Animal Health Research Journal Vol. 8, No. 1, March 2020 pp. 40-48

ISSN: 2356-7767

# Parasitological and pathological studies on *Microsporidian* infection in African sharptooth catfish (*Clarias gariepinus*)

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Received in 7/1/2020
Accepted in 11/2/2020

**Abstract**
Two hundred and forty African sharptooth catfish (*Clarias gariepinus*) were examined for the presence of *Microsporidian sp*. in this study. Fish were collected through one year from the fish market at Al fath center in Assiut Governorate. Forty-six (19.2%) out of 240 catfish were infected by *Microsporidian sp*. Infection was reported as whitish pinhead macroscopic cysts beside microscopic cysts embedded in the musculature of fish. The mature spores were ovoid to pyriform with large vacuole at their ends. Non-significant high infection was detected in summer (33.3%) followed by spring (26.7%) and autumn (10%), while the lowest one was observed during winter (6.7%). The effect of body weight revealed that, non-significant high prevalence rate (26.5 %) was occurred in middle group (301- 400g). Female fish seems to be more sensitive to infection (23.5%) than male (13.5%). The histopathological examination revealed degeneration and necrosis in muscle of infected fish, besides presence of sporophorocyst within skeletal muscle cells.

***Keywords:*** *Clarias gariepinus, Microsporidian sp, Muscle, Pathology.*

## Introduction

Microsporidia are unicellular protozoans living as obligate intracellular parasites and characterized by single-walled spores. The spores are ellipsoidal or egg shaped have a large posterior vacuole, and they have simple life cycles, after ingestion mature spores, merogony and sporogony, which take place in the cytoplasm of the host cell **(Woo, 1995)**. Microsporidial disease affecting most of invertebrates and vertebrates addition to humans which described in patients with acquired immunodeficiency syndrome **(Fedorko & Hijazi, 1996** and **Lom, 2002**). Fish hosts acquire the infection by ingesting infective spores from infected fish or food **(Klinger and Floyd, 2002)**, they commonly were infecting fishes, insects, crustaceans, and other invertebrate and vertebrate groups from different geographical areas **(Lom and Dykova, 1992; Larsson, 1999; Lom, 2002 and Didier *et al*, 2004).** Many species of Microsporidia are infect fish muscles, these included *Pleistophora*, *Heterosporis*, *Kabatana* and *Microsporidium* **(Dykova, 1995).** Microsporidia infecting the musculature of commercially important fish species have a negative impact on fish product and some of species forms elongated whitish nodules which causes liquefaction of muscle fibers resulting in a characteristic concave body surface and, in extreme circumstances, may result in the death of the host fish **(Yokoyama *et al*., 2002)**. *Microsporidia* are considering chronic debilitating and potentially lethal disease of high morbidity rate **(Eissa, 2002)**. In addition, it can cause direct losses due to mortalities, which in natural conditions can be accompanied by subsequent decline of stocks, and by indirect losses due to reduction of the marketing value of infected fish **(Shaw and Kent, 1999)**. Intranuclear microsporidium infection of haematopoietic cells was associated with acute anaemia **(Elston *et al*., 1987).**

Generally, Microsporidian spores can be de-

tected in hematoxylin and eosin stained sections when they occur in aggregates. However, in light infections when only single spores are present in areas of inflammation, detection by hematoxylin and eosin is difficult **(Peterson *et al.*, 2011).** Some pathological examination revealed degeneration, edema and necrosis in muscles with infiltration of mononuclear cells **(Abdel Mawla and Mohamed, 2010).** Fish Microsporidia are embedded directly in the cytoplasm of host cell which destroy or induce hypertrophy of the infected cell **(Lom and Nilsen, 2003).** Infected cells (xenoma) enlarged to accommodate the proliferating parasite. A xenoma is a hypertrophied cell contains spores and other developmental stages of microsporidia. Those hypertrophic cells may reach macroscopic size as multiple whitish nodules occurred in tissues and giving specific gross pathological lesion **(FAO, 1996)**. As the disease progresses, muscle cells are destroyed and replaced by connective tissue, the damage caused by the parasite makes the fish unfit for human consumption (**Lom *et al.*, 2000).**

Large macroscopic cysts present on the musculature and skin of fish making them unappealing for human consumption, **(woo, 2006)**. Microsporidiosis is associated with a wide range of clinical syndromes in human. It causes diarrhea and systemic disease. Stools are watery, non-bloody and free of mucus associated with nausea, loss of appetite, vomiting, abdominal pain, fever, all of these depending on species of *Microsporidia*, site of infection and the immune status of the host.

