NEW RECORD OF *ANGUILLICOLA NOVAEZELANDIAE* (ANGUILLICOLIDAE), A PATHOGENIC GASBLADDER PARASITE OF EELS (*ANGUILLA ANGUILLA)* IN EGYPT

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Abstract

The present study reports for the first time in Egypt, the isolation and identification of Anguillicola novaezelandiae, a pathogenic parasite of gasbladder of eels (Angilla anguilla). A total of 200 eels were collected from commercial fish farms in Kafr El-sheikh and El-Beharah governorates, subjected for investigation for the spread of infection. The prevalence rate was 10%, with intensity 1-5/fish. The clinical signs and postmortem changes associated with infection were reduced feed intake, reduced viability, slight abdominal distension and red swollen anus. The gasbladder had turbid wall as a result of increased thickness of the wall and fibrosis. Upon incision, brownish mass of worms and bloody exudate containing eggs and larvae in the lumen were noticed. Cyclops and Daphnia were examined for the presence of the larval stages. Cyclops were found to be infected with the the larval stages and acted as intermediate host. The worms are darkly coloured, cylindrical and tapering at both ends. The most morphological characters of the adult worm were very small teeth about 32 in number and slight neck constriction. Also, three large oval rectal glands and small rectal gland which overlapped by the large ones. The histopathological changes in gasbladders naturally infected with A. novaezelandiae were a great reduction of lumen size due to the presence of adult and larval stages of worms. The blood feeding activities of adult worm and the migration of larvae leading to degenerative, inflammatory, hemorrhagic, edematous and proliferative fibrosis which greatly thickened the wall of gasbladder. The larvae were surrounded by compact connective tissue layers giving rise to nest like arrangement.

INTRODUCTION

Anguilliculture is one of the most important warm water fish species cultured allover the world. Eel (*Anguilla anguilla*) could become an important species in Egyptian commercial aquaculture in the near future. The dracunculoid nematodes of the genus Anguillicola are widely distributed blood feeding gasbladder parasites, recorded from various eel species of family Anguillidae as definitive host (Moravec and Taraschewki, 1988; Molnar, 1994; Woo, 1995; Wurtz and Taraschewski, 2000; Kirk, 2003 and Moravec *et al.*, 2005) .

Genus Anguillicola comprises five species (Moravec *et al.*, 1994). Anguillicola species is a native parasite in Japanese eel (A. japonica), then introduced into Europe

through importation of live Japanese eels from East Asia causing serious problems in European eel populations since the early 1980s and later introduced into North Africa (Kirk, 2003 and Moravec *et al.*, 2005) .

The economic impact of anguillicolois is basically due to blood sucking worms which occlude the gasbladder with severe histopathological alterations in wall of the organs due to larval migration which inturn raise plasma cortisol (Wurtz and Taraschewski, 2000 and Kirk, 2003).

Additional cost of *anguillicolosis* may reduce swimming speed and probably increase their susceptibility to predators and also, impair the success of spawning migration to the sea (Woo,1995). Eel farms have suffered reduction in growth rates, emaciation and mortalities up to 65% (Sinderman, 1993).

Anguillicolosis has been shown to reduce gasbladder oxygen levels in European eels, possibly due to pathological damage to the gas glands (Molnar, 1994 and Wurtz and Taraschewski, 2000). Mass mortalities of eels due to *anguillicolosis* have been recorded (Kennedy and Fitch, 1990; Molnar *et al.*,1991; and Kirk, 2003).

A. crassus has become the most studied introduced eel parasites in Egypt (El-Dosoky, 2001 and El-Sayed, 2003). According to the best of our knowledge, this is a new record of *A. novaezelandiae* among the cultured eels in Egypt. So, the present investigation was aimed to through the light on *A. novaezelandiae* infection among eels in Egypt.

MATERIALS AND METHODS

Fish:

200 eels (*Anguilla anguilla*) were collected from commercial fish farms (15-250gm), in Kafr El-sheikh and El-Beharah governorates and transferred alive or freshly dead to the laboratory.

Clinical picture:

The clinical signs and postmortem changes were performed as described by Lucky (1977). The gasbladders of all eels were removed and examined macroscopically for the presence of Anguillicola. The prevalence and intensity were recorded according to Bush et al. (1997).

Parasitological Examination:

The detected nematodes were washed in physiological saline solution, relaxed in refrigerator then cleared in lactophenol and mounted in polyvol. The isolated nematodes were identified according to Moravec and Taraschewski (1988) and Moravec *et al.* (1994).

