Light And Electron Microscopic Study On The Myxosporean (*Henneguya branchialis*) Infecting Suprabranchial Organ Of Cultured African Catfish (*Clarias gariepinus*) In Sharkia Govenorate, Egypt

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ABSTRACT

Four hundreds African catfish (*Clarias gariepinus*) were collected for investigating the infection of the suprabranchial organ. The total percentage of *Henneguya branchialis* infection was 9.5%. The highest percentage of infection (14%) was recorded in spring, while the lowest (5%) was reported in winter. No *Henneguya* infection was recorded in fish weighting below 35 gm. The prevalences of infection in fish weighting 36 to 100, 101 – 150 and over 150 gm were 8.6, 11.8 and 13.7%, respectively. Clinically, the heavily infected fish showed decreased feed intake, lethargy, increased mucus secretion and swimming near the surface of water. The cysts were generally spherical to ovoid in shape, white to yellowish in color and of variable size with milky contents. The total number of cysts per infected fish ranged from 1 to 12.

Microscopically, the *H. branchialis* mature spore, that caused the disease, was fusiform and measured around 15 X 5.2 µm. They showed anterior two equal banana–shaped polar capsules which measured about 7.1 X 2 µm, occupied about half the spore-body. The scanning and ultrastructural details were illustrated. Histipathologically, cysts of *H. branchialis* were embedded in the suprabranchial organ and surrounded by a thin layer of connective tissue infiltrated with leukocytes. The pathological effects and control of the parasite were discussed.

INTRODUCTION

Freshwater aquaculture is one of the most important and the fastest growing sector of agriculture in Egypt during the last two decades. Parasitic diseases constitute the most important hindrances leading to a decreased marketability and high mortality among the cultured fish. Fish culturists, allover the entire world reported significant mortalities caused by protozoans *(1)*.

Myxosporidea are frequently described in freshwater, brackish and marine fishes and have a great importance in Ichtyopathology *(2-4)*. The genus *Henneguya* was described for the first time by Thélohan *(5)*. *Henneguya* is enigmatic and economically important group of parasites for the aquaculture and fisheries industry *(6-8)*. Also, *Henneguya sp.* is highly specialized metazoan parasites of aquatic hosts *(8)*.

Eiras (3) mentioned 146 described species from the genus *Henneguya*. However, lack of information about the epidemiology and ultrastructure of the parasite, makes it difficult to plan the control strategy of the protozoal diseases.

Understanding the etiology of the parasitic disease is of crucial importance as it determines the choice of a potential treatment. Therefore, the only elements needed for an effective parasite diagnosis, at the farm level, are a light microscope and a basic knowledge of parasite-taxonomy. The present study aimed to throw light on pathological effects of *Henneguya* on the hosts, with special attention for the ultrastructure description of the parasite.

MATERIAL AND METHODS

Four hundred (100 per season) cultured (male and female) African catfish (*Clarias gariepinus*) were collected, alive, throughout the year. They were1cm long and 1gm body weight. The fish were transferred to the laboratory for recording the signs, necropsy and parasitological findings *(9)*.

Impression smears for light electron microscope examination were prepared from

cyst of the suprabranchial tissues, air dried, fixed in methanol and then stained with Giemsa stain. The infected cysts were immediately fixed in Bouin's solution, dehydrated in ascending grades of ethanol and cleared in xylene, embedded in paraffin wax and processed routinely for light microscopy. All sections were stained with hematoxylin and eosin (H&E) ***(10)***.

Cysts were fixed in 5% glutaraldehyde buffered with 0.1M sodium cocodylate pH 7.4 at 4C for 24 hr and dehydrated in ethanol. After critical point of drying, the samples were coated with gold and examined using scanning electron microscope. For ultrastructure examination, the isolated spores and fragment of the cyst were fixed in 2.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 5 h at 4°C, washed overnight in the same buffer at 4°C, and postfixed in 2% OsO4 buffered with the same solution for 4 h at the same temperature and then processed by standard methods references for transmission electron microscopy. Samples were embedded in Epon. Ultrathin-sections, 90 nm thick, were prepared using a diamond knife, post-stained with uranyl acetate and lead citrate for examination with a transmission electron microscope ***(4, 7, 11)***.

RESULTS

One *Henneguya* species(*Henneguya branchialis*) was encountered at a percentage of 9.5% (Table, 1). A higher percentage of infection was recorded in spring (14%), followed by summer (11%), then autumn (8%) and the least (5%) in winter (Table, 1).

