Some Studies On Systemic *Dermocystidium* sp. Infection In Some Cultured Freshwater Fishes

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ABSTRACT

This is first study that carried out in Egypt on the distribution and prevalence of systemic infection caused by *Dermocystidium* in some cultured fishes and factors affecting the epizootiology of that disease. The clinical signs of naturally infected *Claris garpinus*, *O.niloticus* and *Ctenopharyngoton idella* with *Dermocystidium* spp.were observed as thin walled roundish oval or elongated shaped micro and macrocysts found in skin and all fins and present of *Dermocystidium* spores freely with mucus in gills. Visceral granulmatus infection was recorded only in *O.niloticus* and *C.idella* with signs of severs emaciation. Kidney, liver, spleen and intestine were found enlarged and contain thick walled longish yellow macrocystes. Microscopic examination of cyst contents revealed the presence of numerous spores characteristic of *Dermocystidium* species.

Attempt to culture *Dermocystidium* spores in liquid thioglycollate medium was successes. In addition, zoospores formation occurred in water contain antibiotics at 25°C within 4days. Healthy *O.niloticus* and *C.garpinus* were infected experimentally by zoospores of *Dermocystidium* originated from visceral *Dermocystidium* infection at 15°C and 25°C. Mortality and morbidity, clinical signs, postmortem changes in experimentally infected fish were recorded. It has been concluded that *O.niloticus* single exposed to infection were susceptible to systemic infection by *Dermocystidium* spp. and *C.garpinus* not susceptible. The clinical picture of the diseased *O.niloticus* was affected by environmental temperature.

INTRODUCTION

Dermocystidium infection is a fungal disease affecting fish .As its name suggests, *Dermocystidium* infection is a disease that affects the skin of the fish and can be found on the gills, fins or body. *Dermocystidium percae* described early as microsporidium *Haplosporidia* which parasitize common Perch *Perca fluvialillis* in Germany ***(1)***. The species observed produce oval cysts in the subcutis of the Perch, fins, head and between pectoral fins. Visceral granulomatous infection occur in *Gold fish* ***(2)***, *Carp* ***(3)*** and *Oreochromis hybrids* in Israel ***(4)***. Systemic infections in fish were associated with intense granulomtous lesions mainly epitheloid, which gradually increasing necrotic core. Infection first developed in the kidney, next in the spleen and later in other organs, hemorrhagic dropsy often occurred and kidney and spleen became enlarged. In Tilapia infection the disease is epizootic thus far has been detected only once in over wintering stock in March. The disease believed to be a world wide distribution as described by many authors but not known in Africa and recorded in Tilapia farmed in Israel ***(4)***.

Three kind of *Dermocystidium* cysts were recorded ***(5)*** elongated cysts, round ball like and egg- like micro cysts .Longish cysts of *Dermocystidium* species found in gills of *Anguilla anguilla* ***(6)*** and skin of *Tetraodon palembangensis* ***(7)***. Roundish and dumbbell – shaped cysts with thicker walls of *Dermocystidium* species of *Perch* cysts occurred in the skin of all fins but most often in abdominal fins ***(8)***. *Dermocystidium* like organism occurring freely with the epidermis and mucus of *Juvenile salmon* that was associated with low level mortalities under culture condition was previously described ***(9)***. *Dermocystidium salmonis* forms small white cysts on the gills of *Chinook salmon* but can also infect skin and spleen and mortalities have been reported ***(10)***. Examination of cyst contents revealed the presence of numerous spores typical of the genus *Dermocystidium* ***(11)***.Spores were spherical with marginated

sporal cytoplasm and a nucleus, which was displaced by the characteristic central refractile body or vacuoplast and can be detected both in wet mount or Giemsa stained smears under light microscope *(2)*. *Dermocystidium* diagnosis was done by cultured the specimens in fluid thioglycollate medium contains antibiotics and examined under light microscope after 4-7 days with drop of lugol's iodine to detect bluish black spherical spores *(2)*. When the life cycle of *Dermocystidium* was studied a zoospores stage was revealed. Zoospores and water –borne transmission of *D. salmonis* has been carried out in the laboratory *(12)*. Development of small cyst from an experimental infection by the zoospores of *Dermocystidium* spp. Was Produced in water at 25°C and *perch* kept in an aquarium at 17°C *(8)*.