Zooplankton sampling:

At the eel sampling sites, samples of zooplankton were taken with sieves, fixed in 4% formalin, mounted in polyvol and examined for detection of the larval stages in the intermediate hosts.

Histopathological examination:

Tissue specimens were taken from the infected gasbladder, fixed in 10% neutral buffered formalin and processed for histopathological examination according to Roberts (2001).

RESULTS AND DISCUSSION

Eel is one of the most commercially important fish species in Egypt. The trans-boundary movement of living aquatic animals is well recognized as facilitating the introduction of serious infectious diseases into new areas. Currently, several emerging diseases are also recognized. These diseases cause large-scale mass mortalities of cultured species, inducing devastating losses to regional aquaculture production (Kennedy and Fitch, 1990 and Molnar *et al.*,1991). An expanding eel trade allover the world has ensured that the Anguillicola infection is now wide spread (Sinderman, 1993). Anguillicolosis had become a parasitosis wide spread throught out the world causing serious economic damage to eel populations (Lefebvre et al., 2004 and Moravec *et al.*, 2005). *A. crassus* and *A. novaezelandiae* were brought to Europe in the early 1980s, possibly with the importation of infected Japanese eels from Taiwan (Kirk, 2003 and Moravec *et al.*, 2005) .

Genus Anguillicola comprises the following five valid species: *A. globiceps, A. australiensis, A. crassus, A. novaezelandiae and A. papernai* (Moravec *et al* .1994). Numerous studies have delt the spread and development of *A.crassus* and pathological changes associated with it (Molnar, 1994; El-Dosoky, 2001; El-Sayed, 2003 and Kirk, 2003). On the other hand, few reports have addressed with the *A. novaezelandiae.*

Parasitological examination of eels revealed that the prevalence rate of A. novaezelandiae infection was 10% with intensity 1-5 parasites per infected fish. Saroglia *et al.*(1985) mentioned that A. novaezelandiae is quickly spreading in Italy and occurring there mainly in fish farms. Lefebvre *et al.*, (2004) reported that the prevalence of *A. noveazelandiae* was low among *A. australis* with intensity 1-5 parasites per fish. More common infestation intensities are 10 or fewer per eel (Kennedy and Fitch 1990). However, the prevalence rate due to *A. crassus* may reach to 100% (Molnar, 1994 and Kirk, 2003). The differences of infection may be related to localities, source of water, season, abundance of intermediate hosts and fish species (Woo, 1995; El-Sayed, 2003; Kirk, 2003 and Moravec *et al.*, 2005).

Concerning to the clinical signs and postmortem changes (Fig., 1) associated with anguilliculosis were represented as reduced feed intake, reduced viability, emaciation, slight abdominal distension and red swollen anus. The gasbladder had turbid wall as a result of increased thickness of the wall and fibrosis. Gasbladder infected with worms looks like sausage appearance and stomach like to touch. Also, the wall of gasbladder showed intensive pigment accumulation. Upon incision, brownish mass of worms and bloody exudate containing eggs and larvae in the lumen were also noticed. The pneumatic duct became dilated and red. Most of these signs and postmortem changes were almost similar with the findings noticed by Moravec and Taraschewki, (1988); Kennedy and Fitch, (1990); Sinderman, (1993); Molnar (1994); Moravec *et al.*, (1994); Woo, (1995); El-Dosoky, (2001); Kirk, (2003) and Lefebvre *et al.*, (2004) .

Water samples showed that Cyclops only were found to be infected by the larval stages of *A. novaezelandiae* and acted as intermediate hosts (Fig., 2). However, freshwater copepods are suitable hosts: Cyclops (*cyclopoid copepods*), Diaptomus, Eurytemora (a brackish water copepod), Daphnia (water fleas), Gammarus (amphipods), and Asellus (isopods). Species of Cyclops appear to be the most suitable hosts (Kennedy and Fitch 1990). Infection involves the ingestion of the 3rd stage larvae either in an intermediate or paratenic hosts (Kirk, 2003; Lefebvre *et al.*, 2004 and Moravec *et al.*, 2005). The third stage larva penetrates the intestinal wall of the eel into the gasbladder, molt and becomes adult worm (Molnar, 1994). They lay eggs in the lumen of gassbladder. The eggs and the second stage larvae pass through the ductus preumaticus and reach to aquatic environment with feaces (Moravec and Taraschewski, 1988; Molnar *et al.*, 1991; Wurtz and Taraschewski, 2000 and Moravec *et al.*, 2005).

