Some Epizootological Aspects Of Hole In The Head Disease Affecting Cultured *Oreochromis Niloticus*

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ABSTRACT

This is the first study that carried out in Egypt to get information on several factors affecting the epizootiology of hole in the head disease (HITH) among cultured *Oreochromis niloticus.*

Prevalence of *Spironucleus* intensity was determined in naturally infected *Oreochromis niloticus.* The majority of infected fish had moderate grade of infection in smallest fish ranged body weight 2-10 grams and high mortality and morbidity percentage. *Spironucleus* has significantly higher occurrence and density in the hind part of the intestine.

Seasonal variation and prevalence of *Spironucleus* showed that high infection of *O.niloticus* with *Spironucleus* in winter and autumn with prevalence rate 90 % and 85 % respectively.

Cultivation of *Spironucleus* has been attempted on two cultured media minimum essential medium (MEM) 10% and Axenic culture medium with success. Growth of *Spironucleus* was very rapid over five days in MEM 10% bovine serum (BS) and Axenic cultured medium but gradually decreased till day 15 of cultivation where they did not survive. Experimental infection were done to study the susceptibility of *O. niloticus* and *Cl. gariepinus* to *Spironucleus* infection by intraperitoneal injection and oral inoculation at 15°C and 20°C water temperatures. Also study the stocking density and transmission by cohabitation at the two different water temperatures.

The present study was succeeded in isolation, identification and cultivation of *Spironucleus* causative agent of HITH infected *O. niloticus.* Some important epizootiology information of disease was study by experimental infection of cultured *Oreochromis niloticus* and *Cl. gairepinus* at two different temperatures.

INTRODUCTION

Hole in the head disease (HITH) has been reported from tropical species of fish including freshwater and marine *(1- 4).* The causative agents was Hexamita (*Spironucleus*) .It is common in cultured tilapia *(5)* as well as in commercially reared South American cichlids in Israel, but, has not been reported from cichlids in Africa *(6).*

For many years hexamitids, *Hexamita spp.* and *Spironucleus spp.*, have frequently been reported in vertebrates, particularly in fish. This suggests a potentially important role of these parasites in the fish culture industry. But the disease are still in need of further investigation into their geographical distribution, host range, life cycle, host-parasite relationship, pathogenicity, diagnosis, prevention, treatment, and control *(7).*

The Diplomonadida (Hexamitidae) are very small, less than 30 micron in length. Most of the early descriptive studies made using light microscopy and diagnoses by light microscopy is helpful in confirming the presence of diplomonad flagellates .Some taxonomic progress was made as preliminary ultra structural work (SEM and TEM) on a diplomonad from juvenile rainbow trout in Northern Ireland *(8).*, Ultrastructural features used to distinguish between trophozoites of the genera Hexamita and genera Spironucleus are the position of habitat and shape of nuclei *(9, 10).*

Cultivation of diplomonads has been attempted with various degrees of success. Axenic (pure) cultures are advantageous for biological and physiological investigations *(11).* The life cycle of *H. salmonis* was observed *in vitro* by cultivation in MEM supplemented with calf serum and antibiotics *(12).*

There are two stages in the diplomonad life cycle, trophozoites and cysts. The trophozoite is the motile stage that actively feeds and multiplies, and for parasitic species, this is the stage that is most readily observed, usually in the intestinal lumen .The cyst is the resistant stage of life cycle in which the cell can survive outside of the host *(13).* Trophozoites and cysts pass out of the host with the feces, and are ingested orally by the host. Although the faecal oral transfer of diplomonads cysts is the major route of transmission in fish, infection via the trophozoites from skin lesions in Atlantic salmon *(14),* and the rectal route of transmission through cyst and trophozoites were also suggested *(9, 15 , 16).*

The present study was initiated since there are no published reports on the *in vitro* cultivation of the organism and experimental infection with *Spironucleus* in *O.niloticus* culture. This study focuses on the course of infection, the transmission between fish, and the susceptibility of *Claris gariepinus* to infection with *Spironucleus* isolated from *O.niloticus* at 15°C and 20°C water temperature.

