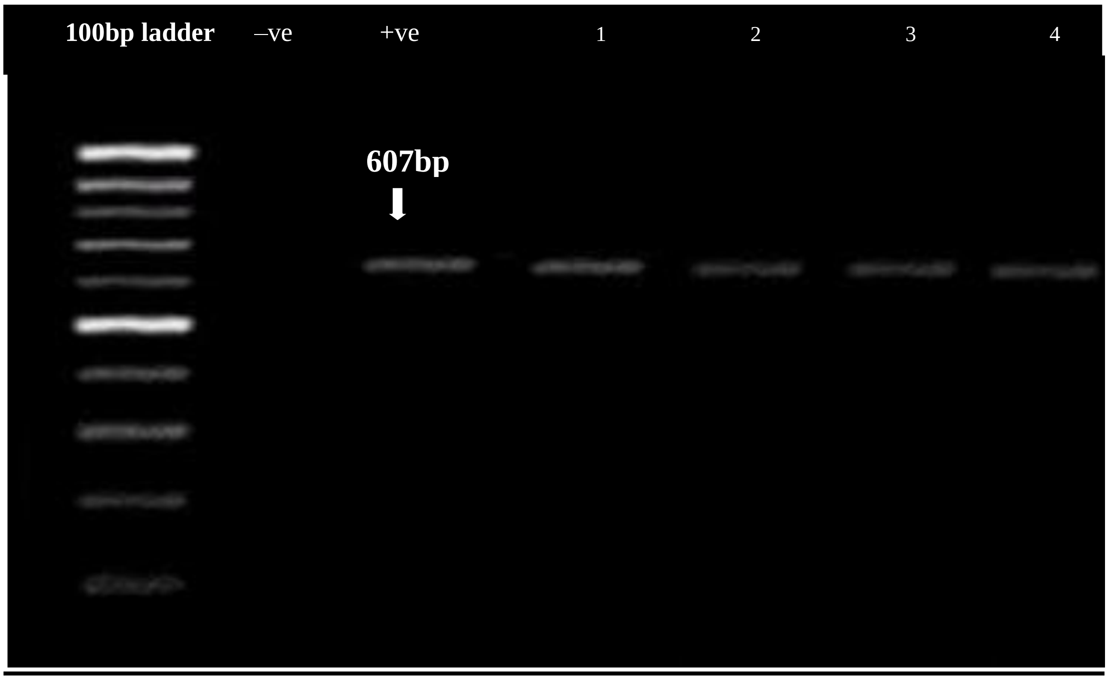


*Figure (15): Ethidium bromide stained 2% agrose showin PCR amplified product of Enterocytozoon bieneusi. M: marker; -ve: negative control; +ve: positive control; lanes 1-5: positive samples.*

## 9) Concomitant enteric parasites in microsporidia-positive patients:

In the present study, the associated parasitic pathogens in microsporidia-positive samples were detected by direct microscopic examination and acid fast trichrome staining technique. It was found that 21(43.8%) of microsporidia-positive patients suffered from concomitant parasitic infections. *Entamoeba histolytica* (7/49, 14.2%) was the commonest parasite detected in microsporidia-positive patients followed by *Giardia lamblia* (6/49, 12.2%), *Cryptosporidium parvum* (5/49, 10.2%), *Enterobius vermicularis* (4/49, 8.1%) and *Strongyloides stercoralis*. *Cyclospora* (1/49, 2%) and *Hymenolepis nana* (1/49, 2%) were the least parasites detected in microsporidia-positive patients (Table 15, Table 16 and Figure 16).

***Table (15): Concomitant enteric parasites in microsporidia-positive patients.***

| Total | Patients without other enteric parasites with Microsporidia | | Patients with other enteric parasites with Microsporidia | |
|---|---|---|---|---|
| 49 | No | % | No | % |
| | 28 | 57.2% | 21 | 42.8% |

***Table (16): Distribution of concomitant enteric parasites in microsporidia-positive patients.***

| Parasite \ cases | **No** | **%** |
|---|---|---|
| *Cryptosporidium parvum* | 5/49 | 10.2 |
| *Cyclospora* | 1/49 | 2 |
| *Entamoeba histolytica* | 7/49 | 14.2 |
| *Enterobius vermicularis* | 4/49 | 8.1 |
| *Giardia lamblia* | 6/49 | 12.2 |
| *Hymenolepis nana* | 1/49 | 2 |
| *Strongyloides stercoralis* | 2/49 | 4 |