The worms are darkly colored, cylindrical and tapering at both ends. The buccal capsule is small, feebly sclerotized, with very small teeth about 32 in number. It is characterized by expansion of oesophages at its posterior half. The valvular apparatus of oesophagus is well developed. The nerve ring situated below the neck constriction, while the excretory pore lies near the junction of oesophagus and intestine. Intestine is dark ,straight and broad .There are three large oval rectal glands and small rectal gland which overlapped by the large ones. Tail is conical and pointed (Fig., 3).

Female: It measures 8.22-29.30 mm long with maximum width 0.72-3.70 mm. The length of oesophagus is 0.52-0.92 mm ,while the maximum width 0.14-0.23 mm. The buccal capsule measures 0.01-0.02 mm long and 0.01-0.03 mm width. The excretory pore opens at 0.15-0.19 mm from the anterior extremity. The vulva is

prominent, elevated and situated in the posterior part of body at 2.1-5.6 mm from the posterior extremity. Ovarial tubes not reaching to level of oesophagus anteriorly. The uterus containing eggs and larvae. The rectal glands measures 0.25- 0.32 X 0.14-0.23 mm. The tail is conical and pointed. It measures 0.16-0.30 mm (Fig., 3) .

The first stage larvae measures 0.22-0.24 with maximum width of 0.015 mm (Fig. 4). The second stage larvae measures 0.2- 0.4 mm length (Fig. 4). The third stage larvae is darkly colored and measures 0.5 to 0.8 mm in total length and 0.026 mm width. A rounded cephalic end with four papillae, 2 amphids and two vertically arranged labia. The mouth is a split-shape with two teeth. The tail is short and conical (Fig. 4).

Similar morphological description has been recorded by Moravec and Taraschewski (1988), Moravec *et al.*, (1994) and Lefebvre *et al* (2004), based upon buccal capsule size ,the number of teeth and head end with neck construction just in front of nerve ring. They were identified for the first time in Egypt as A. novaezelandiae. Moravec *et al.* (1994) recorded that the morphology of larval stage of A. novaezelandiae was similar to that of larvae of the related species *A. crassus*.

The completion of the life cycle is dependent upon predator-prey interactions (Kirk, 2003). It is possible for an eel to acquire the parasite by preying on other infected eels (Kennedy and Fitch 1990). Experimental studies have demonstrated that osmoconformation with the blood plasma of the eel hosts enables the majority of the parasite population to survive and complete their life cycle in both marine and brackish water, although the infection levels are lower than in freshwater (Kirk, 2003).

Anguilliculosis produced severe pathomorphological changes in the gas bladder wall that they resulted in complete dysfunction of the organ. Based upon the histopathological examination (Fig., 5,6,7,8,9 &10) of the gasbladder naturally infected by A. novaezelandiae showed the presence of adult worms and larvae in the lumen leading to a severe reduction of its size (Fig., 5 & 6). Severe congestion of blood capillaries in the wall of gasbladder was seen (Fig., 7, 8 & 9). The blood feeding activities of adult worms and the migration of larvae leading to degenerative, inflammatory, hemorrhagic, edematous and proliferative fibrosis which greatly thickened the wall of gasbladder (Fig., 5,6,7,8 & 9). A large number of melano-macrophages were detected in the wall of gas bladder especially around the invaded larvae (Fig., 10). Hyperplastic changes in the epithelial cells lining the internal surface of the gas bladder and form folds leading to sever decrease in the lumen size. Also, inflammatory edematous lesions developed as a result of larval migrations. The larvae were surrounded by compact connective tissue layers giving rise to nest-like arrangement (Fig., 10) .

Similar pictures of histopathological observations were described by Molnar et al. (1991), Molnar (1994), Wurtz and Taraschewski, (2000), El-Dosoky, (2001), El-Sayed (2003) and Kirk, (2003). The accumulation of macrophages around the larvae constitutes the first step in the restriction of larval migration. Roberts (2001) noticed that the body defense was presented by presence of melanomacrophages. Molnar (1994) mentioned that anguillicolosis impair the eel natural resistance and under unfavorable environmental conditions may lead to their death.

Several studies of eels have demonstrated significant pathological effects resulting both from the migration of larvae in the gas bladder wall and from blood feeding by the adult worms (Molnar, 1994; Wurtz and Taraschewski, 2000; El-Sayed, 2003 and Kirk, 2003).