MATERIALS AND METHODS

1. Naturally infected fishes

A total number of 320 cultured *Oreochromis niloticus* were collected from different fish farms at different localities in Egypt during the period of March 2008 till the end of February of 2009. The collected fishes were examined clinically *(17).*

2.Isolation and identification of *Spironucleus*

To study association between infection and fish weight, fish were weighed before examination for parasite and examined externally for diplomonad flagellate *Spironucleus* (Hexamitidae) infection. Smears were made from skin lesions and various sites along the gastrointestinal tract and blood .The intestine of each fish was divided into 3 parts (anterior, mid and hind part) and the infection intensity in each part was recorded *(18)* and imprints were made from internal organs (liver, kidney, spleen, and gall bladder). Microscopic examination of wet mounts under bright field and phase contrast X40 were used in detailed examination. Stained Smears with Giemsa were used for examination and identification *(19).*

3.Determination of infection intensity in fish

Squash preparation of the intestine was taken and mixed with 3 drops of water on slide, cover slipped and examined with a light microscope (40X). Estimation of infection intensity was recorded as follows *(18)*: free (-) *Spironucleus* not detected in the sample; Low (+) more individual of *Spironucleus* detected in the sample and the average number per microscope field being less than 10; Moderate (++) with average number 10- 50 and High (+++) average number > 50.

4.*In vitro* culture of *Spironucleus*

Two different media were used for cultivation of diplomonads Spironucleus from *O. niloticus*. MEM *(12)* (SIGMA) containing 10% bovine serum (SIGMA), and 29.3% sodium bicarbonate solution (7.5%w/v) (SIGMA). The second culture medium was Axenic culture medium *(11)* containing 2% (w/v) Trypticase, 1% (w/v) yeast extract, 0.5% (w/v) maltose, 0.1% L-cysteine, 10 mM K phosphate buffer and 10% foetal bovine serum (FBS) . To prevent microbial growth the previous *(11, 12),* supplemented the media with 2000 unit's penicillin/ml (SIGMA), 50 µg gentamicin/ml (SIGMA), and 100 unit's nystatin/ml (SIGMA). Intestinal tract from positive heavily infected fish were taken and cultured in selected medium. The pH adjusted at 7.5, and incubated at 15°C in the dark. Samples were taken from medium for monitoring the approximate density of *spironucleus* trophozoites for the first 30 min

after culturing, and then every day, using an inverted microscope for 15 days. Cultured media with large numbers of active motile flagellates with a long survival period was considered succeeded.

5.Experimental infection of fish with *Spironucleus* agents

Spironucleus cultivated in MEM were harvested at 5 days of maximum growth by centrifugation at 3000 rpm for 5 minutes. Spironucleus was washed with PBS three times at pH 7.2 and resuspended in PBS and counted *(7)*. Fish were anaesthetized by MS222 and by using micropipette tips attached to plastic micropipette fish were orally inoculated with 1ml contains $5X10^{6}$ cells of spironucleus *(7)*. Another group was intraperitoneally (IP) injected with $2X\ 10^{6}$ cells of spironucleus.

6- **Experimental fishes**

A randomly selected (328) apparently healthy *O. niloticus* (average body weight of 60 ± 5 g) and sixty *Cl. gariepinus* with average body weight of 100 ± 5 g. All fishes were maintained in 24 glass aquaria (100 × 80 × 40 cm). They were acclimatized in the aquaria for two weeks and fed on the basal diet twice a day. The aquaria were supplied with well-aerated de-chlorinated tap water and water temperature was maintained at 20°C.

7. Experimental design:

1- Two routes of infection were used, oral and intraperitoneal (IP) inoculation, in two fish species (*O. niloticus* and *Cl. gariepinus*) then kept at 15°C or 20°C.

2- The influence of stocking density on morbidity and mortality of *O.niloticus* experimentally infected with *Spironucleus* was achieved by IP inoculation of two fish group of different numbers (20 and 40 fish) at 20°C.

3- Experimental infected *O. niloticus* and uninfected *O. niloticus* were kept in the same aquaria to study the influence of cohabitation on morbidity and mortality of fish experimentally infected with *Spironucleus* at 15°C and 20°C.

The control fish (each contained the same number of fish group) were orally inoculated or IP injected with sterile PBS and were kept at the same conditions. All groups of fish were observed for 8 weeks and the Cumulative mortality rate was recorded. Moribund fish were subjected to laboratory examination and parasitic re-isolation. The number of fish used, groups and subgroups are shown in Table 1.