This study spot light on the prevalence, sex, weight and seasonal variation of *Microsporidian* species parasitism infecting African sharptooth catfish (*Clarias gariepinus*) as well as the histopathological alterations in infected muscles.

## Materials and Methods

### Fish:

Two hundred and forty specimens of sharptooth catfish (*Clarias gariepinus*, Burchell, 1822) of different sexes and weights (divided into three groups as 100-300 gm, 301-400 gm and 401-600 gm) were collected from the fish market at Al fath center in Assiut Governorate through one year (60 fish /season). Fish were immediately transported, on ice, to the laboratory of Fish Medicine and Management Division; Fish were weighed and subjected to external clinical examinations for detection of any abnormalities according to **(Austin and Austin, 1987).**

### Parasitological examination:

Macroscopic examination of muscles was carried out for detection of any visible *Microsporidia* cysts. Squash preparations of muscles were examined microscopically for studying the spore morphology. Smears were air dried at room temperature, fixed in methyl alcohol, stained in 1:9 Giemsa solutions for 20 min. and examination under the oil immersion lens **(Narasimhamurti and Kalavati, 1979)**. Measurements of spores were taken using the ToupTekToupView ® Software, Version: 3.7, calibrated to a stage micrometer. Dimensions that include length and width of spores were measured in micrometer (μm) according to **(Dykova, 1995)**.

### Histopathological examination:

Infected and uninfected specimens of skeletal muscles were fixed in 10% neutral buffered formalin for histopathological studies. Paraffin embedded sections were cut by microtome as four-micron thickness and stained with haematoxylin and eosin (H&E). In addition, selected sections were stained with Giemsa. The histological preparations were examined under light microscope **(Lom and Dykova, 1981)**.

### Statistical analysis:

Results were analyzed using (Prism 5, version 5.01, Graph pad software Inc.). The differences were considered significant if the (P-value <0.05) by using unpaired t-test or one way ANOVA test (tukey's compare all pairs of column).

## Results

### Clinical signs and gross findings:

Infected fish demonstrated various non-specific clinical signs and gross lesions including paler or darker skin than normal, erosions, ulcers, hemorrhagic and/ or black spots or fin rot, in some infected fish were observed. Numerous macroscopic pin head white cyst throughout the skeletal muscles also detected,

usually spherical, cyst like formation. Bulge from the muscle. (Fig. 1, a).

**Morphological findings:**
In Squash preparations from infected muscles showed the presence of mature spores were mostly ovoid to pyriform in shape with characteristic one polar capsule and large posterior vacuole at the posterior end appeared as a pale area and extend to middle of the spores. (Fig. 1, b & c). They are measuring 6±0.5 µm in length x 3± 0.7 µm in width. Spores causative agent was identify as *Glugea anomala.*

**Epidemiological findings:**
Percentage of infection in examined fish was summarized in Table1: out of 240 examined fish, 46 (19.2%) were found infected with *Microsporidia*. The highest infection rate in examined fish was in summer 33.3%, followed by spring 26.7% and autumn 10%, while the lowest one was observed during winter 6.7%.
Concerning weight of examined fish, prevalence was distributed as following: 22.2 % in the group 100-300g, 26.5 % in the group 301-400 g and 9.8% in the group 401-600 g (Table 2 and Chart 1). Fish weight was not a statistically significant factor for infection with *Microsporidia.*
According to effect of sex on the infection rate, females *Clarias gariepinus* had a highest infection rate 23.5% than male 13.5%, (Table 3 and Chart 1), although fish sex was not a statistically significant factor for infection.

**Histopathological findings:**
Skeletal muscle bundles suffered from degenerative changes expressed by intermuscular odema and vacuolation. The necrotic muscle bundles replaced by fibrous tissue (Fig.2). The lesions were confirmed by presence of Microsporidian spores collection surrounded by a thin layer of connective tissue invading skeletal muscle bundles (Fig.3). In addition to H&E, Sporophorocyst containing sporoblast and spores within skeletal muscles and ruptured sporophorocyst vesicles indicated by Giemsa stain (Fig.4).

**Discussion**
Microsporidial disease is characterized by a severe inflammatory reaction in branchial microsporidia in rainbow trout, with variable (generally lesser) degrees of systemic organ involvement, high mortality rates, and prolonged recovery periods during which production efficiency is severely affected **(Speare *et al.,* 1998)**.