In Egypt, information on the distribution and prevalence of *Dermocystidium* species affecting cultured fish is required to determine and clearfing the threats to the health of cultured fish .This work provides data on the prevalence and mortalities in some cultured fish and information on the results of our investigation on the morphology of the organism, in addition to factors affecting epizootiology of that disease.

MATERIALS AND METHODS

1- Natural infected fishes

Three cultured fish species have been selected for the present study *Oreochromis niloticus (O.niloticus), Ctenopharyngodon idella (C. idella)* and *Claris garpinus (C.garpinus)*.

A total number of 300 fishes, one hundred from each species, weighted two body weight rang 10-20 gm and 100-120 gm. Fishes were collected from three private fish farms in Kefir El Sheikh Governorate, water temperature in fish farms were recorded. The fish were transferred to lab and maintained in glass aquaria contain dechlorinated water. All fish were clinically examined for any clinical manifestation and visible lesions or cysts. Postmortem examination was done and any abnormalities were recorded. Wet preparation from external and internal visible cysts and organs were freshly examined under microscope for *Dermocystidium* spores and measured using phase contrast microscopy with an ocular micrometer calibrated with a stage micrometer.

2-Isolation and identification of *Dermocystidium* spp

Tissue samples measuring approximately 5-10 mm from gills, skin, fins visible cysts, liver, kidneys and spleen were placed in fluid thioglycollate medium (Difco) containing 200 units of mycostatin (Nystatin), 500 units penicillin and 500 dihydro-streptomycine per ml of medium *(13)*, incubated at 25°C for 4-7 days in the dark. Identification of the fungal growth was performed by microscopic examination of wet preparation and examined after mounting the stain with Lugol's iodine solution *(14)*.

3-Cultivation for experimental infection in sterile dechlorinated water

Spores of *Dermocystidium* isolated from visceral infected *O.niloticus* cysts was inoculated in sterile dechlorinated water contains antibiotics (500 mg streptomycin /ml) and inoculated at 25°C for 4-7 days then examined for zoospores formation every day when zoospores formed and released into water, move with flagella then collected and orally inoculated in experimental healthy fish. Some of the spores left for complete life cycle under observation *(8)*.

4-Experimental infection

A total of 40 *O.niloticus* and 40 *C. garpinus* weighting 80-100 gm were obtained from healthy lot of private fish farm were held in eight glass aquaria with an air supply and dechlorinated water *(15)*. Each species were classified into four groups 10 fish each and maintained at 15±1°C and 20±1°C respectively adjusted with thermostatic heater . The fish fed on commercial pellets, a week prior to and through out the period of the study. Each two aquaria of fishes at 15°C and 20°C were experimentally orally inoculated with mobile zoospores. On the other hand, the rest of

aquaria were left as control .All fish were observed over two months for any mortalities and clinical abnormalities. All fish that died during experiment were examined and the other fish left till the end of the experiment were scarified and examined similarly for any *Dermocystidium* cysts formed or spores and mycological reisolation.

RESULTS

Forms of *Dermocystidium* spp. in natural infected fishes

Only 25 cultured *C.garpinus* out of 100 examined, 37 cultured *C. idella* out of 100 examined and 66 cultured *O. niloticus* out of 100 examined revealed *Dermocystidium* species, infection representing 25, 37 and 66% respectively Table (1). Dermal infection was recorded in all positive examined fishes as different size of thin-walled roundish oval or elongated shaped micro and macrocystes found almost anywhere in the skin and all fins (Fig .1&2) but in gills *Dermocystidium* spores found freely with mucus only no cyst observed.

Visceral granulomatous infection was recorded only in *O. niloticus* and *C.idell* with prevalence 35% and 20% respectively. The fishes with dermal and visceral infection were emaciated. Mortality rate reached 10% in *O.niloticus* at range temperature (22-26°C) and no mortalities in *C.idella* and *Cl.garpinus*.

Internal examination of *O.niloticus* and *C.idella* showed widely disseminated cysts in the kidney, spleen and liver. The intestines of *O. niloticus* were enlarged and contain thick walled longish yellow macrocysts. (Fig.3) Occurrence of *Dermocystidium* species were recorded with no difference in fish species

Microscopic examination of cysts content revealed that cyst contained typical appearance of *Dermocystidium* spores. They were round, have a refractile central inclusion situated slightly eccentrically (Fig.4) opposite to the inclusion was the nucleus with emarginated sporal cytoplasm. Spores sizes measured using phase contrast microscope were highly variable from 6-8 µm. (n=30) in diameter to 12-15 µm. (n=30) according to fish species, cyst age and infected organs.