Table 1. Experimental design

Group	Fish species	No of fish	Rout of infection	Water Temperature °C
I	*O. niloticus*	10	IP	15
	O. niloticus	10	IP*	15
	Cl. gariepinus	10	IP	15
	Cl. gariepinus	10	IP*	15
II	*O. niloticus*	10	IP	20
	O. niloticus	10	IP*	20
	Cl. gariepinus	10	IP	20
	Cl. gariepinus	10	IP*	20
III	*O. niloticus*	10	Oral inoculation	15
	O. niloticus	10	Oral inoculation*	15
	Cl. gariepinus	10	Oral inoculation	15
	Cl. gariepinus	10	Oral inoculation*	15
IV	*O. niloticus*	10	Oral inoculation	20
	O. niloticus	10	Oral inoculation*	20
	Cl. gariepinus	10	Oral inoculation	20
	Cl. gariepinus	10	Oral inoculation*	20
V	*O. niloticus*	20	IP	20
	O. niloticus	40	IP	20
VI	*O. niloticus*	20	IP*	20
	O. niloticus	40	IP*	20
VII	*O. niloticus*	6	IP	15
		6	Uninfected	
	O. niloticus	6	IP	15
		6	Uninfected	
VIII	*O. niloticus* *	6	IP	15
		6	Uninfected	
	*O. niloticus**	6	IP	20
		6	Uninfected	

RESULTS

Clinical signs

Naturally infected fish were showing darkening in color, excessive body mucus, lethargy, tendency to hang in corners or to remain in isolation with other fish and some of their trailing yellowish mucus. In severe cases reduction of muscle tissue which give the pinched appearance behind the head. Skin lesions especially on the body and the head, in the region of lateral system may be expanding to make holes.

Post- mortem examination

Naturally infected fish were showing enteritis, yellowish intestinal mucus, intestinal hemorrhages, enlargement of spleen and focal liver yellowish nodules.

Parasitological examination

The morphology of the trophozoite stage of *Spironucleus* species isolated mainly from hind part of intestine of *O.niloticus* were transparent elongated pyriform body and considers the smallest diplomonad flagellates;

with a body range 12.5- 20.5 mm long and 5.0-11.2 mm wide. The parasite is anterior tapering and intertwined elongate compact nuclei S-shaped and wrap around each other at their narrow anterior ends. The body of *Spironucleus* is emerging three anterior flagella, and one posterior flagellum **(Fig 3).** *Spironucleus* significantly higher occurrence and density in the hind part of the intestine than elsewhere in all examined fish weight except in heavily infected fish all intestinal tract showed degree of infection. Smallest fish were moderately infected density in spited highly mortality (80%). Internal organs (liver, gall bladder, kidney and spleen) showed moderate intensity of infection in heavily infected intestinal fish with body weight > 150 g and no systemic infection in smaller infected fish.

Based on morphological examination (n= 20) isolated *Spironucleus*, was identified as the following :-
Phylum: - Metamonada
Subphylum:- Eopharyngia (incl. diplomonads)
Order: - Diplomonadida
Suborder: - Diplomonadina
Family: - Hexamitidae
Subfamily: - Hexamitinae
Genus: - *Spironucleus*
Species: - *Spironucleus spp.*

Seasonal variation and prevalence of *Spironucleus*

The results indicated a high infection of *O. niloticus* with *Spironucleus* in winter and autumn with prevalence rate 90% and 85% respectively. The infection rate showed decrease in spring and summer where it reached 45% and 55 % respectively as showing in Table 2.

Table 2. Seasonal variation and prevalence of *Spironucleus* infected *O. niloticus*

Season	Total No. of examined fish	No. of infected fish	%
Winter	80	72	90
Summer	80	44	55
Spring	80	36	45
Autumn	80	68	85

Determination of infection intensity in fish

The intensity of infection in *O. niloticus* showed that the majority of infected fish had moderate grade of infection in smallest fish ranged body weight 2-10 grams and high mortality (80%) and morbidity (85%). The largest fish more than 150 grams showed heavily grade of infection intensity with low mortality 20% and high morbidity reached to 100%. The intensity of infection in *O. niloticus* of body weight 10-50 and 50-150g showed light and moderate grade of infection with low mortality and morbidity percentage as showing in Table 3.