Chronically infected eels with Anguillicola have been shown to have pronounced thickening of the gas bladder wall and formation of fibrotic granulomatous parasitic nodules. Molnar (1994) mentioned that the oedematous reaction is a general signs of anguilliculosis which attributed to hypoproteinemia from the blood sucking activities of adult worms. Repeated larval invasion of the gasbladder wall results in hemorrhage and injury to connective tissue and blood feeding by adult can cause mechanical injury to the epithelium (Wurtz and Taraschewski, 2000). Also impaire the capacity of eels to complete spawning migration (Kirk, 2003).

It could be concluded that A. novaezelandiae, a pathogenic parasite in the lumen of gasbladders, is a new species recorded in Egypt and may cause mortalities in feral and cultured eels. Cyclops plays an important role in the infection of eels as intermediate hosts. The gasbladder of infected eels are irreversibly damaged.

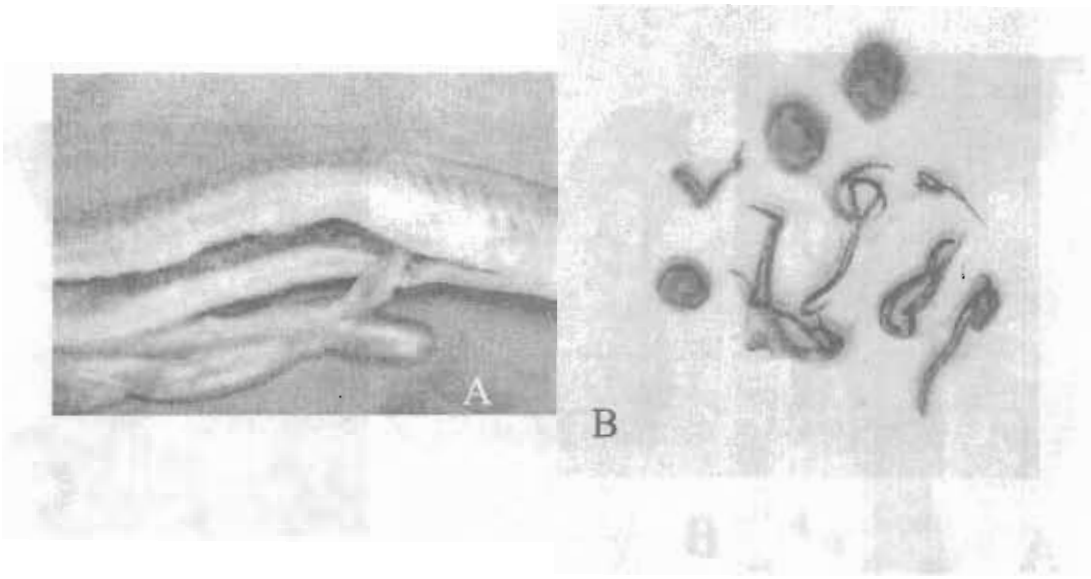

Fig. 1. (A) *Anguilla anguilla* naturally infected with *Anguillicola novaezelandiae* showing gasbladder turbid wall. (B) *A. novaezelandiae.*