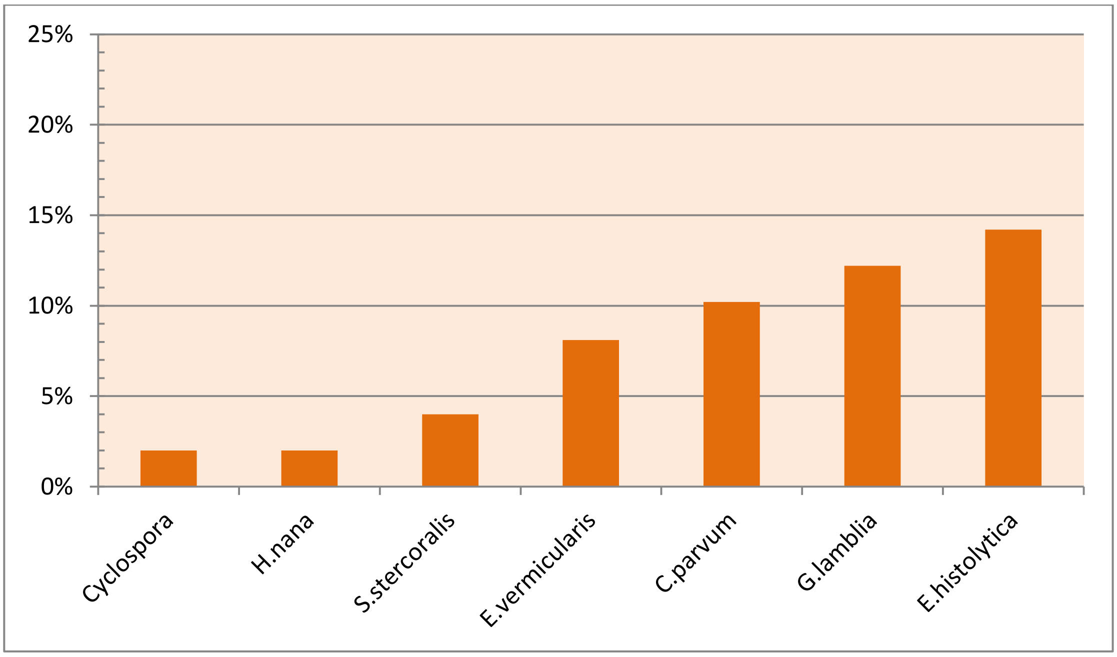


***Figure (16): Concomitant enteric parasites in microsporidia-positive patients***

## 10) The relation between water supply and the rate of infection:

With respect to the source of water supply of examined patients, Table 5, Table 17 and Figure 17 showed that the rate of microsporidial infection was higher in patients who had underground water supply (23.6%) than patients who had tap water supply (11.8%). Statistically, the presence of microsporidia was significantly more common among population used underground water; as Pearson $Chi^2$ = 7.441 and P value (2-sided) = 0.006 for D.F. = 1.

*Table (17): The relation between water supply and the rate of infection.*

| Cases / water supply | Total | Positive cases | | Negative cases | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| **Tap water** | 186 | 22 | 11.8 | 164 | 88.2 |
| **Underground water** | 114 | 27 | 23.6 | 87 | 76.4 |