Table 3. Association between infection and fish weight

Fish weight (g)	No. of examined fish	Morbidity		Mortality		Intensity of infection
		No.	%	No.	%	
2- 10	80	68	85	64	80	++
10-50	80	38	47.5	12	15	+
50-150	80	24	30	10	12.5	++
> 150	80	80	100	16	20	+++

+ Light ++ moderate +++ heavily

***Spironucleus* cultures results**

The parasite cell cultures multiplied rapidly in MEM 10% BS culture medium and Axenic culture medium. Growth of *Spironucleus* was very rapid over five days in MEM 10% BS but gradually decreased till day 15 of cultivation where after they did not survive and no cells were observed alive. In the cultured medium the dividing trophozoites looked promising within 10 hours which resembled bifurcated poles (V-shape cells) were found in the highest number on the fifth day of cultivation. In addition, the dividing trophozoites which were round to oval shape were also observed in the culture medium (Fig 4). The dividing trophozoites did not always move forward in one direction, but sometimes in an opposite direction and were less active only the last few days of cultivation.

Experimental infection

1.Susceptibility of *O.niloticus* and *Cl. gariepinus* to *Spironucleus* infection by intraperitoneal injection at 15°Cand 20°C water temperatures

O.niloticus were susceptible to *Spironucleus* infection by intraperitoneal injection route at 15°Cand 20°C water temperatures and the cumulative mortality rate was 100% within 8 weeks (Table 3) but *Cl.gariepinus* were not susceptible to *Spironucleus* infection isolated from *O. niloticus* .Most of moribund *O. niloticus* showed clinical symptoms after 2 week from injection as emaciation, abdominal distension, ulcerated skin, small holes on the head and around the eyes, loss of appetite and granulomatous lesions in internal organs (liver and kidney).Smears from ulcerative skin and from various sites along the gastrointestinal tract of these fish showed the presence of flagellate *Spironucleus*.

2.Susceptibility of *O. niloticus* and *Cl. gariepinus* to *Spironucleus* infection by oral inoculation at 15°Cand 20°C water temperatures

O.niloticus were susceptible to *Spironucleus* infection by oral inoculation rout at 15°Cand 20°C water temperatures and the cumulative mortality rate was 60 and 80% respectively within 8 weeks (Table 4) but *Cl.gariepinus* were not susceptible to *Spironucleus* infection by oral inoculation .Most of moribund *O.niloticus* showed clinical symptoms after one week from oral inoculation as emaciation, abdominal distension ,loss of appetite also fish developed a blister below the dorsal fin and above the right lateral line; the blister grew to a noticeable bump, fish died after the blister ruptured at 8 week .post mortem finding showed whitish to yellowish granulomatous nodules in the liver and spleen. Smears from skin blister and from various sites along the gastrointestinal tract of these fish showed the presence of flagellate *Spironucleus*.

3.Experimental infection to study Influence of stocking density

Stocking density affect the susceptibility of *O. niloticus* to *Spironucleus* infection at 20°C over 8 weeks (Table 5).

4. Experimental infection to study Transmission by cohabitation

The parasites were found in the skin lesion of all experimental infected fish and the uninfected fish at 8week from the begining of cohabitation, however it was present in the mucus of uninfected fish with no lesion at 2 week. The course of infection in fish by cohabitation followed a similar pattern of fish infected by IP injection at 8 week. None of the 10 fish in each control group died or were infected with *Spironucleus* as shown in Table 6.

Table 4. Influence of temperature on morbidity and mortalities of *O. niloticus* and *Cl. gariepinus* fish experimentally infected with *Spironcleus at* 15°C and 20°C water temperature.

Group	Fish species	No of fish	Rout of infection	Water Temperature °C	No of dead fish within 8 weeks	Cumulative Mortalities %
I	*O. niloticus*	10	IP	15	4	40
	O. niloticus	10	IP*	15	0	0
	Cl. gariepinus	10	IP	15	0	0
	Cl. gariepinus	10	IP*	15	0	0
II	*O. niloticus*	10	IP	20	10	100
	O. niloticus	10	IP*	20	0	
	Cl. gariepinus	10	IP	20	0	0
	Cl. gariepinus	10	IP*	20	0	0
III	*O. niloticus*	10	Oral inoculation	15	6	60
	O. niloticus	10	Oral inoculation*	15	0	0
	Cl. gariepinus	10	Oral inoculation	15	0	0
	Cl. gariepinus	10	Oral inoculation*	15	0	0
IV	*O. niloticus*	10	Oral inoculation	20	8	80
	O. niloticus	10	Oral inoculation*	20	0	0
	Cl. gariepinus	10	Oral inoculation	20	0	0
	Cl. gariepinus	10	Oral inoculation*	20	0	0