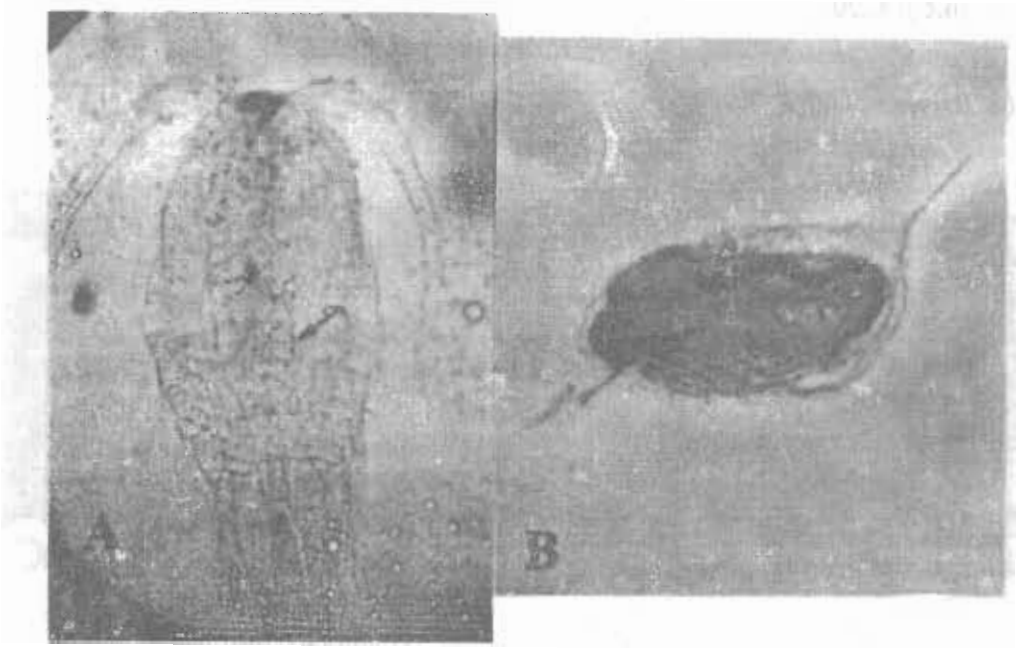

Fig. 2. Intermediate hosts (A) Cyclops showing larval stage (arrow) (B) Daphnia.

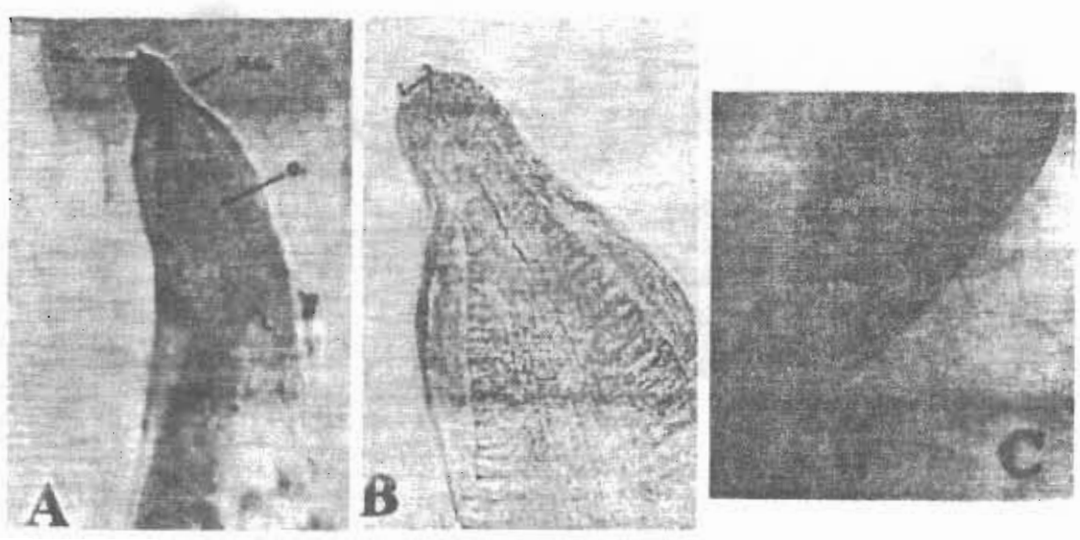

Fig. 3. Adult *A. novaezelandiae*

(A) anterior end showing buccal capsule (b.c.), oesophagus (o.) and neck constriction (n.c.). X120.

(B) anterior end showing neck construction (arrow). X300.

(C) Posterior end. X120.

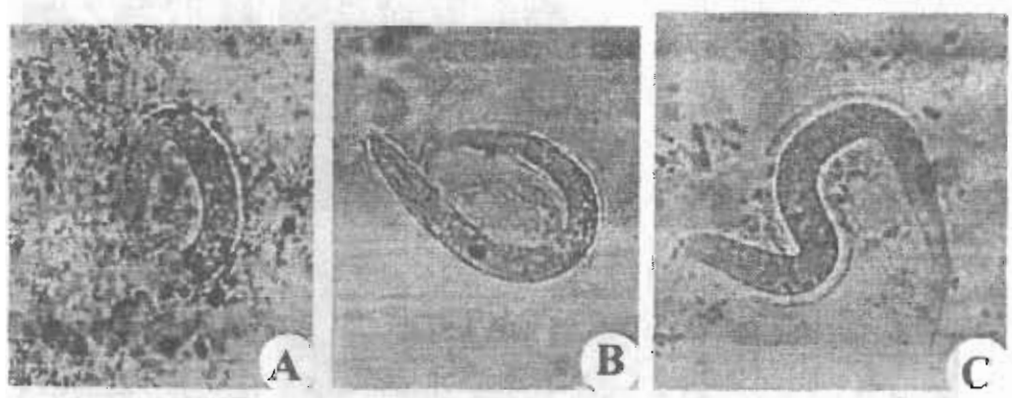

Fig. 4. Larval stages of *A. novaezelandiae* (A) 1st satge (B) 2nd stage (C) 3rd stage . X200.