Table 1.Prevalence of Henneguiasis in different seasons.

Season	Total no. examined fish	Infection rates	
		infected	%
Spring	100	14	14
Summer	100	11	11
Autumn	100	8	8
Winter	100	5	5
Total	400	38	9.5

There was a relationship between the prevalence of infection and body weight of the examined fish . No *Henneguya* infection was found in fish weighting below 35 gm (Table, 2). The prevalence of infection in fish weighting 36 to 100, 101 – 150 and over 150 gm was 8.6, 11.8 and 13.7%, respectively . The heavy infection led to a decrease in feed intake, lethargy, increased mucus secretion and swimming near the surface of water. The cysts were generally spherical to ovoid, white to yellowish, variable in size (1–2.5 mm in diameter) and firm in consistency with milky material. They were attached to the tips of the secondary suprabranchial organs of the infected fish (Fig.1). The total number of cysts per infected fish ranged from 1 to 12. Each cyst contained numerous *Henneguya branchialis* mature spores.

Table 2.Prevalence of Henneguiasis prevalence in relation to fish lengths and weights.

Fish weight (gm)	Total no. examined	Infection rate	
		infected	%
Below 35	45	0	0
36 – 100	162	14	8.6
101 – 150	135	16	11.8
Over 150	58	8	13.7

On microscopic examination, the morphology of the *Henneguya branchialis* mature spores that cause the disease fusiform in shape and measured around 15 X 5.2 µm (Fig. 2) . They showed anterior two equal banana – shaped polar capsules which measured about 7.1 X 2 µm. The latter occupyied about half the spore body. The sporoplasm contained iodinophilus. oval to rounded vacuole. The posterior end of the spore was prolonged with two extending processes forming a tail which measured around 2 µ in length.

The induced lesions varied according to the intensity of infection. Cysts of *H. branchialis* were embedded in the suprabranchial organ. They were surrounded by a thin layer of

connective tissue. Each cyst showed an outer thick cartilaginous layer of which formed the suprabranchial organ followed by capsule containing dilated blood vessels with bronchiole epithelium. Necrotic areas of cartilaginous tissue surrounded of the cyst. The severe infestion induced a cyst like sac filled with the parasitic elements, surrounded with atrophied cells and proliferatied cartilaginous structures (Fig. 3-5).

The scanning electron micrograph of the external feature of *H. branchialis* spores showed smooth spore valves with characteristic ridged posterior body surface . The sutural edge folds were prominent with thickned lateral sutural crest (Figs . 6-8).

The ultrastructural data using transmission electron microscopic (TEM) study on the early developmental stage (generative cells), elucidatea the presence of mature spore and pansporoblasts (developing stage) (Figs . 9-13). The grenerative cells were spherical have large nuclei which usually occupied the whole volume of the cell (Fig. 9). Two types of generative cells may be recognized. One type with the nucleus containing a distinct nucleolus and another with the nucleus lacking anucleolus. The developing generative cells grow progressively in size , accumulating several tiny mitocondria with few tubular cristae, numerous ribosomes and smooth endoplasmic reticulum in their cytoplasm (Fig. 10) .

The earliest pansporoblast stage was represented by the enclosure of a generative cell by an envelope cell. Subsequently, the cellular division resulted in pansporoblast with sporont progeny cells, which were all contained within the envelope cell. A three cell stage pansporoblast resulted from such a division (Fig. 11), while five cell stage, with distinctly differentiated cells, was limited by two unit membranes. Each five cells stage was a sporoplasm cell contained within a developing cell. Both were surrounded by two valvogenic cells (Fig . 12) .

The longitudinal section through the polar capsules showed that the polar filament is arranged into seven coils in each polar capsule (Fig. 13). The longifudrnal section through immature polar capsule in the sporoblast of *H. branchialis,* showed double layered polar capsule and a cap at the spore tip besides flattened valvogenic cell nucleus , fine granular matrix and polar filament arrangement, besides inner and outer layers of the capsular wall (Fig . 14).