Forms of *Dermocystidium* in liquid thioglycollate medium

Culture of spores cysts and infected materials on liquid thioglycollate medium containing antibiotics at 25°C for 4-7 days collected and macerated with scalpel blade on a glass slide, then examined under light microscope after stained with lugol's iodine. The Dermocystidium spores were enlarged and stained bluish –black (Fig .5).

Forms of *Dermocystidium* in sterile dechlorinated water

The origin of the spores was *O.niloticus* infected organs. The spores were incubated in sterile dechlorinated water containing antibiotics at 25°C. The spores grew in size till reach approximately 20 µm in stained smears with methylene blue and the cytoplasm became granular. After 4 days, the spores divided into two daughter cells and the divisions continuous until numerous cells were produced within the spore wall. Zoospores bodies appeared within the envelope and released into water and moves quickly with long flagella. The Zoospores collected and used in experimental infection and some of them left to complete life cycle. The zoospores flagellum disappeared and formation of round cells with refractile inclusion were observed (Fig . 6).

Table 1. Prevalence of *Dermocystidium* spp. in natural infected fishes.

Fish species	No. of exam. fish	Infected samples	Percent %
C.garpinus	100	25	25%
C.idella	100	37	37%
O.niloticus	100	66	66%

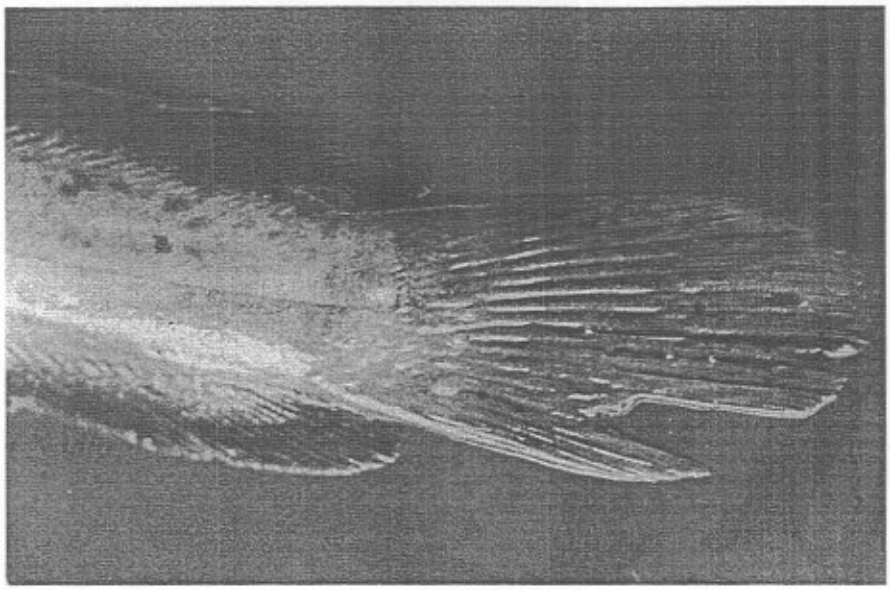

Fig. 1. *Cl.garpinus* fish natural infected with *Dermocystidium spp*. showing different size of roundish oval shaped micro and macrocystes cysts found almost in the skin and all fin.(Arrows)