Fig. 5. Gasbladder of *A. anguilla* showing numerous of larvae of *A. novaezelandiae* in the lumen, congested capillaries and cellular infiltration . X150.

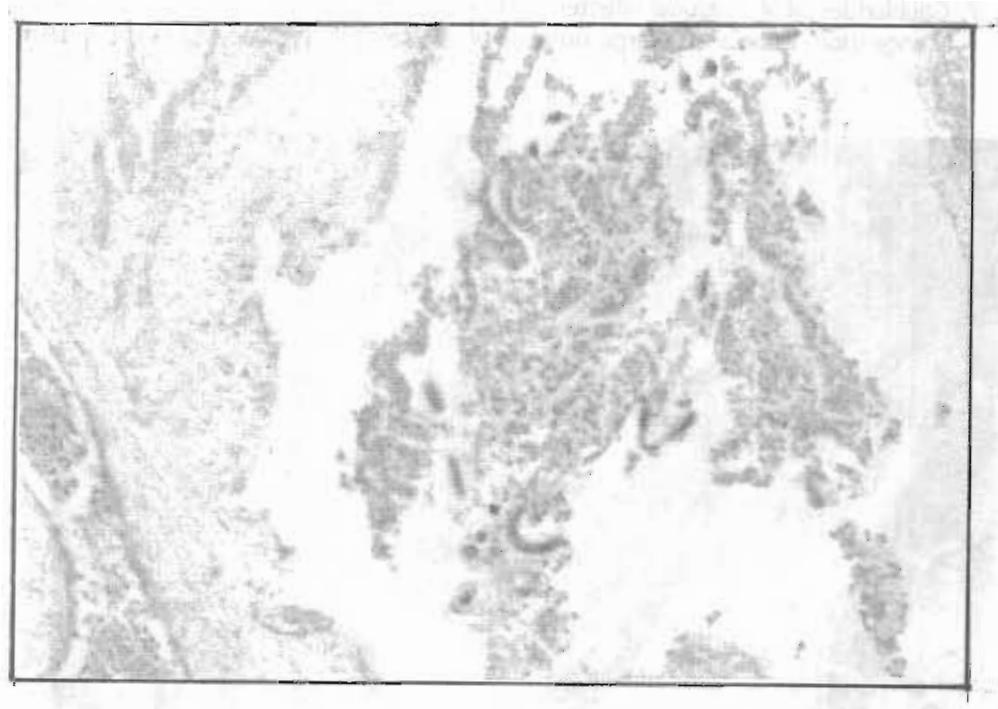

Fig. 6. Gasbladder of *A. anguilla* infected with *A. novaezelandiae* showing inflammed wall, edema, sever congestion and erythrocytes and larvae in the lumen. X300.