Examination of the infested fish revealed presence of pin head whitish cyst, these are nearly similar to the findings reported by **Eissa, (1995) and (2002); Yokoyama, *et al*., (2002) and Abdel Aal, (2002)**. **El- Khatib (2002**) confirmed our finding of microsporidiosis as cysts who detected it in gills and muscle of *Mugil sp*, *Sebastes marinus* and *Clarias gariepinus*.

Until recently, little pertinent information on *Microsporidia* epidemiology in *Clarias gariepinus* has been reported in Assiut Governorate. In our study overall infection rate of *Microsporidia* in examined fish was 19.2%, which similar to **El- Khatib, (2002)** who recorded the infection in *Clarias gariepinus* 20%. Our result was lower than that obtained by **Eissa, 1995** (43%), and higher than that obtained by **Abdel Aal (2002)** and **Ibrahim and Ezz El din (1999)** was 8.6% and 3.5% respectively, in different species of fish. Some species of *Microsporidia* have even a wider host range but other limited to one host **(Lom and Dykova, 1992)**. **Woo (1995**) mentioned that the site selection of *Microsporidian* within the hosts depends on their requirements for a particular cell type within which they can develop; and *Microsporidian* species seen to be highly cell specific. Cyclic patterns of the parasite may due to the climatic conditions, food conditions, host behavior, and host immunity **(Grassly *et al*., 2005)**.

The parasite was identified as *Microsporidia* sp on the basis of its spore morphology. The future classification of Microsporidian parasites will require a combination of morphological and molecular data **(Yokoyama *et al*., 2002)**.

Concerning to season, non-significant high infection was reported in summer (33.3%) which agreed with **Eissa (1995), Ebrahim& Khattab (2000) and Abdel Aal (2002)**, **Abdel**

**Mawla and Mohamed, (2010)**, the development of *Microsporidia* is dependent on the water temperature as mentioned by **(SeongJoonjoh *et al*., 2007)**. The infection was affected by the environmental temperature which affecting the maturation and enhance the generation period of the parasite **(Woo, 1995 and Ibrahim &Ezz El din, 1999)**. Low environmental temperatures inhibit or suppress *Microsporidian* development **(Sveen *et al*., 2012)**. According to weight of examined fish, non-significant high infection was associated with middle group (301-400g) and the lowest one in large weight group (401-600g). **Becker *et al*., (2005)** reported that the smaller fish are more susceptible to *Microsporidia* sp, typically larger (almost market size) succumb to disease in salmon species. Generally, transmission factors of *Microsporidia sp* that fall under the umbrella of host factors are such as host species, fish size, population size or nutritional status **(Hedrick, 1998)**.

The present study found that female fish seems to be more susceptible to *Microsporidia* infection than males, this results is in accordance with **Woo, (1995)**. That is probably due to immunological differences among host sexes and different degrees of resistance to infection by parasites as was suggested by **(Shaw and Kent, 1999**).

In our study, histopathological examination of skeletal muscles revealed presence of smaller groups of spores embedded in muscles of infected fish. Similar observation was recorded by **(Lom and Dyková 2005 and Lovy *et al*., 2007)** who found that *Pleistophora* sp. embedded in skeletal muscle cells of fish and make growth xenoma.
The parasitic nodule which detected in the present work were encapsulated by fibrous layer. **Weissenberg (1976)** mentioned that a common reaction against invading microsporidia is development of layers around the dividing parasites within the xenomas. Recent morphological evidence has suggested that the host endoplasmic reticulum is the source of membranes forming the parasitophorous vacuole during xenoma formation **(Lovy *et al*., 2006).**

The degenerative and necrotic changes of skeletal muscles, suggested that the muscles are the primary site of infection for *Microsporidia* which similar to the result reported by **Sanders *et al*.,( 2010) and Nicholas *et al*., (2015)** who reported that massive infection by proliferative stages and spores cause severe inflammation and rupture of myocytes. In genus *Heterosporis*, the whole development is completed within a special thick membrane, called sporophorocyst of parasitic origin as all stages of the life cycle were present inside it **(Bruno *et al*., 2006)**.

**Conclusion**

This study provided with information on the prevalence, weight and seasonal variation of *Microsporidian* species infecting African sharptooth catfish *Clarias gariepinus* as well as the histopathological alteration in infected muscle, thus continuous investigation are needed. Additionally, microsporidiosis can cause a great economical loss in infected fish as a result of the destruction of muscle tissue in infected fish which giving visible lesions grossly make the flesh unfit for human consumption. Moreover, it must be recommended examination in exported and imported fish to avoid this parasitism in fish.