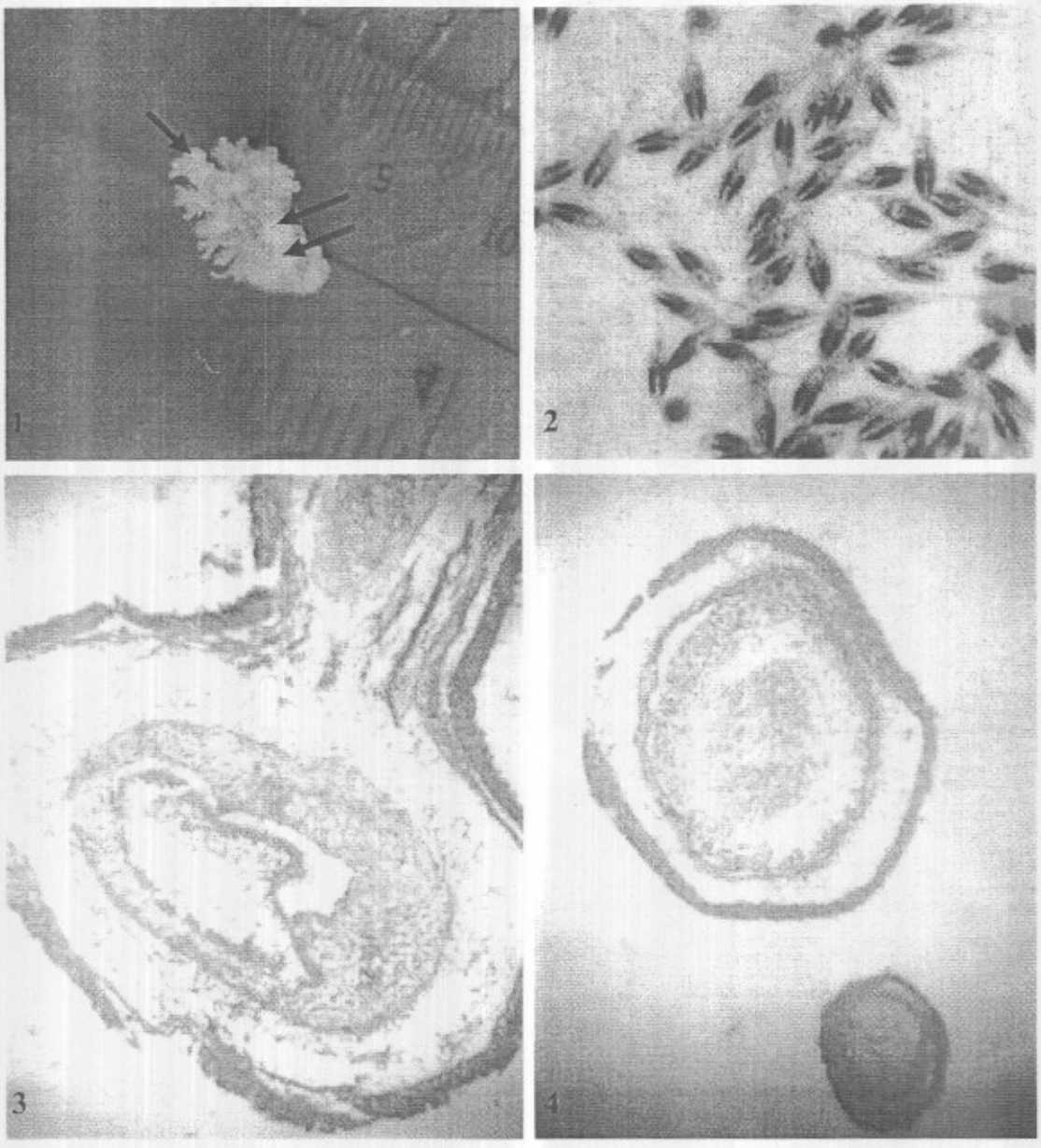

Fig. 1. Suprabranchial organ of catfish showing macroscopic cyst of *H. branchialis*.

Fig.2. Mature spores of *H. branchialis* showing tail bifurcation, darkly stained polar capsules and whitish unstained iodinophilous vacuoles, Giemsa stain. (X 100).

Fig.3. Longitudinal section of the suprabronchiole of the secondary respiratory organ of catfish, *C. gariepinus* showing *Henneguya branchial* cyst surrounded by thick cartilaginous layer, invaded by few inflammatory cells. H&E. (X10)

Fig.4. longitudinal section of the suprabronchiole of the secondary respiratory organ in catfish, *C. gariepinus,* showing the cyst of *H. branchialis* with destruction, sloughing and necrosis of the cartilgenous tissue. H&E. (X4).