* Control group

Table 5. Influence of stocking density on morbidity and mortalities of *O.niloticus* experimentally infected with *Spironucleus*

Group	Fish species	No of fish	Rout of infection	Water Temperature °C	No of dead fish within 8 weeks	Cumulative %Mortalities
V	*O. niloticus*	20	IP	20	16	8
	O. niloticus	40	IP	20	18	90
VI	*O. niloticus*	20	IP*	20	0	0
	O. niloticus	40	IP*	20	0	0

* Control group

Table 6. Influence of cohabitation on morbidity and mortalities of fish experimentally infected with *Spironucleus*

Group	Fish species	No of fish	Rout of infection	Water Temperature °C	Morbidity % within 8 weeks	Cumulative %Mortalities
VII	*O. niloticus*	6	IP	15	100	75
		6	Uninfected			
	O. niloticus	6	IP	20	100	100
		6	Uninfected			
VIII	*O. niloticus* *	6	IP	15	0	0
		6	Uninfected			
	*O. niloticus**	6	IP	20	0	0
		6	Uninfected			

* Control group

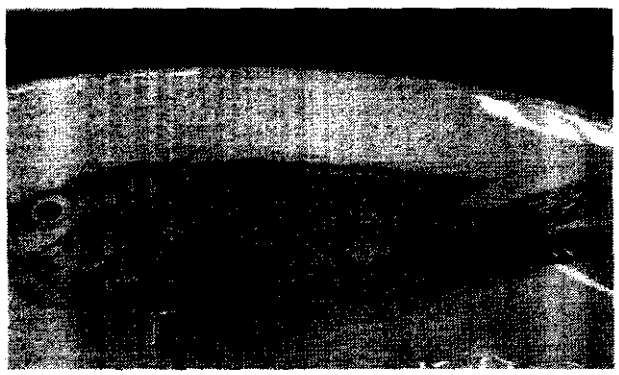

Fig 1.Natural infected *O.niloticus* with Spironucleus showing emaciation and sever skin ulceration on lateral side.

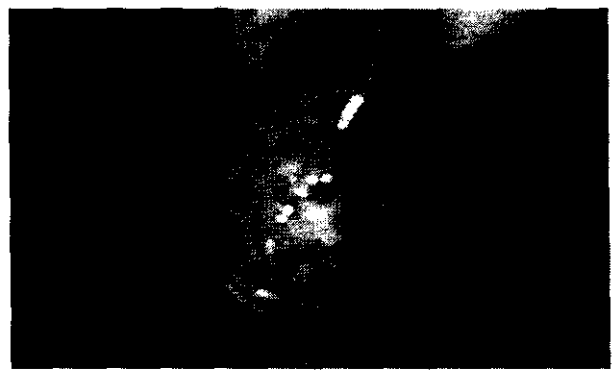

Fig 2. Natural infected *O.niloticus* with external *Spironucleus* showing head, skin blisters and ulceration.

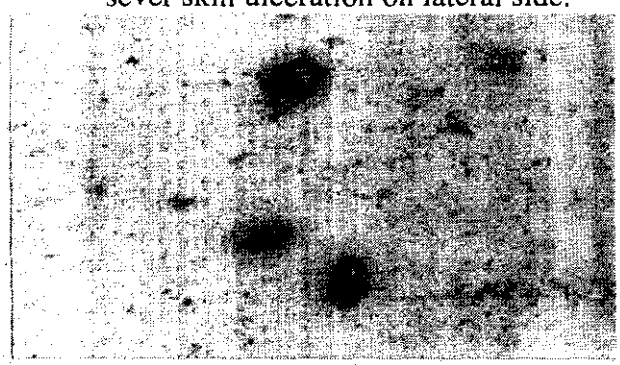

Fig 3. Giemsa stained *spironucleus from* the intestinal lumen, pyriform in shape with two nuclei (40X).