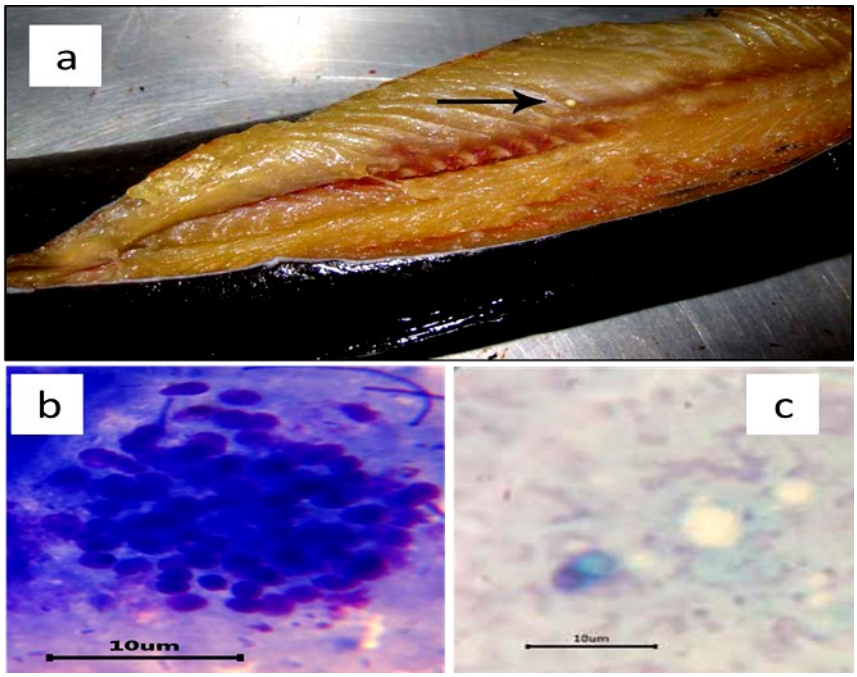



**Fig. (1):** (a) *Clarias gariepinus* fish naturally infected with *Microsporidia* showing pin head white cysts in muscle (Arrow). (b) Opened *Microsporidi*a cyst, Giemsa stain (X 40) (c) *Microsporidia* spores, Giemsa stain (X 100).

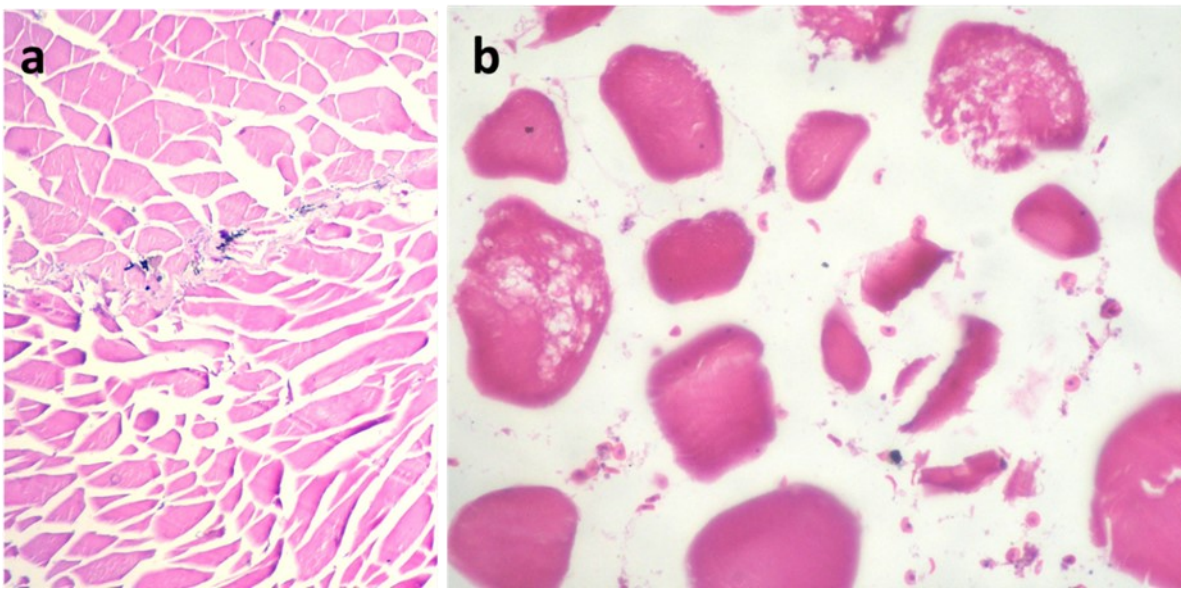



**Fig. (2):** (a) Non infected skeletal muscles showing normal muscle bundle appearance (H&E X100).
(b) Skeletal muscle bundles showing degenerative changes expressed by intermuscular odema and vacuolation ( H&E X400).