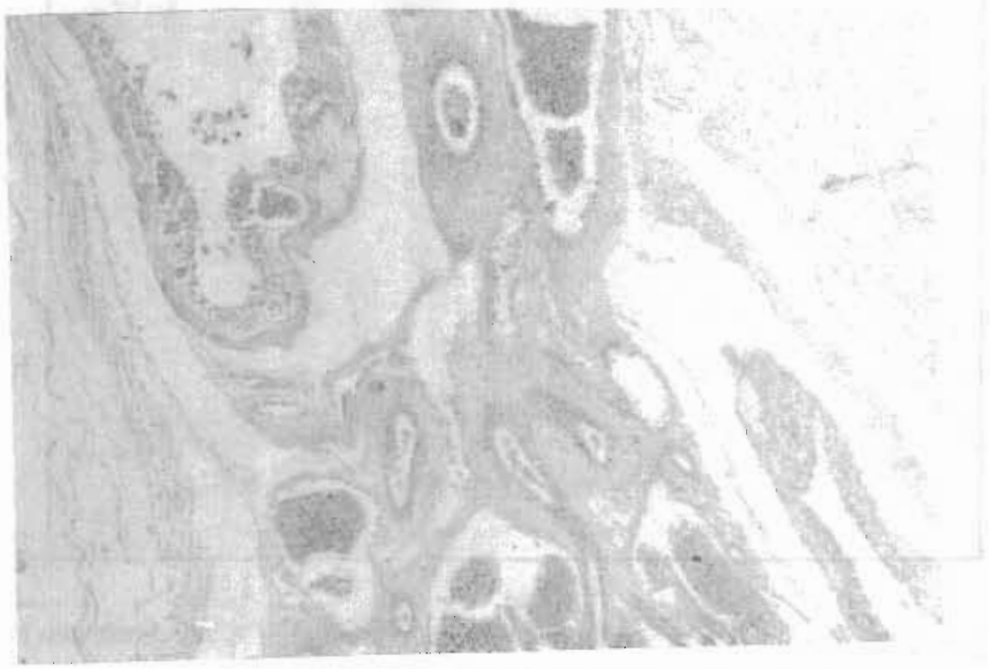

Fig. 7. Gasbladder of *A. anguilla* infected with *A. novaezelandiae* showing sever blood congestion, edema and large number of larvae and erythrocytes in the lumen. X60.

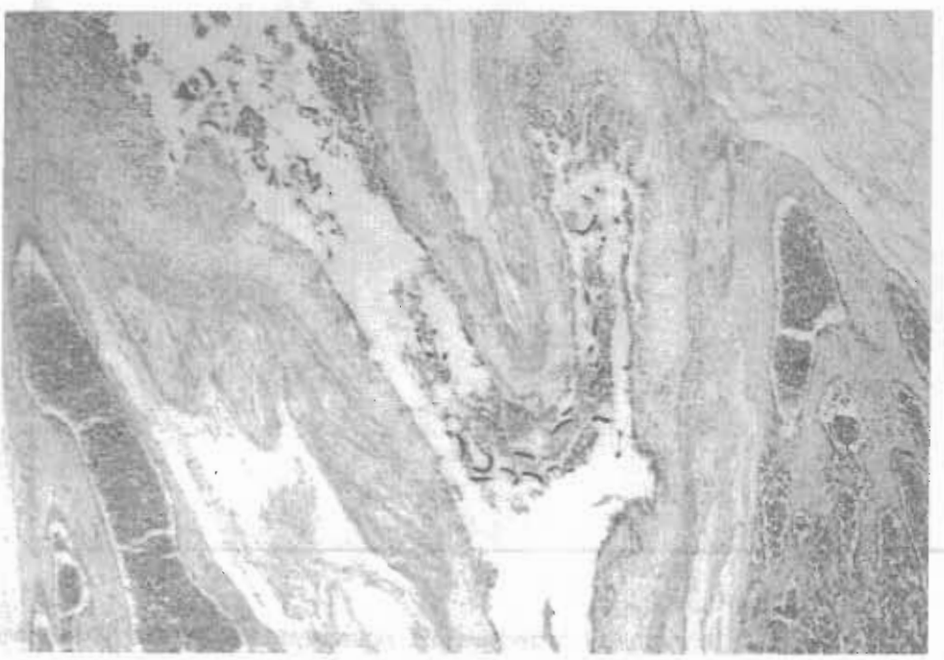

Fig. 8. Gasbladder of *A. anguilla* infected with *A. novaezelandiae* showing filling of the lumen with erythrocytes, larvea and congested blood capillaries, oedema and sloughing of the epithelial cells linning. X150.