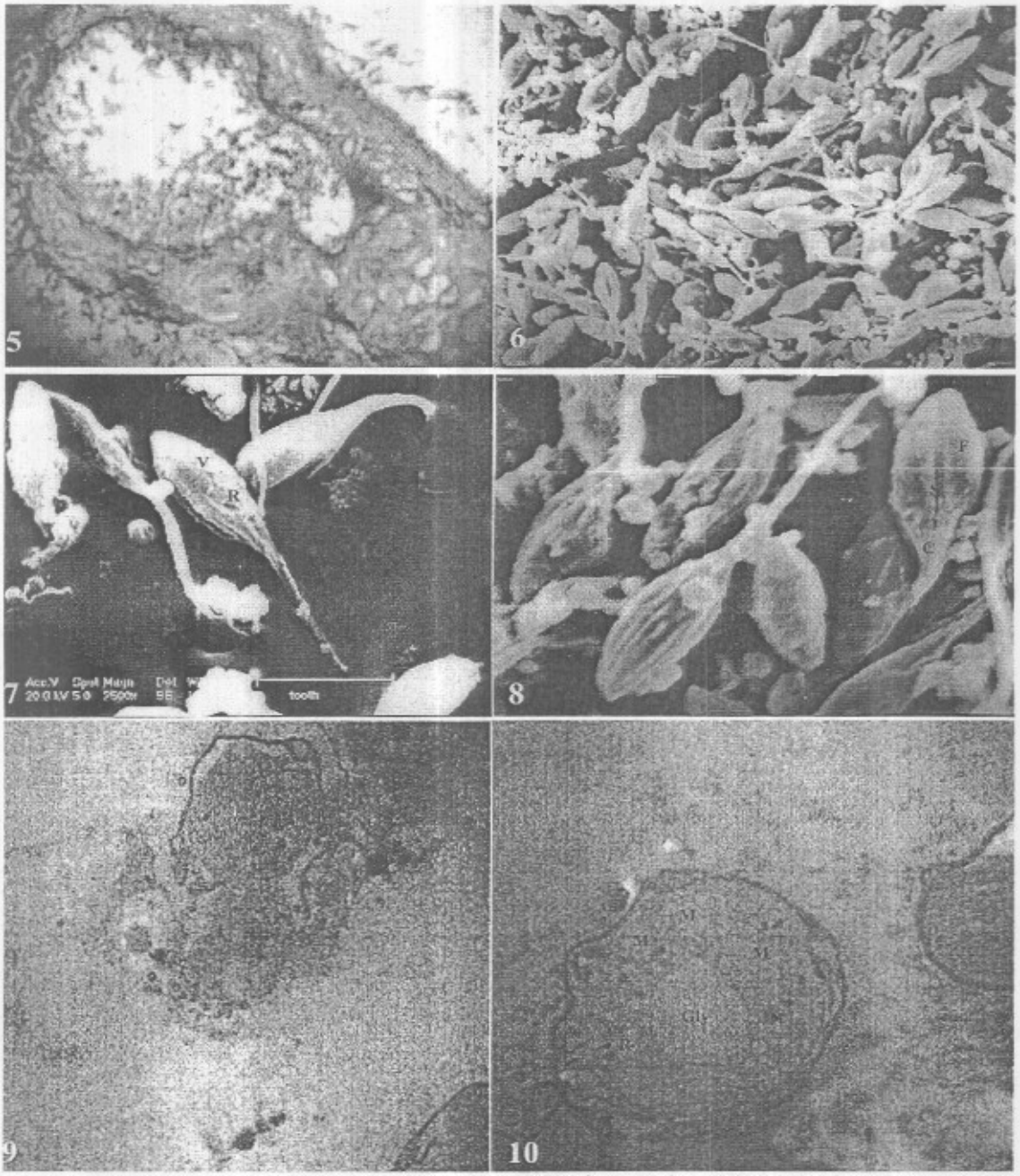

Fig. 5. Suprabranchial organ of *C. gariepinus* showing cyst of *H. branchialis* with hyperplastic cartilagenous layers of the suprabranchial organ. H&E.(X 10).

Fig. 6. Scanning electron micrographs (SEM) of *H. branchialis*, (X 1000).

Fig.7. Scanning electron micrographs (SEM) of *H. branchialis* appearing the lenticular shaped spores with smooth spore-valves (V) and characteristic ridges (R) at the posterior part in adorso–ventral view, (X 2500).

Fig.8. Scanning electron micrographs (SEM) of *H. branchialis* showing the sutural edge Fold (F), thickened lateral sutural crest (S) and the union between two caudal processes (C), (X 3500).

Fig. 9. Electron micrograph of different developmental stages in *H. suprabranchiae*. (X41315).

Fig. 10. Primordia of growing generative cell showing a smaller nucleus (N), many mitochondria (M), ribosomal rich cytoplasm (R), glycogen (Gly) and valvogenic cell (Vc). (X51643).