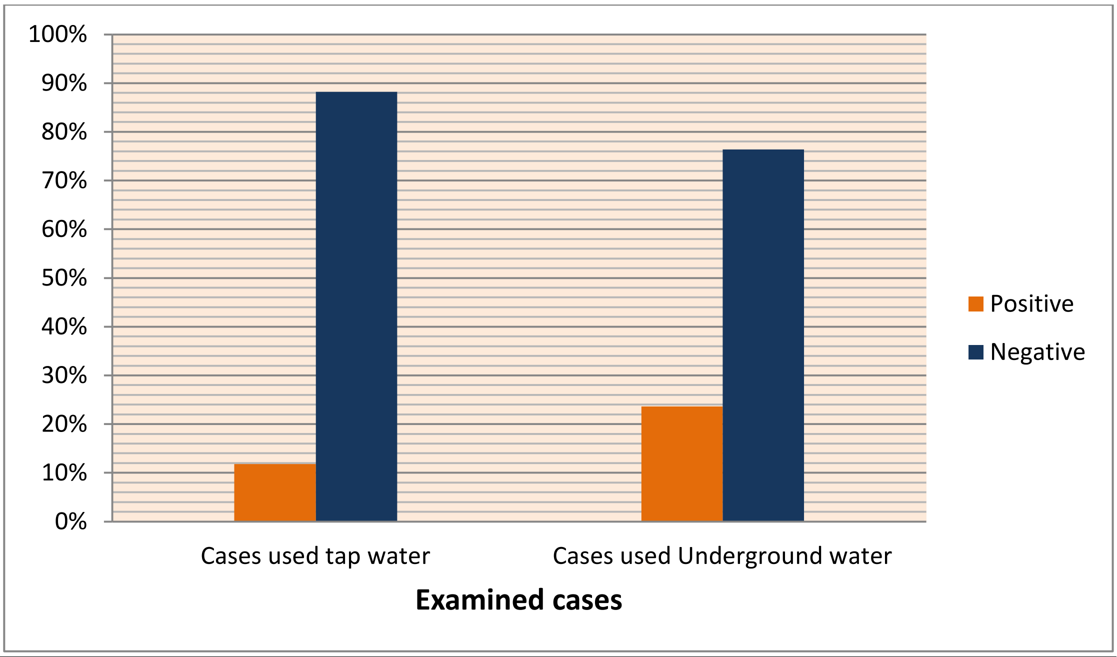


*Figure (17): The relation between water supply and the rate of infection*

## 11) Associations between residence and the presence of microsporidia:

With respect to the habitat of examined patients, the microsporidial infection was detected in 15(12%) patients who lived in urban areas and in 34(19.2%) patients who lived in rural areas (table 12 and figure 15). statistically, the presence of microsporidia was significantly more common among rural population; as Pearson $Chi^2$ = 9.047 and P value (2-sided) = 0.003 for D.F. = 1 (Table 5, Table 18 and Figure 18).

***Table (18): The relation between residence and the rate of infection.***

| Cases / habitat | Total | Positive cases | | Negative cases | |
|---|---|---|---|---|---|
| | | No | % | No | % |
| **Urban cases** | 124 | 15 | 12 | 109 | 88 |
| **Rural cases** | 176 | 34 | 19.2 | 142 | 80.8 |

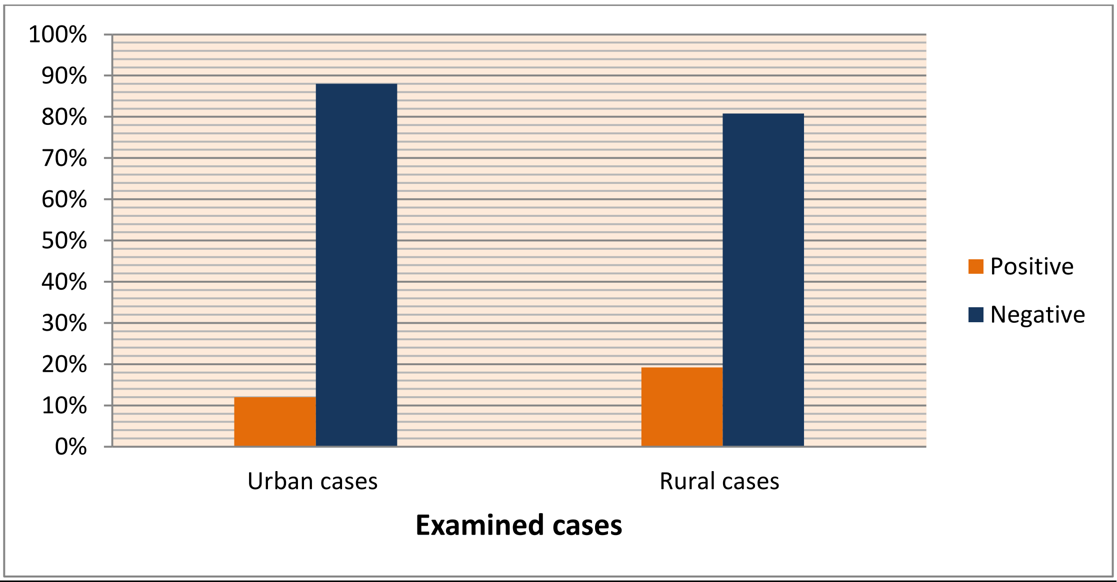


***Figure (18): The relation between residence and the rate of infection.***