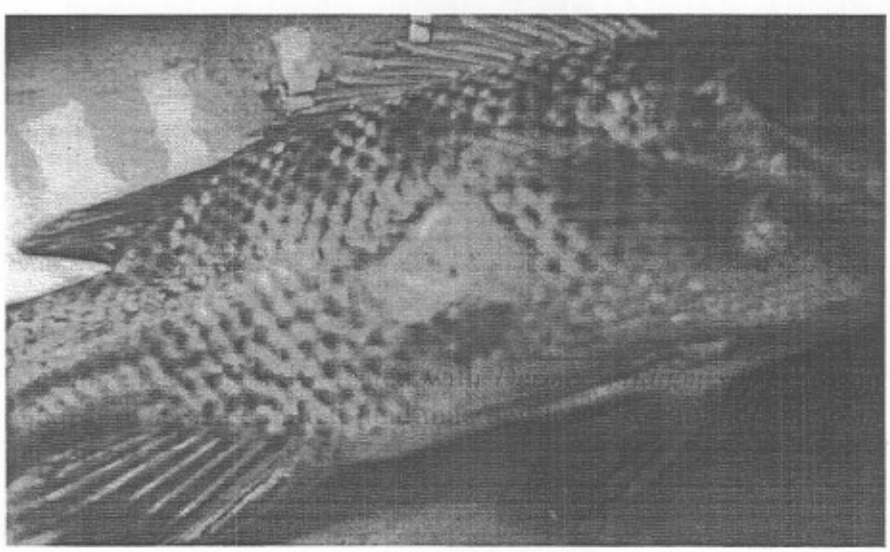

Fig.2. *O. niloticus* fish natural infected with *Dermocystidium spp*.showing ulceration of skin. (Arrows)

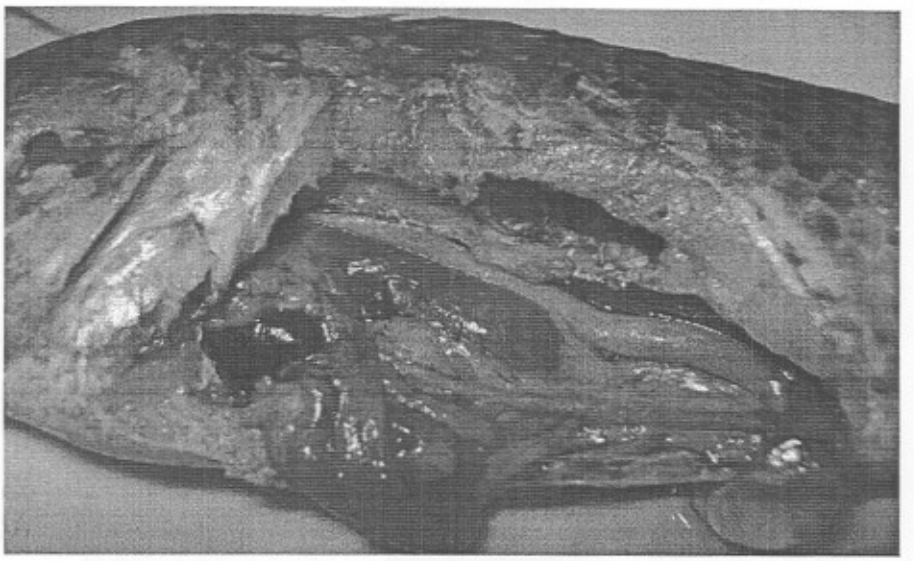

Fig. 3. *O. niloticus* natural infected with *Dermocystidium spp*.showing Intestine enlarged and contain longish yellow macrocystes. (Arrows)

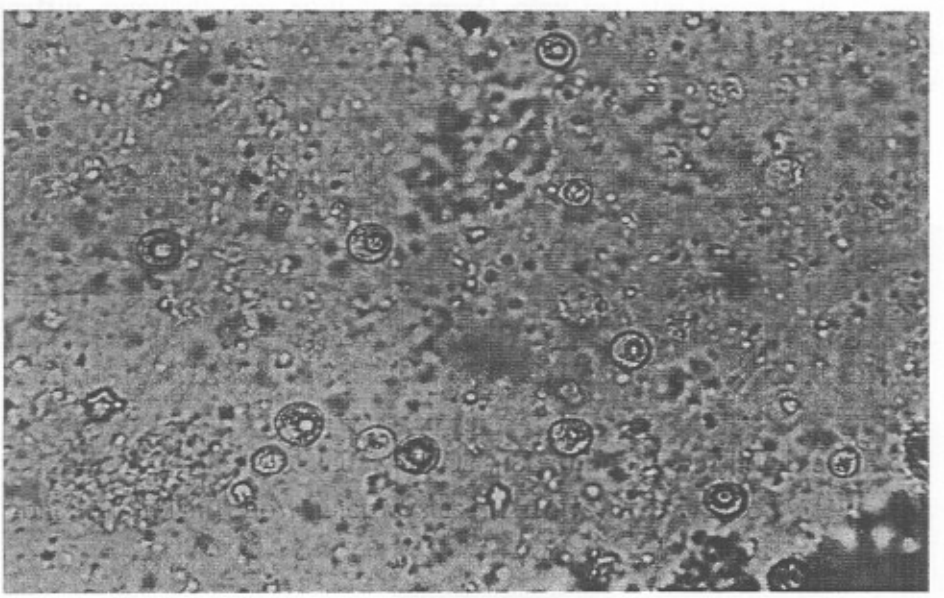

Fig.4. Microscopic examination of *Dermocystidium* spores which appeared as roua with refractile central inclusion situated slightly eccentrically opposite to the inclusion is the nucleus with marginated sporal cytoplasm X40.