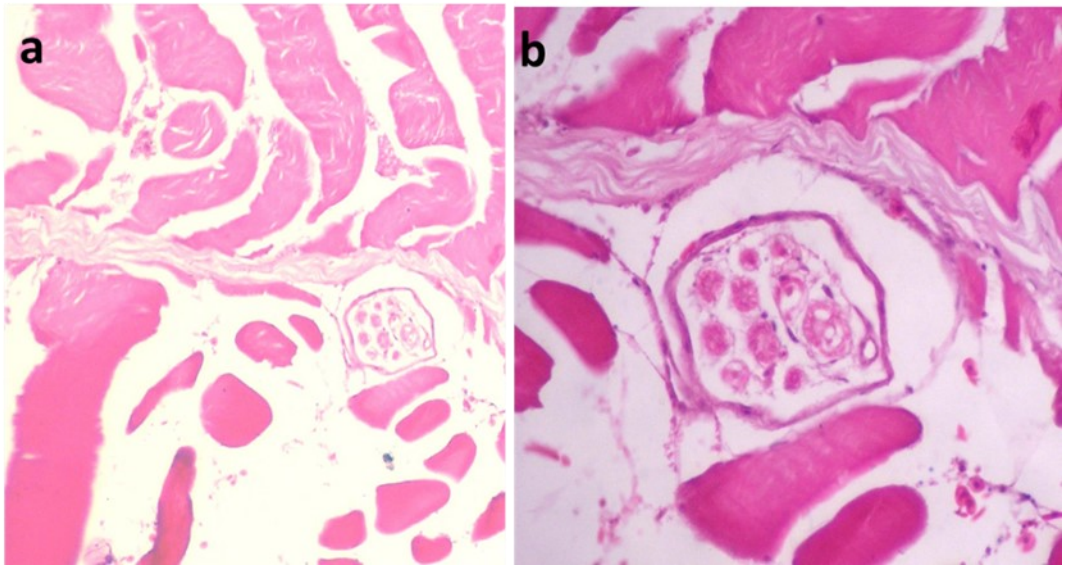



**Fig. (3):** (a-b) collection of spores of *Microsporidia* surrounded by a thin layer of connective tissue invading skeletal muscle bundles. (a) (H&E X100), (b) (H&E X 400).

