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MORPHOBIOLOGICAL AND IMMUNOLOGICAL STUDIES ON *PROCAMALLANUS LAEVICONCHUS* WORMS IN CATFISH (*CLARIAS GARIEPINUS)*

(With 2 Tables and 6 Figures)

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دراسات وصفية حياتية ومناعية على ديدان البروكاملانس ليفيكونكس في اسماك القراميط (كلارس جارابينس)

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تعتبر ديدان البروكاملانس ليفيكونكس من أهم الديدان الأسطوانية آلتي تصيب القراميط وتؤثر على الحالة المناعية لهذه الأنواع من الأسماك. تم في هذا البحث دراسة دورة الحياة والنمو لدودة البروكاملانس ليفيكونكس حيث تم إجراء العدوى التجريبية لقشريات الكوبيبودا ميزوسيكلوبس لوكارتى (كعائل وسيط) بالطور اليرقي الأول لهذا النوع والذي يخترق جدار القناة الهضمية آلي تجويف الجسم في منطقة الرأس صدرية حيث تدخل في العديد من التطورات فنجد اليرقة تزيد في الحجم وتخضع لانسلاخين عند درجة حرارة ٢٢ إلى٢٤م قبل آن تصل آلي الطور الثالث المعدي للعائل النهائي. تم الحصول على الطور اليرقى الثاني بعد ٤ أيام من عدوى القشريات والطور اليرقى الثالث من ٦-٨ أيام بعد العدوى ذاتها. استخدم الطور اليرقي الثالث لعدوى العائل النهائي وهى اسماك القراميط (كلارس جارابينس) حيث يمر هذا الطور داخل العائل النهائي آلي انسلاخين وينتج عنهما الطور اليرقى الرابع من ١٢- ١٦ يوم بعد العدوى. تم الحصول على الديدان الذكر بعد ٢٥ يوم والأنثى بعد ٣٠ يوم من عدوى العائل النهائي. بالنسبة للاستجابة المناعية آلتي حدثت أثناء العدوى التجريبية اتضح آن البروتين الكلى قد قل بفروق معنوية بعد العدوى وذلك عند مقارنتها بالمجموعة الضابطة. وقد أوضح الجلوبيولين زيادة معنوية خلال التجربة. خلايا الدم البيضاء أوضحت زيادة ملحوظة خلال أيام التجربة.

SUMMARY

The present study deals with the development of *Procamallanus laeviconchus* through experimental infection of copepods crustacean

(*Mesocyclops leukarti)* as an intermediate host with first stage larvae and *Clarias gariepinus* as definitive host with the third stage larvae. The first stage larvae penetrate the haemocoel of the intermediate host where further development takes place. The larvae increase in size and undergo two moults, one at 4 days post-infection to produce the second stage larvae and the other at 6-8 days post- infection to produce the third stage larvae. Experimental infection of the definitive host with the third stage larvae leads to the appearance of the fourth stage larvae at 12-16 days post infection and adult male and female at 25 and 30 days post infection. The morphological characters of each developmental stage were discussed in details. Moreover the immune response of infected *Clarias gariepinus* showed significant decrease of total blood protein and significant increase of leukocytic count and globulin.

Key words: *Morphology, Immunology, Procamallanus Laeviconchus, Catfish, Clarias gariepinus.*

INTRODUCTION

Procamallanus laeviconchus is a wide spread stomach parasite of fish of the family Clarridae in Africa. It is a small larviparous spiruurid worm firstly described by Wedle (1862) from the Egyptian Synodont. Morphological characters and prevalence of this species of nematodes has been reported in Egypt by several authors as Imam (1971), Shalab (1982), Negm El- Din (1987), Imam and Askalany (1990) and El-Naggar (1995). The development of this nematode and their larval morphogensis remain insufficient so the present investigation was planned to study the life cycle through experimental infection of copepod crustacean intermediate host (*Mesocyclops leukarti)* with the first stage larvae and experimental infection of fish definitie host with the third stage larvae. Also study the effect of the infection on the immune response of the definitive host.

MATERIALS and METHODS

(A) Collection of the nematode specimens:

The nematode species *Procamallanus laeviconchus* was collected from stomach of *Clarias gariepinus* taken from Domiat branch and their tributaries and permanent slides for identification were carried out according to Negm El- Din *et al.* (1988). Identification of the collected parasites was done according to the keys of Yamaguti (1961). Only the gravid females

containing moving larvae in their uteri were selected for the experimental infection.

(B) Experimental infection of copepod crustacean (*Mesocyclops leukarti*):

The isolated gravid females of *Procamallanus laeviconchus* containing moving larvae in their uteri were individually placed in small glass dishes filled with filtered Nile water, their bodies were opened by fine needle to evacuate the larvae from the uteri into water. For each vessel 100-200 copepods, *Mesocyclops leukarti* were added. (The copepods were collected from Central Laboratory for Aquaculture Reseaarch, Abbassa). The glass dishes containing the larvae and copepods were kept at a temperature of 22-24C. The copepods were examined daily for the presence of the second and third stage larvae of *P. laeviconchus*. The infected *Mesocyclops leukarti* with infective third stage larvae were used for the experimental infection of *Clarias gariepinus*.

(C) Experimental infection of fish definitive host:

A total number of 30 *Clarias gariepinus* as a definitive host (90-100 g in weight) were kept in 6 glass aquaria (40 x 50x70 cm supplied with fresh declorinated tap water), each contained 5 fish specimens. The fish specimens of each aquarium were exposed to infection by feeding of 50 live infected *Mesocyclops leukarti*. On the other hand 16 *Clarias gariepinus* fish specimens were kept in another aquarium as control. All fish were fed on commercial dry food during the period of experiment. Infected and control fish specimens were examined for the presence of nematodes at 3, 5, 8, 12, 16, 20, 25, and 30 days post-infection. The collected larvae from copepods as well as the larvae and adults from infected *Clarias gariepinus* were fixed in 4% formaldhyde and passed in Polyvenol (Polyvenyl alcohol with phenol) for clearing and mounting.