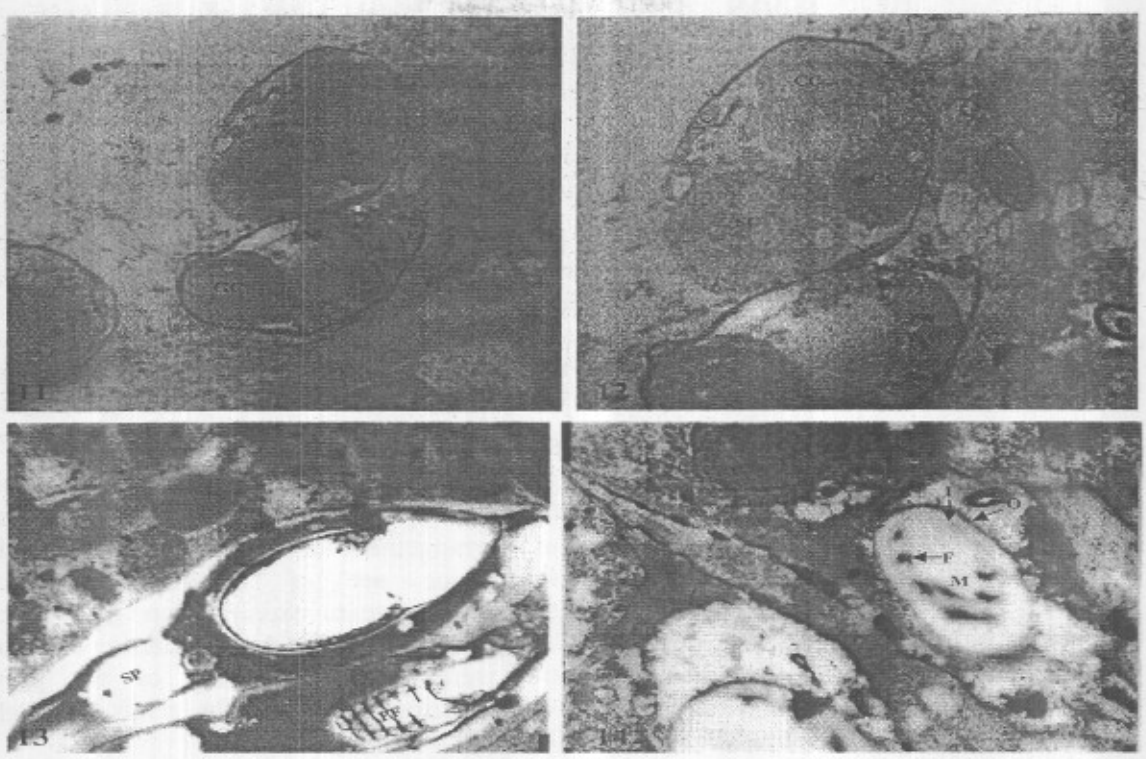

Fig. 11. Showing developing sporoblast resulting from the envelopment of agenerative cell by a pericyte (enveloping cell). Envelope cell (Ec), generative cell (Gc), nucleus (N) and nucleolus (Nu). (X30986).

Fig. 12. Showing developing sporoblast in which two capsulogenic cells (Cc), one sporoplasmic cell (Sc) and two valvogenic cells (Vc). (X51643)

Fig.13. Longitudinal section throughout H. branchialis whole spore showing the polar capsule with polar filam ent (PF) helically arranged in seven coils and sporoplasm (Sp). (X 6701000).

Fig.14. Longitudinal section through H. branchialis immatunre spore showing cap-like structure at the spore tip (C), flattened valvogenic cell nucleus (Vn), polar filaments (F), finely granular matrix (M) and outer and inner layers of the capsular wall (OI). (X 1400998).

DISCUSSION

Several heavy myxosporean infections of cultured fish were reported to be pathogenic and predispose the host to other infections *(1, 4, 8, 12)*. There are about 1350 species of myxosporeans distributed in 52 genera, most of them parasitize freshwater fish *(13)*.

Henneguya is among the most cosmopolitan of all the Myxosporidian parasites infecting wide range of fish species and can infect any organ but does not infect human *(1, 8, 11)*.

