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STUDIES ON *CRYPTOSPORIDIUM NASORUM* IN FISH *(TILAPIA ZELII)* IN EGYPT

(With 5 Figures)

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دراسات عللا طفيل كربتوسبورديم نازورم في سمك البلطي زيلى في مصر

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تم دراسة نسبة الإصابة بطفيل كربتوسبورديم نازورم بين عدد ١٢٠ سمكه من نوع البلطي زيلي من مزرعة كينج ماريوت بمصر. وقد تم فحص مسحات من أمعاء الأسماك بعد صبغها بصبغة الزيل نيلسون المعدلـه . أثبتـت الدراسـة أن ٢٠% مـن الأسـماك كـانت مصابـة بطفيـل الكربتوسبورديم نازورم. وقد تم إجراء عدوى معملية لخمسة عشر سمكه من نوع البلطي زيلـي بخمسين ألف بذرة معديه من كربتوسبورديم نازورم لكل سمكه. كما تم عدوى عشـرة سـمكات أخرى بمائة ألف بذره معديه لكل سمكه. وكان إكتشاف البذور في السمكات المعدية بعد ٤-٥ أيام من العدوى وبإختبار الهستوباثولوجي للأمعاء كان طور الشيزونت قد أكتشف بعد ٢-٣ يوم مـن العدوى والبويضه المخصبه ٣-٤ أيام من العدوى. لم يعثر على البذور بواسطة فحص مسـحات من أمعاء الأسماك التي كانت تستخدم كمجموعة ضابطه وكذلك لم يعثر على الأطوار الداخليـة في أمعاء نفس المجموعة عن طريق اختبار الهستوباثولوجي. كما ثبت من هذه الدراسـة عـدم نجاح عدوى خمسة عشر فأرا أبيضا (عمر يومين) بمائة ألف بذرة معدية من الكبرتبوسـبورديم نازورم معزولة من سمك مصاب بالطفيل . كما لم تنجح عدوى خمس فئران بيضاء (عمر عشرة أيام) بعد إصابتهم معمليا بمائة ألف بذرة من الكربتوسبورديم نازورم وقد حددت هذه الدراسـة أن الجزء الأوسط من المعده (الجوجينم) هـو المكـان الـذي يتـم فيـه النمـو الداخلـي لطفيـل الكربتوسبورديم نازورم في سمك البلطي ، كما أوضحت عدم إمكانية عـدوى الثدييـات بطفيـل الكربتوسبوريديم المعزول من الأسماك.

SUMMARY

The incidence of *Cryptosporidium nasorum* was investigated among 120 *Tilapia zelii* obtained from a fish farm, King Mariote, Egypt. Intestinal smears were prepared, fixed and stained with Modified Zeihl-Nelson (M.Z.N).Twenty percent of fish were infected with *C. nasorum*. Fifteen

fingerlings of *Tilapia zelii* fish were each infected experimentally with 50,000 *C. nasorum* oocysts and ten fingerlings were each given 100,000 oocysts. Oocysts were detected by mucosal smears at 4 to 5 days postinfection (dpi). Schizonts were identified at 2 to 3 dpi and zygotes were found 3 to 4 dpi. No oocysts were detected by examination of smears and no endogenous stages were found in non-infected *Tilapia zelii*. Histopathological examination of fifteen mice (Two days old) experimentally infected with 100,000 *C. nasorum* oocysts isolated from infected *T. zelii* fish failed to induce infection. The same result was obtained in five (ten days old) mice inoculated with 100,000 oocysts. This study confirmed the tropism of *Cryptosporidium* species to the jejenum of the *T. zelii* fish. It also showed that infection of mammals with the fish *Cryptosporidiosis* did not ocuur.

Key words: *Fish – Tilapia zelii – Cryptospooridium - Egypt*

INTRODUCTION

Since Tyzzer (1907) described *Cryptosporidium* for the first time in mice, research has proceeded on different facets of this parasite, such as pathogencity, diagnosis, cross transmission and zoonotic importance. Now *Cryptosporidium* infections have been recorded in over 170 different host species originating from some 50 countries ranging in location from tropical to temperate zones throughout the world (O'Donoghue, 1995). The author added that *Cryptosporidium* infections have been detected in nine species of fish including marine and fresh water species. The objectives of this study were to present data on :

1) The prevalence of *C. nasorum* infecting cultured fish *T. zelii* in Egypt.
2) The endogenous life cycle of experimental infection of *C. nasorum* to non infected fish.
3) The possibility of infection of mammals with *Cryptosporidium* species originating from fish.

MATERIAL and METHODS

1- Prevalence of *C. nasorum* in *T. zelii* Fish :

Wet preparations of mucosal scrapings were taken from ileum, jejunum and colon of 120 cultured fish, *(T. zelii)* from different localities in Egypt. (King Mariote, Portsaid and Ismalia). The smears were left to dry,

fixed with methanol and stained with M.Z.N. according to Henriksen and Pohlenz (1981) and Baxby et al (1984).