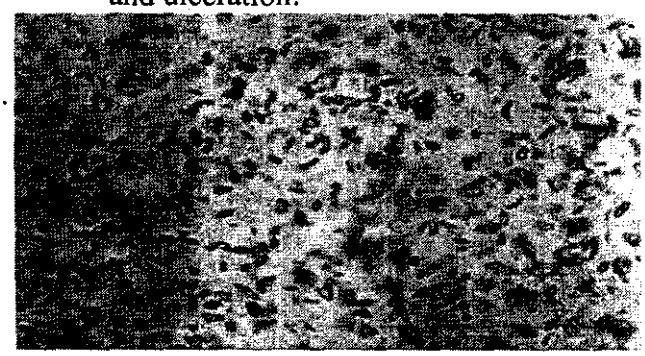

Fig 4.Wet preparation from MEM culture medium after 3 days of cultivation showing different dividing shaped of *Spironucleus* trophozoites (40X).

Fig 5. Natural infected *O. niloticus* with systemic *Spironucleus* showing dark liver, spleen and yellowish mucoid exudate from intestinal content.

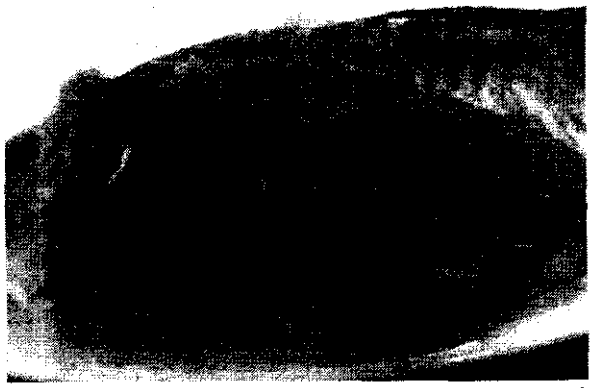

Fig 6. Experimental infected *O. niloticus* with *Spironucleus* showing yellowish nodules in the liver and yellowish mucoid content of intestine.

DISCUSSION

Intestinal infection of fish by flagellate of the genus Hexamita is often associated with high mortality *(18)*.Systemic *Spironucleus* infections have been reported in salmonids as well as cichlids, namely angel fish *Pterophyllum scalare* and discus *Symphysodon discus*, and are possibly associated with hole-in-the head disease *(10 , 20)* and lip tumor *(21)*. Infections by *Hexamita* (*Spironucleus*) are common in cultured tilapia **(5)** as well as in commercially reared South American cichlids in Israel, but, have not been reported from cichlids in Africa *(6)*.

In our study, the most obvious symptoms were darkening in color, excessive body mucus; skin lesions especially on the head and body, in the region of lateral system may be expanding to make holes. Similar lesions were described cichlids *(20)*.

Spironucleus salmonis occurs primarily in smaller trout fishes than larger and that provides an evidence for a size related infection of rainbow trout by *S.* salmonis *(22)*. This is caused by an inadequately developed immune system. These finding were nearly similar to our results.

Also our study showed that systemic spironucleosis in *O. niloticus* in the liver, spleen and kidney beside intestine with no record in heart or blood. *S. vortens* was absent *(20)* in the blood or heart of cichlids, while was found in the blood, heart and other internal organs in other ornamental Fishes *(2 , 23)*.

Regarding susceptibility of local breeds (*O. niloticus* and *Cl. gariepinus*) to spironucleosis under our environmental conditions, no previous studies concerned with it was recorded. Our results indicated that single exposure to *Spironucleus* through either IP injection of (2 x10^6) or oral inoculation of cultivated *Spironucleus* in MEM-10% medium of (5 x10^6) initiated *Spironucleus* infection. In angel fish (*pterophyllum scalare*), cichlids and *salmonis* similar susceptibility was recorded *(7, 20, 24)*. On the other hands, different parasite inoculate (100 000, 50 000 or 30 000 parasites per fish) produced similar onset and disease patterns in Atlantic salmon *(25)*.

In addition to dosage (parasite number), route of infection is another factor inducing the progression of disease. In our study faster beginning of mortalities in fish oral inoculated with *Spironucleus* cultivated in MEM 10%medium than those IP injected .These results suggest that natural infection via ingestion of *Spironucleus* resulting in disease .On the other hand angelfish orally inoculated with a dose (5 x 106) of *S. vortens* were in appetent for three days after infection, with no other clinical sign but intraperitoneally injected with dose(2 x10^6) of the *S. vortens* were sick, and died within three weeks after infection. The disease may become latent for

long periods of time when the fish are orally infected with a low number of parasites *(7)*. However, the parasites could facilitate the disease and become lethal to the host if they reach a hematogenous route.