The total percentage of *H. branchialis* infection was 9.5%. Nearly similar percentage (10%) was recorded in African-catfish *(14)*. However, a higher prevalence (23 to 88%) was reported *(8, 11, 15, 16)* in different fish species. The current study revealed a high prevalence (14%) in spring. Similar results were documented *(6, 17)*. The peak of infection was during the spring. others *(14)* found the highest rate during the summer. On contrary 100% prevalence was recorded in winter *(15)*.

Dealing with the relationship between the prevalence of infection and body weight of the examined fish, our results showed that no *Henneguya* infection was recorded in fish weighting below 35 gm. The prevalence of infection was recorded in fish weighting 36 to 100, 101 – 150 and over 150 gm was 8.6, 11.8 and 13.7%, respectively. These results agree with previous study which showed *(16)* significantly low rate of infection in smaller fish. However, the differences in the prevalence may be attributed to locality, fish species, weight and length of fish, season and water quality *(1)*.

Henneguya spreads through the water. When the cyst ruptures, millions of *Henneguya* spores are released. The spores are drifted in the water to infect a new host where they attach with a grappling hook-like organ called a polar filament. Once attached to a new host, the organism forms a new cyst and begins to multiply.

Clinical signs of henneguyasis were generally nonspecific. Only the heavily infected fish, showed a decrease in feed-intake, and increased mucus-secretion, besides respiratory distress. The cysts are generally spherical to ovoid in shape, white to yellowish in color and variable in size. They attach to the tips of the accessory branchial organ. Similar signs and lesions were recorded *(1, 6, 12, 14, 15)*. Rupture of the branchial cysts caused intense hemorrhage and facilitated invasion of the secondary opportunistic pathogens *(1)*. However, myxozoans infecting the gills were characterized by a specific site selection *(18)*.

The identification of the species depend on the spore-size, shape and ultrastructural arrangement of its components, besides the major set of taxonomic criteria for myxosporean classification *(19)*. the most stricking morphological criteria of this parasite were similar to those of the previously described species of the genus *Henneguya (1, 19)*.

Microscopically, the infected fish showed intercellular cyst-like structure filled with mature spores, showing long spermatozoa-like-tail. The cyst-wall, surrounding the parasitic elements, showed fibrous connective tissue and leukocytic infiltration. Similar picture was noticed *(1, 6, 7, 14, 15, 16, 20)*.

The most important method for controlling the disease is the removal of dead and moribund fish as the experience with the treatment of parasite is lacking or rare potentiating the immune response against the parasitic infections and the development of vaccine against the most important pathogenic parasites are promising.

It could be concluded that *Henneguya* sp. induced destructive effects in the suprabranchial organ. It showed the highest prevalence in spring, and affected the general health of the fish.

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الملخص العربى

دراسة باستخدام الميكرسكوب الضوئي والألكتروني على الميجسوسبورين (هينيجويا برنكياليز) والتى تصيب عضو فوق الخيشيوم لأسماك القط الأفريقي المرباة بمحافظة الشرقية – مصر

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قسم أمراض الأسماك - المعمل المركزي لبحوث الثروة السمكية بالعباسة – مركز البحوث الزراعية

اجريت هذه الدراسة على عدد ٤٠٠ سمكة من القرموط الأفريقي والتى تم تجميعها على مدار العام بواقع ١٠٠ سمكة فى الموسم. كانت نسبة الأصابة الكلية بطفيل الهينجويا ٩,٥ %. أشارت الدراسة ان أعلى نسبة للأصابة كانت فى الربيع (١٤%)، يليها موسم الصيف (١١%) ثم الخريف (٨%), بينما كانت أقل نسبة فى الشتاء (٥%). لم تظهر اى اصابة فى الأعمار الصغيرة والتى ووزنها أقل من ٣٥ جم. بينما كانت نسبة الأصابة فى الأسماك التى تزن ٣٦-١٠٠، ١٠١-١٥٠ ، وأكثر من ١٥٠ جم هى ٨,٦، ١١,٨ و ١٣,٧% على التوالى. سجلت الدراسة أعراض مصاحبة للأصابة الشديدة على هيئة امتناع عن التغذية، زيادة فى افراز المخاط ، خمول والعوم قريبا من سطح الماء. تسبب الطفيل فى ظهور حويصلات على العضو المصاب على شكل بيضاوي او كروى ذو لون أبيض مصفر ومختلف الأحجام ويحتوى على مادة لبنية، ويتراوح عددها من ١ - ١٢ لكل سمكة. تم وصف الطفيل باستخدام الميكروسكوبين الضوئي والألكتروني (الماسح والنافذ) وكذلك التغيرات الباثولوجية المصاحبة للأصابة ومناقشتها.