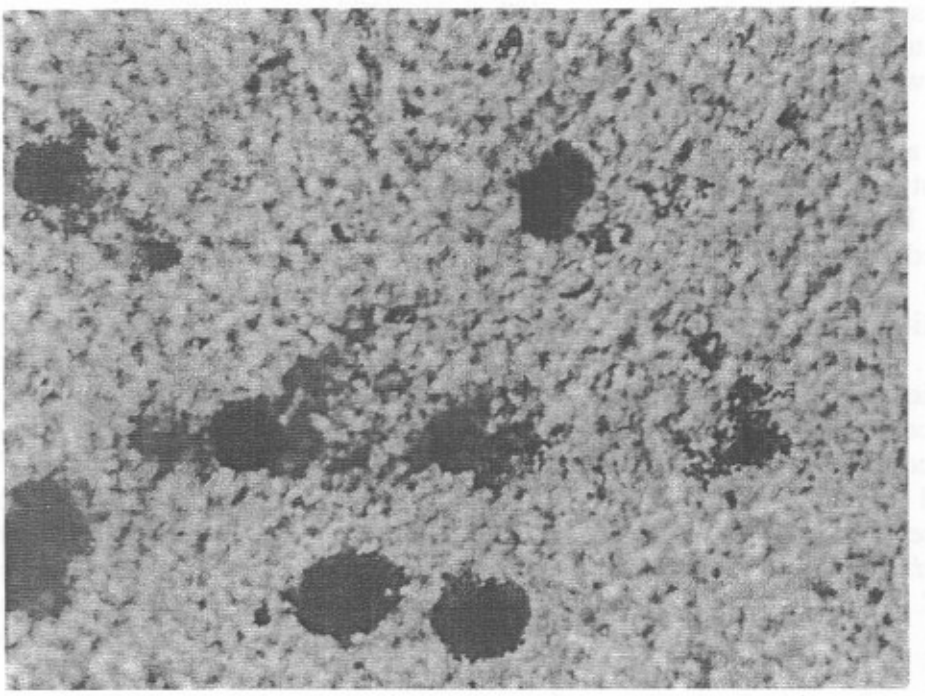

Fig. 5. Cultured spores in liquid thioglycollate medium were enlarge and stained bluish–black with Lugol's iodine solution X40.

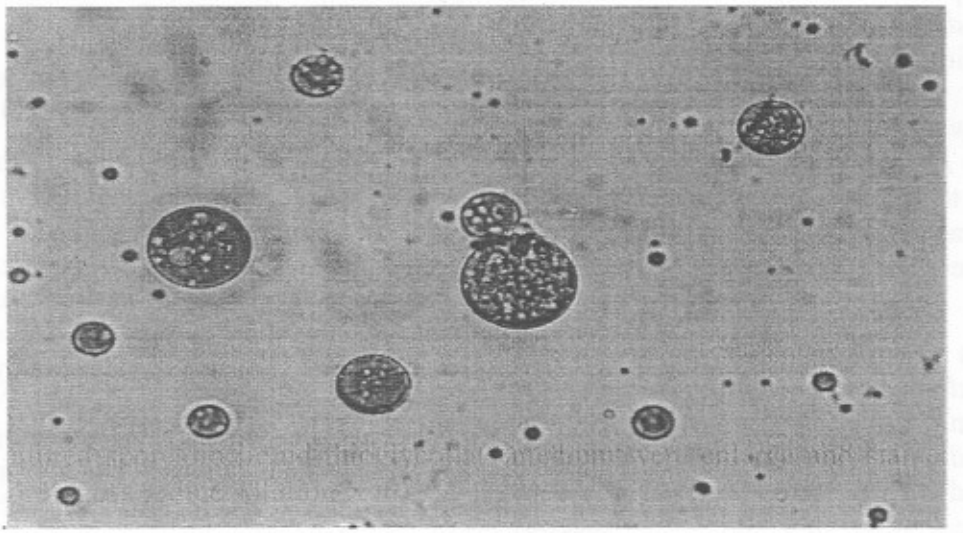

Fig. 6. Wet preparation of spores in water culture after 4 days staining with lactophenol cotton blue showing sporangia contain spores divided into two daughter cells and the large size of zoospores X40.