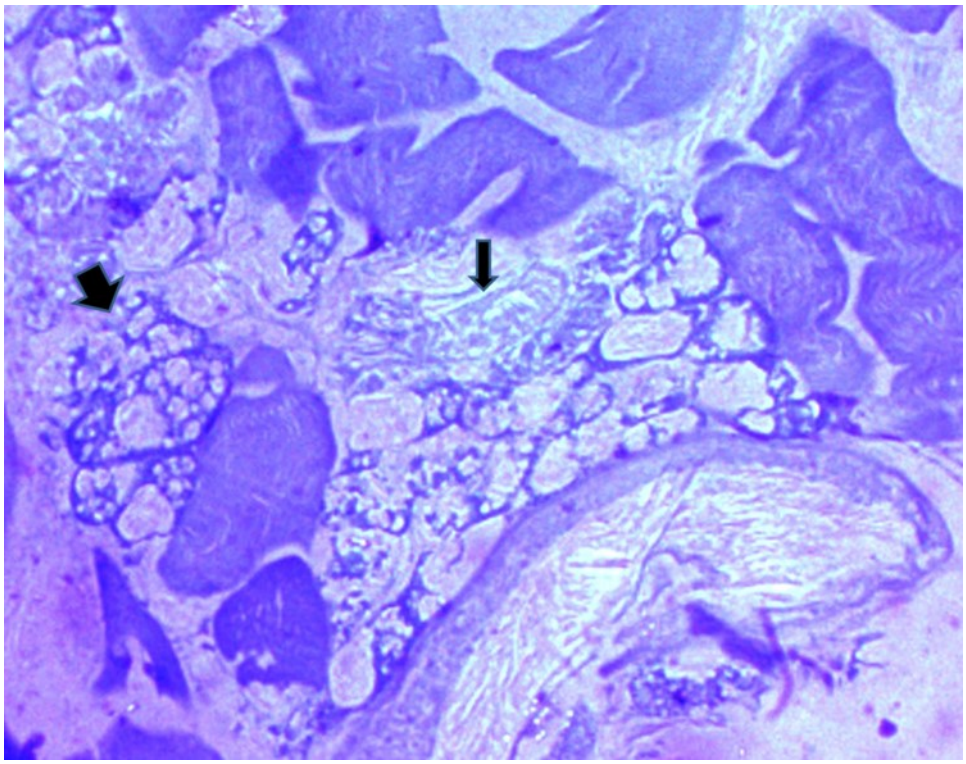


**Fig. (4):** Sporophorocyst within skeletal muscles containing sporoblast and spores (indicated by the narrow arrow) and ruptured sporophorocyst vesicles (indicated by the wide arrow (Giemsa X400).

**Table (1).** Seasonal prevalence of infection with Microsporidia parasites in examined muscle of *Clarias gariepinus.*

| Season | Autumn (n=60) | Winter (n=60) | Spring (n=60) | Summer (n=60) | Total (n=240) |
|---|---|---|---|---|---|
| **Infected fish** | 6 | 4 | 16 | 20 | 46 |
| **Percentage (%)** | 10 | 6.7 | 26.7 | 33.3 | 19.2 |

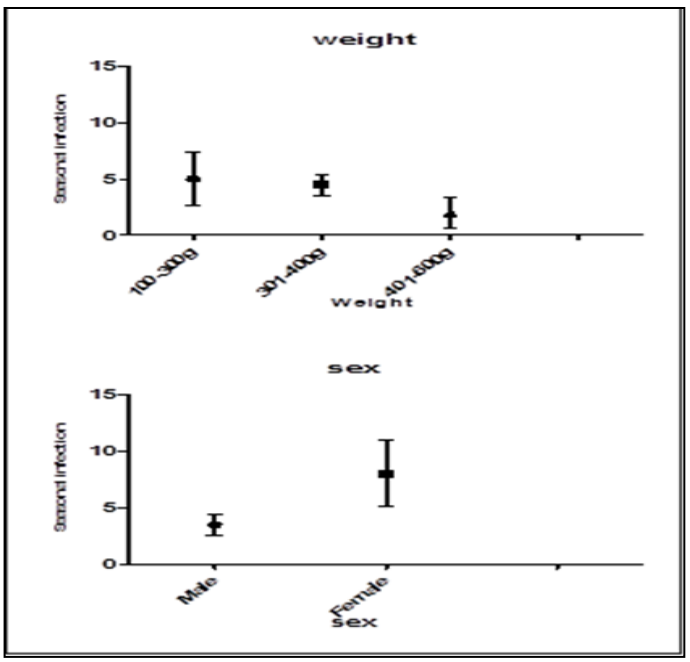
**Table (2).** Weight susceptibility to microsporidia infection in examined *Clarias gariepinus* in various seasons.

| Season / Weight | 100-300g (n=90) | 301-400g (n=68) | 401-600g(n=82) |
|---|---|---|---|
| **Autumn (n=60)** | 2 | 4 | - |
| **Winter (n=60)** | - | 2 | 2 |
| **Spring (n=60)** | 10 | 6 | - |
| **Summer (n=60)** | 8 | 6 | 6 |
| **Total (n=240)** | 20 | 18 | 8 |
| **Percentage** | 22.2% | 26.5% | 9.8% |

**Table (3).** Sex susceptibility to microsporidia infection in examined *Clarias gariepinus* in various seasons.

| Season / Sex | Male (n=104) | Female ( n=136 ) |
|---|---|---|
| **Autumn (n=60)** | 2/24 | 4/36 |
| **Winter (n=60)** | 2/26 | 2/34 |
| **Spring (n=60)** | 4/26 | 12/34 |
| **Summer (n=60)** | 6/28 | 14/32 |
| **Total (n=240)** | 14/104 | 32/136 |
| **Percentage** | 13.5% | 23.5% |

**Chart (1).** Graph Shows body weight and sex susceptibility to Microsporidia infection in examined *Clarias gariepinus*. Columns represent average seasonal infection in examined *Clarias gariepinus*. Data expressed by Mean ±SEM, where there is no significant different at $p<0.05$ using one-way ANOVA and using unpaired t test.



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