In the present study, fish susceptibility to *Spironucleus* was affected markedly by water temperature .Namely the cumulative mortalities, prevalence and infection intensity was higher at 20°C than 15°C in *O.niloticus* and no susceptibility to *Spironucleus* was noticed in *Cl. gariepinus*. The variations in susceptibility to *S. barkhanus* showed significant differences between salmon families may be due to a genetic basis as was shown in cryptobiosis *(25)*.

Regarding the susceptibility of *O. niloticus* to *Spironucleus* infection at 20°C with different stocking density results showed that overcrowdness increased the susceptibility of *O. niloticus* infection to *Spironucleus* over 8 weeks (Table 4). This can be attributed to death of half of the infected fish during first two weeks so number of remaining fish is nearly equal that of uncrowed ones. On other hand, at 20°C, the higher mortality and morbidity in crowded *O. niloticus*. Bad water conditions or overstocking, and infrequent water changes increase susceptibility to Hexamitid infection *(25)*.

Our present cohabitation experiment indicated that *Spironucleus* could direct transmission is possible. The parasite was detected in the skin mucus of all experimental fish at 8 week. This result may be mimic an infection occurring in nature. The trophozoites and cysts of *Spironucleus* pass out of the host with the feces, and are ingested orally by the host *(7, 25)*. It has been suggested that although the faecal oral transfer of diplomonads cysts is the major route of transmission in fish, and the rectal route of transmission through cyst and trophozoites were also possible *(9, 15 ,16)*.

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الملخص العربى

بعض الآوجة الوبائية لمرض ثقب الرأس الذى يصيب أسماك البلطى النيلى المستزرعة

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هذه اول دراسة تجرى فى مصر لدراسة بعض العوامل التى تؤثر فى وبائية مرض ثقب الرأس الذى يصيب أسماك البلطى النيلى المستزرعة.

تم دراسة نسبة الأصابة بطفيل السبيرونيكلس المسبب المرضى لمرض ثقب الرأس وشدة الاصابة فى الأسماك المصابة طبيعيا .وقد وجد ان شدة الأصابة بطفيل السبيرونيكلس فى الأسماك الصغيرة والتى يتراوح وزن الجسم فيها بين ٢-١٠ جرام من الدرجة المتوسطة وأن نسبة الأسماك المصابة ونسبة النفوق كانت كبيرة.وقد وجد أن أكثر الأماكن أصابة فى الأسماك بطفيل السبيرونيكلس هو الجزء الأخير من الأمعاء.

وكانت الأختلافات الموسمية قد شهدت أرتفاع فى نسبة أصابة أسماك البلطى النيلى المستزرعة بطفيل السبيرونيكلس فى فصل الشتاء والخريف بنسبة أصابة تصل إلى ٩٠% و ٨٥ %على التوالى .

تم محاولة زراعة طفيل السبيرونيكلس على نوعين من الميديات وهم ميديا الميم المضاف إليها ١٠% مصل بقرى والميديا الثانية الأكسنك ميديا مضاف اليها ١٠% مصل جنين بقرى وقد تم الزرع بنجاح.وكان نمو الطفيل سريع حتى اليوم الخامس فى ميديا الميم والأكسنك ميديا حتى ١٥ يوم أما بعد ذلك لم يبقى طفيل فى الميديا حى.

تم عمل عدوى صناعية بطفيل السبيرونيكلس لدراسة قابلية أصابة أسماك البلطى النيلى المستزرعة و القراميط الأفريقية بالطفيل بطريقتين مختلفتين عن طريق الحقن البروتونى وعن طريق الدفع الفمى عند درجة حرارة الماء ١٥ و ٢٠م° .

أيضا تم دراسة تأثير شدة التزاحم فى الأحواض على الأصابة الصناعية بطفيل السبيرونيكلس وكذلك دراسة التعايش بين الأسماك المصابة صناعيا والسليمة على العدوى والأصابة عند درجتى حرارة مختلفة .

وقد تم بنجاح فى هذه الدراسة عزل وتصنيف وزراعة المسبب المرضى لمرض ثقب الرأس الذى يصيب أسماك البلطى النيلى .وتم دراسة بعض العوامل الوبائية بعمل أصابة صناعية بالطفيل فى أسماك البلطى النيلى المستزرعة و القراميط الأفريقية عند درجات حرارة مختلفة.