Experimental infection of *O.niloticus* and *C.garpiuns* with zoospores of *Dermo - cystidium* at 15 and 25°C

O. niloticus were susceptible to *Dermocystidium* spp at 25°C where the cumulative mortality rate reached 100% and the morbidity percentage were 60% within 6 weeks and less susceptible at 15°C where they had no mortalities and lower morbidity percentages (10%). Most of moribund O. niloticus showed emaciation. Internal examination of moribund fish revealed presence of internal macro and microcystes in kidney, liver, spleen and intestine. Microscopic examination of infected organs and cysts appeared the presence of characteristic *Dermocystidium* spores with refractile central inclusion body situated slightly eccentrically and nucleus with emarginated sporal cytoplasm. *C.garpiuns* was not susceptible for infection by *Dermocystidium* spp. of *O.niloticus* origin at 15 and 25°C.

DISCUSSION

Dermocystidium species have been known to be the cause of gill and skin infections in fish but only recently they have appeared as cause of systemic disease in *Atlantic Salmon* ***(16)*** and in *Oreochromis hybrids* ***(4)***. Some early reports include species of *Dermocystidium* as parasite of *Oysters* ***(17)***, many species of freshwater fish and some amphibians ***(18)***. However, the *Oyster* pathogen was in fact a protozoan in the subphylum Apicomplexa ***(19)*** and erected a new genus to distinguish from the amphibian and fish parasites ***(20)***.Later it was few reports, have been recorded dermal Dermocystidium in Egypt ***(21)***.

This is the first record of a systemic *Dermocystidium* species from *O. niloticus* and *C.idella* in Egypt. Also we recorded *Dermocystidium* infection in *C.garpinus, C.idella* and *O. niloticus* as external fungal disease affect skin, fins and gills. Recently *Dermocystidium* have been appearing as cause of systemic disease ***(22)***. In the present study visceral granulomatous infection occur in *O. niloticus* and *C.idella* in kidney ,spleen ,liver and intestines as elongated cysts causing enlarged organs, this results were noticed in *tilapia* farmed fish ***(4)*** in *Gold fish* ***(2)*** also *Carps****(23)***, in *Atlantic Salmon* ***(22)***.

Dermal infection of *Dermocystidium* species in this study differ in their cyst wall, shape and microhabitat preference of the cysts in the fish surfaces and present of free spores in gill mucus this agree with previous observation in *Perch* and *ruff* in Finland and Estonia ***(8)***.

Regarding susceptibility of local cultured fish (*O.niloticus, C.garpinus* and *C.idella)* to *Dermocystidium* under our environmental conditions, no previous studies concerned with it. Our results indicated that percentage of fish infected with *Dermocystidium* and mortalities was higher at range temperature 22-26°C. High prevalence of *Dermocystidium percae* in small *Perch* in late summer and spring but was previously recorded ***(8)***, it has been found that *Chinook Salmon* suffer from severe infection only when the water temperature is less than 15°C ***(5)***.

The diagnostic features of the *Dermocystidium* species appeared as white cysts with different size at fins, gills and skin which contain round uninucleated spores with refractile center inclusion situated slightly eccentrically, the cytoplasm was thin ring except at one side where it is thicker to accommodate the nucleus, this agrees with previous investigations ***(5)***, ***(8)***, ***(12)*** and ***(19)***.

In this study we noticed that in the occurrences of *Dermocystidium* species, no host size preference was found, this result agreed with ***(8)***. Attempt to *in vitro* culture of *Dermocystidium* in thioglycollate medium contains antibiotics was success. Also formation of zoospores of *Dermocystidium* in water within four days at about 25°C occurred in dark places .The spores grew in size to more than 20 µm in diameter and the spores then divided into daughter cells and the division proceeded until numerous cells were produced within the spores wall (envelope), the zoospores bodies began to move with flagella and by rupturing the envelope they were released into the water. This are consistent

with previously cited investigation ***(8)***.Our results indicated that single exposure of *O.niloticus* and *C.garpiuns* to the fungal zoospores of *O.niloticus* origin initiated *Dermocystidium* infection in *O.niloticus* and no infection in *C.garpiuns* at 15°C and 25°C. Similarly it has been mention that approximately twelve species of *Dermocystidium* have been described in freshwater fishes in Eurasia and Western North America ***(23)***.