2- Experimental infection of Fish:

a) Experimental Design:

Three groups of fingerlings (*T. zelii)* were reared in the Parasitology Dep. lab., Faculty of vet. Medicine, Cairo Univ. Egypt. The fish were free from *Cryptosporidium* or any other intestinal parasitic infection. Group A (15 fish) were inoculated with 500,000 *C. nasorum* oocyst, group B (10 fish) inoculated with 100,000 *C. nasorum* oocysts and group C (15 fish) remained as negative controls. One fish was killed every day until the third day, then every other day for preparation of mucosal scrapings and histopathological sections from ileum, jejunum and colon.

b) *C. nasorum* inocula :

Inocula were prepared from infected feces of *T. zelii* with *C. nasorum*. The feces were left to sediment in a 2 litre cylinder. The sediment was sieved and centrifuged at 1500 g. The sediment was centrifuged with 2 M Sucrose (1:1) at 310 g for 5 minutes. One third of the supernatant was taken and washed with P.B.S. (Zierdt, 1984 and Current et al., 1983). Fat was removed from the sample with formaline-ether sedimentation (Adam et al., 1971) and then the sample washed with P.B.S. to remove ether. Potassium dichromate 2.5% was added to the oocysts solution (1:1). The number of *Cryptosporidium* oocysts was calculated using haemocytometer (Fig 1). Inocula were stored in refrigerator at 4°C until use. Oocysts from the postassium dichromate solution were washed several times with P.B.S. and adjusted back to the original volume just before the experimental infection.

3- Experimental infection of mice

Fifteen, 2-day-old mice (Exp. 1) and five, 10 day old mice (Exp. 2) were inoculated with 100,000 *C. nasorum* oocysts for each mouse. Fecal smears were taken from each group every day. Two mice were killed every day post infection in Exp. 1 and one mouse every day postinfection in Exp. 2. Mucosal scraping from ileum, jejunum and colon. In addition samples were taken from the same organs for histopathological examination to follow the infectivity of *C. nasorum* in mice.

RESULTS

1- Prevalence of *C. nasorum* in fish:

Twenty four fish (20%) out of 120 examined showed *C. nasorum* oocysts in intestinal mucosal smear. The oocysts were 6-7 μm in diameter taking the red colour with different affinities with green background (Fig. 2).

2- Experimental infection of fish:

Group A and group B showed oocysts in the mucosal smears at 4-5 DPI. First schizonts were identified in histopathological sections of jejunum 2-3 DPI. They were typically spherical lying on the brush border and measured 3-μm in diameter (Fig 3). Second schizonts stage were recorded 3 DPI in jejunum, mushroom in shape and measured 4-6 μm in diameter (Fig 4). The zygotes were recorded 3-4 DPI and appeared spherical in shape and measuring 4-5 μm in diameter (Fig 5).

3- Experimental infection of mice:

Fecal examination of the two infected groups of mice failed to detect oocysts for 20 DPI. Histopathological examination of the ileum, jejunum & colon of the infected mice also failed to recognise the endogenous stages of *Cryptosporidium*.

DISCUSSION

Cryptosporidium species infecting *T. zelii* was demonstrated in this study. It was identified as *C. nasorum* (Hoover et al., 1981). The prevalence of *C. nasorum* in the examined fish was 20%. Hefnawy (1989) recorded that 12% of *Tilipia nilotica* in Assuit province, Egypt were infected with *Cryptosporidium*. Eid and Hilali (1990) also reported the presence of *Cryptosporidium* species in Egyptian fresh water fish. Unfortunately Hefnawy (1989) and Eid & Hilali (1990) did not identify the species of the *Cryptosporidium*, or describe oocysts measures or endogenous developmental stages.

Cryptosporidium species were also detected in different species of fish in different countries. It was recorded in *Cyprinus caprino* fish in Czechoslovakia (Pavlasek, 1983), *Lates calcarifer* spp. in Australia (Glazebrook and Campbell, 1987), *Naso lituratus* and unnamed tropical fish in Canada (Hoover et al., 1981), *Oreochromis* hybrid fish and *Oreochromis aureus* in Israel (Landsberg and Paperna, 1986; Paperna, 1987) and *Salmo trutta* in UK (Rush et al, 1990).

In this study, experimental infection of fingerlings of *T. zelii* was carried out with 50,000 and 100,000 *C. nasorum* oocysts (group A and group B respectively). The schizonts were identified 2-3 DPI and zygotes 3-4 DPI in the jejunum of the infected fish. Oocysts were detected in feces 4-5 DPI. The first schizont stage measured 3 μm in diameter, second schizont measured 4-6 μm in diameter with a mushroom like shape attaching to the brush border of the mucosa, while the zygote measured 4-5 μm .

O'Donoghue (1995) recorded that *Cryptosporidium* developmental stages were found attached to the intestinal mucosa. Since the parasite has been detected in the intestine of most of other fish species except for *cichlids* where they were confined to the stomach mucosa (Landsberg and Paperna, 1986). Ultrastructurally meronts and gamonts of *Cryptosporidium* were found localized at the microvillus zone in *Oreochromis aureus* and *O.niloticus* stomach (Landsberg and Paperna, 1986). The latter added that oocysts were usually enclosed within degenerating or necrotic cells located near the surface. The second schizont stage in this study is closely similar to the schizont recorded by Paperna (1987).