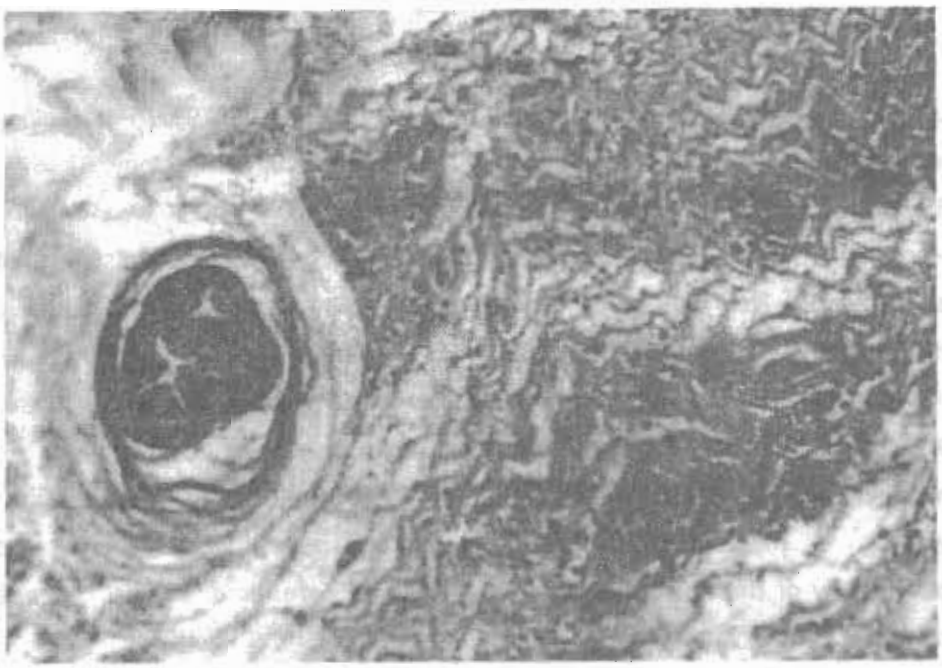

Fig. 9. Gasbladder of *A. anguilla* infected with *A. novaezelandiae* showing congestion of blood vessel and thickening of its wall. X 600.

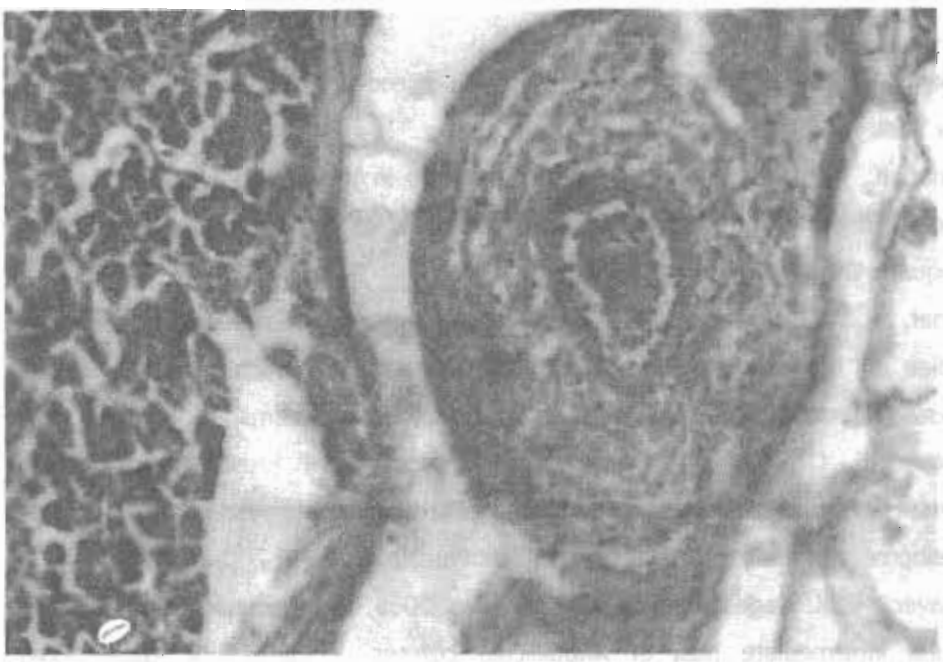

Fig. 10. Gasbladder of *A. anguilla* infected with *A. novaezelandiae* showing larval migration in the wall, surrounded by dense fibrous connective tissue and aggregation of melanomacrophages. X600.

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تسجيل جديد للأنجيليكولا نوفازيلانديا، الديدان المرضية في المثانة الغازية فى أسماك الثعبان في مصر

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أجريت هذه الدراسة على عدد ٢٠٠ من أسماك الثعبان و التى تم تجميعها من المزارع السمكية بمحافظتي كفر الشيخ والبحيرة. كانت نسبة الإصابة بطفيل الأنجيويليكولا نوفازيلانديا 10%، فى المثانة الغازية . أما كثافة الإصابة فكانت ١–٥ دودة لكل سمكة مصابة. تمثلت العلامات الظاهرية و الصفة التشريحية فى الضعف العام و الهزال و احمرار فتحة الشرج و ضعف الإقبال على الطعام وزيادة فى حجم البطن. كما تبين عكارة فى جدار المثانة و بفتحها تظهر ديدان بنية كبيرة الحجم.

بفحص المياه من المزارع السمكية تبين وجود السيكلوب مصابا بالطور اليرقى و التى من الممكن أن تلعب دورا كعائل وسيط فى نقل الطفيل للأسماك. تم وصف الطور البالغ و اليرقى لهذا الطفيل. بالفحص الهستوباثولوجى للمثانة تبين وجود احتقان شديد فى الأوعية الدموية وأنزفة وأوديما و أعداد كبيرة من خلايا الميلانو ماكروفاج و التهاب و تليف حول اليرقات التى اخترقت جدار المثانة. كما تم دراسة دورة حياة الطفيل ومناقشة الأهمية الاقتصادية لهذا الطفيل.