In the present study, fish susceptibility to *Dermocystidium* was affected markedly by the water temperature. Namely the cumulative mortalities, prevalences and infect intensity was higher at 25°C than 15°C *O. niloticus*, similar observation in *D.percae* was reported that warm period of summer showing quick *D.percue* development ***(8)*** and disagree with that observed that fish appear suffer from sever infection, only when the water temperature is less than 15°C ***(24)***.

Concerning clinical signs in experimental *O. niloticus*, they suffer from emaciation.

Emaciation of moribund fish was also seen in the infected *bullheads* ***(11)***. *Dermocystidium* diagnosis in experimental fish were examined microscopically and mycologically, similar results described by several workers ***(8)***, ***(12)***. Systemic infection in experimental *O. niloticus* only and not dermal cases reported may be attributed to *Dermocystidium* and fish species variation ***(3)***. Description of the genus *Dermocystidium,* dividing the known species into three groups according to their morphology and types of infection mostly thanks to light microscope observation ***(25)***.

REFERENCES

1.Reichenbach-Klinke H.-H. (1950): Der Entwicklungskreis der *Dermocystidien sowie* Beschreibung einer neuen Haplosporidienart *Dermocystidium percae n.* sp. Verh. Deutsch. Zool. 2-6 August 1949 in Mainz: 126-132.

2.Landsberg, J. H., and Paperna, I. (1992): Systemic granuloma in *goldfish* caused by a *Dermocystidium*-like aetiological agent. Diseases of Aquatic Organisms, 13, 75-78.

3.Kovac-Gayer, E., Csaba, G., Ratz, F., Bekesi, L. & Szakolczai, J., (1986): Granuloma of common carp (*Cyprinus carpio L.*). Bull. Eur. Ass. Fish Pathol., 6: 72–75.

4.FAO (1996): Parasites, infections and diseases of fishes in Africa An update FAO cifa technical paper number (31) fungal infections.

5.Pronin N. M. (1976): Distribution of *Dermocystidium percae* in the lakes of Trans-Baikallen, and some aspects of epizootiology and aethiology of young *perch's* dermocystidiosis. In Bolezni .

6.Wootten, R. and McVicar, A.H., (1982): Dermocystidium from cultured eels *Anguilla anguilla* L. in Scotland. J. Fish Dis., 5: 215–232.

7.Spangenberg R. (1975): Eine kiemenkrankheit beim aal. Verusacht durch *dermocystidium anguillae n.*sp. Zeitschrift fur die Binnenfischerei Der DDR 22,363-367.

8.Pekkarinen M.and Lotman K. (2003): Occurrence and life cycles of Dermocystidium species (Mesomycetozoa) in the *perch* (*Percafluviatilis*) and *ruff* (*Gymnocephalus cernuus*) (*Pisces: Perciformes*)in Finland and Estonia. J. Nat. Hist. 37: 1155-1172.

9.Höglund, J., Alfjorden, A., and Nikkilä, T. (1997): Infection of juvenile salmon *Salmo salar* with a Dermocystidium-like organism in Sweden. Diseases of Aquatic Organisms 30, 171-176.

10.Allen, R. L., Meekin, T. K., Pauley, G. B., and Fujihara, M. P. (1968): Mortality among *chinook salmon* associated with the fungus *Dermocystidium.* Journal of the Fisheries Research Board of Canada 25(11), 2467-2475.

11.Feist S. W., Longshaw M., hurrell R. H. and Mander B.(2004): Observations of *Dermocystidium* sp. infections in

bullheads, Cottus gobio L., from a river in southern England .Journal of Fish Diseases,Volume 27 : 4, Page 225-231.

12.Olson, R.E., Dungan, C.F. and Holt, R.A., (1991): Water-borne transmission of *Dermocystidium salmonis* in the laboratory. Dis. aquat. Org., 12: 41–48. Parazity Ryb: Sverdlovsk, 104-117 (in Russian, English summary).

13.Ray S.M. (1966): A review of the culture method of detecting *Dermocystidium marinum* with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc., 54, 55-69.

14.McVicar, A. H., and Wootten, R. (1980): Disease in farmed juvenile *Atlantic salmon* caused by Dermocystidium sp. In: W.Ahne (Ed), Fish Diseases . Springer-Verlag, Berlin.