Few Pathological changes were detected in the intestine of the infected fish with some cellular infiltration in the lamina propria. The same results were recorded by Glazebrook and Campbell (1987) in barramundi fish. Landsberg and Paperna (1986) found small pockets of necrotic epithelial cells containing oocyst in the stomach mucosa of *cichlids*.

Paperna and Vilenkin (1996) recorded a new species resembles *Cryptosporidium* by electron microscope in stomach mucosa of gourami trichogasterleeri. It was named *Piscicryptosporidium*. It differed from all other known species of *Cryptosporidium* in that their sporulating oocysts sink into the basal part of the gut epithelium or into the lamina propria.

Cryptosporidium nasorum isolated from fish failed to induce infection in mice. This result excludes the possibility of infection of mammals with fish *Cryptosporidiosis*. Levine (1984) recorded that no attempts had been made to transmit *Cryptosporidium* from birds, reptiles or fish to other species other than the same origin. Upton (1985) found that *Cryptosporidium* oocysts from calf origin failed to induce infection in fish. This study completes the results of Graczyk et al. (1996). Who decided that *C. parvum* is not transmissible to fish, amphibians or reptiles.

In this study isolation and descripition *C. nasorum* from Egyptian fish *(Tilapia zelii)* were recorded for the first time according to the available literature. It also showed the endogenous stages of *C. nasorum* in fish and completed the life cycle. This study has also determind the tropism of *C.*

nasorum to jejenum of *T. zelii* and excludes the possibility of cross transmission between fish and mammals with *Cryptosporidia* species.

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Fig. 1 *C. nasorum* oocysts after purification from fecal debris (X 400).

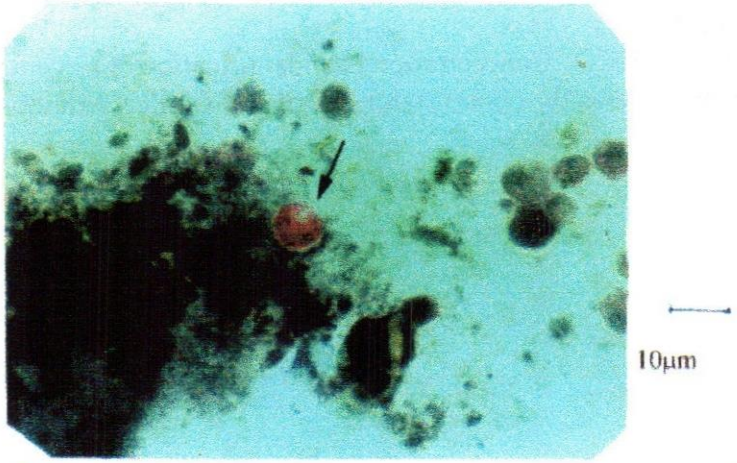

Fig. 2 *Cryptosporidium nasorum* oocysts in feces of *Tilapia zelii* stained with M.Z.N (X 1000).

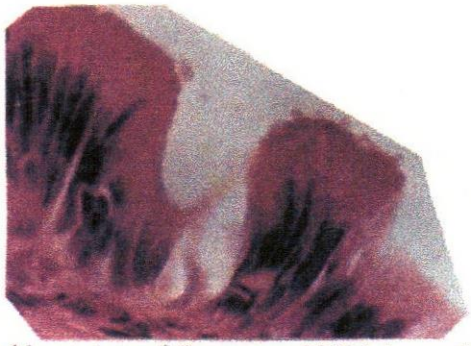

Fig. 3 First schizont stage of *C. nasorum* 2DPI lying on the brush border of jejunum (3 µm in-diameter) (X 1000).

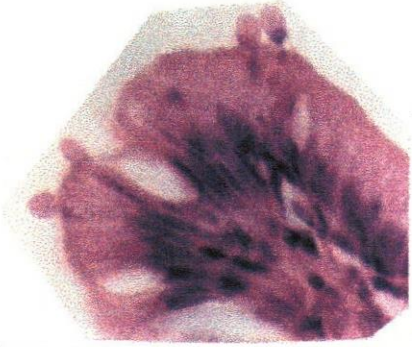

Fig. 4 Second schizont stages of *C. nasorum* 3DPI with mashroom like shape and dimension of 6X4 µm (X 1000).

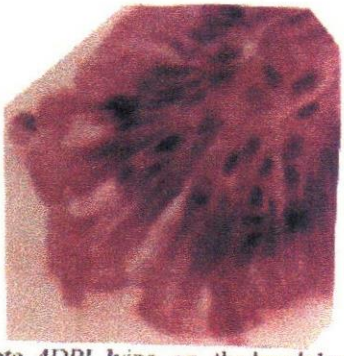

Fig. 5 *C. nasorum* zygote 4DPI lying on the brush border of jejunum (4X5 µm in-diameter) (X 1000).