15.Inness, W. T.(1966): Exotic aquarium fishes .19th Ed. Aquarium Incorporated New Jersy,pp.7,12,24-25,29-30.

16.Nash G., Southgate P., Richards R. H. (1989): A systemic protozoal disease of cultured *salmonids.* J. Fish Dis. 12: 157-173.

17.Mackin, J. G., H. M. Owen & A. Collier. (1950): Preliminary note on the occurrence of a new protistan parasite, Dermocystidium marinum n. sp.in Crassostrea virginica (Gmelin). Science 111:328–329.

18.Reichenbach-Klinke H.-H. And Elkan E.(1965): The principle diseases of lower vertebrates , Book 1 Diseases of fish, T.F.H publications inc. LTD.page 50-53.

19.Perkins F.O. (1996): The structure of *Perkinsus marinus* (Mackin, Owen and Collier,1950) Levine, 1978 with comments on the taxonomy and phylogeny of Perkinsusspp. J. Shellfish Res. 15:67–87 .

20. Levine, N.D., (1978): *Perkinsus gen. nov.* and other new taxa in the protozoan phylum Apicomplexa. J. Parasitol., 64: 549.

21.Shaheen, A. A. (2000): Some Studies On Dermocystidiosis among *Oreochromis niloticus* Fish. SCVMJ, III(1),177-184.

22.Hedrick, R.P., Friedman, C.S. & Modin, J., (1989): Systemic infection in Atlantic salmon *Salmo salar* with a *Dermocystidium*-like species. Dis. aquat. Org., 7: 171–177.

23.Cervinka, S., Vitovec, J., Lom, J., Hoska, J., and Kubu, F. (1974): Dermocystidiosis - a gill disease of the *Carp* due to *Dermocystidium cyprini n.* sp. Journal of Fish Biology 6, 689-699.

24.Wood, J. W. (1974): Diseases of *Pacific salmon* –their prevention and treatment ,2nd ed.Dept .Fish .,Hatcheries Div .,State of Washington.82pp.

25.Lom J., Dyková I. (1992): Protozoan parasites of fishes. Developments in Aquaculture and Fisheries Science, 26. Elsevier, Amsterdam, London, New York, Tokyo.

الملخص العربى

بعض الدراسات على الإصابة بالدرموسيستديم الداخلى فى بعض أسماك المياة العذبة المستزرعة

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هذه هى أول دراسة أجريت فى مصر لمعرفة مدى أنتشار مرض الدرموسيستديم الداخلى والعوامل المؤثرة على وبائية المرض فى بعض الأسماك المستزرعة القرموط الأفريقى والبلطى النيلى والمبروك الفضى. وكانت الأعراض الإكلينيكية للأسماك المصابة بالمرض طبيعيا عبارة عن وجود حويصلات رفيعة الجدار بيضوية وطولية على الجلد والزعانف مع وجود بوغيات حرة فى مخاط الخياشيم .أما أصابة الأعضاء الداخلية كان فى اسماك البلطى النيلى والمبروك الفضى فقط بالإضافة إلى هزال واضح فى الأسماك المصابة. الأعضاء المصابة كانت الكلى والكبد والطحال والأمعاء وكانت كبيرة فى الحجم مع وجود حويصلات طولية صفراء ؛ فحص محتوى الحويصلات ميكروسكوبيا أوضح وجود البوغيات المميزة لفطر الدرموسيستديم.

محاولات زراعة الحويصلات على ميديا الثيوجلوكولات السائلة كانت ناجحة ،بالأضافة الى أمكانية أنتاج الزوسبور فى الماء المعقم المضاف اليه مضاد حيوى عند درجة حرارة ٢٥ م° لمدة ٤ ايام. تم أجراء تجربة صناعية بإعطاء اسماك بلطى نيلى وقرامبط افريقى سليمة الزوسبور مرة واحدة عند درجة حرارة ١٥ م° و ٢٥ م° وتمت متابعة نسبة الوفيات والأعراض الإكلينيكية والصفات التشريحية ونسبة وشدة الإصابة لهذه الأسماك .وقد أوضح هذا البحث ان أسماك البلطى تصاب بفطر الدرموسيستديم الداخلى وأن الأعراض الإكلينيكية للمرض تتأثر بدرجة الحرارة وأسماك والقرامبط افريقى لاتصاب بالفطر المعزول من أعضاء اسماك البلطى.