(D) Immunological studies:

Blood samples from the experimental infected and control fish specimens were collected during the days of the experiment 3, 8, 12, 16, 20 and 30 days. Each blood sample was placed into two dry clean tubes. One tube contained 3.8% sodium citrate as anticoagulant and the other was used for serum preparation. The whole blood was used for determination of total and differential leuckocytic count (Luky, 1977), while the obtained serum was used for estimation of total protein (Marshall, 1989), albumin and globulin (Schaperclaus, 1992).

RESULTS

(A) Morphological criteria of the collected adult *Procamallanus laeviconchus:*

It is a small larviparous spirurid worm, the cuticle is thick with distinct transverse striations. The mouth opening is quadripapillated and its margin is provided with fine cuticular membrane. The buccal capsule is chitinous followed by oesophagus which is shorter in male than female and divided into short anterior muscular part and long posterior glandular one. The female is larger than the male. Uterus of gravid female is filled with first stage larvae. The tail is conical in shape. The tail of the male is short, curved ventrally, and provided with narrow alae. There are eight pairs of pedunculated precloacal papillae of which two small pairs surrounding the cloaca and three to four postcloacal pairs. Spicules are short, unequal and weakly sclerotized. The right one is slender and longer than the left one which is very short and difficult to be seen (Fig 1 A, B,C and D).

(B) Experimental infection of copepod crustacean *Mesocyclops leukarti:*

After ingestion by the copepods, the liberated first stage larvae 1 penetrate the gut wall to the haemocoel, and in the cephalothoracic area further development took place. The larvae increase in size and undergo two moults at temperature of 22-24°C for reaching the infective third stage larvae. The early full-developed third-stage larva liberated from the cuticle of the second moult was detected in a copepod crustacean *Mesocyclops leukarti* 6-8 days post- infection. About 50% of copepods were infected in the first day but due to the mortality, this rate decreased by the time when the third stage larvae were present. The intensity of infection ranged between 1-3 larvae per *M. leukarti*. Most of the infected *Mesocyclops leukarti* were seen in the bottom of the vessel, being less motile than uninfected ones. All *Mesocyclops leukarti* remained alive 8 days post infection and were used for the experimental infection of fish definitive host.

(C) Experimental infection of fish definitive host:

The fourth stage larvae were found at 12-16 days post infection. Single male specimen was recovered at 25 days post infection. A male and female specimens were recovered at 30 days post infection. During the development of *Procamallanus laeviconchus* in the definitive host, some morphological changes were observed of which the buccal capsule was enlarged, the glandular portion of oesophagus gradually increase in length

and become longer than the muscular one and conical processes on the tail tip of the larval stages becomes shorter and blunt.

Morphology and larval development:

1- First stage larva:

The body of the first-stage larvae is transparent, slender with slightly transversely striated cuticle (0.325- 0.393 mm in length and 0.022-0.029 mm in width). The head is rounded with a small dorsal cuticular tooth. Oral papillae are indistinct. The mouth is formed by a short, thin; feebly sclerotized tube. The oesophagus is undivided, without a distinct lumen. The posterior end of the oesophagus contains glandular tissue. The oesophagus leads to the intestine through a small valve. Intestine is wide light colored, with fine granulations. The tail is conical, slender, with a sharply pointed tip (Fig 2).

2- Second stage larva:

The second stage larvae were observed in the haemocoel of the copepods four days post infection. The body is still light coloured, larger in size than that of the first stage larva (0.396- 0.450mm in length and 0.032-0.036 mm in width). The cuticle is slightly transversely striated, the cephalic end is rounded, the mouth is formed by short fine tube opening into the oesophagus. The anterior end of the oesophagus is covered with thick, hyaline bell-shaped structure surrounded by several drop-like glandular formations.The posterior glandular portion of the oesophagus becomes longer but a distinct division between the two oesophagus parts is not yet apparent.The intestine is wide and straight The tail resembles that of the first-stage in that it is rather long and sharply pointed However, it is relatively shorter, representing 30-33% (Fig3)

3- Infective third stage larva in copepods:

The third stage larvae obtained on days 6 and 8 post infection (0.999 mm in length and 0.047mm in width). The mouth opening is rounded. The buccal capsule is almost colorless or slightly golden, elongate, thick-walled and continuous without a distinct separation into anterior and posterior portion. The buccal capsule leads to the oesophagus through a distinct oesophageal cup. The oesophagus is distinctly divided into an anterior, almost cylindrical muscular portion with a strong cuticular lining and a somewhat shorter posterior glandular one. Oesophagus leads to the intestine through a small valve. The intestine is wide, orange-brown in color and containing

numerous granules. The tail is conical, its tip bears four caudal processes (Fig.4).

4- Fourth stage larva from the definitive host:

The fourth stage larvae obtained at the 12-16 days post infection with striated cutical (1.33-2.06 mm in length and 0.067- 0.0113mm in width). Their morphology is very similar to that of the third one whereas the main differences being the markedly wide and longer buccal capsule, the size of the body is larger and longer glandular oesophagus than the muscular one. The intestine is straight and brown coloured. The tail is short and conical, with short and blunt caudal processes (Fig 5 and 6).

(D) Effect of the experimental infection of the larval stages and adults of *procamallanus laeviconchus* on the immune status of *Clarias gariepinus:*

The total leukocytic counts of the infected fish showed significant increase, neutrrophils, eosinophils and monocytes showed significant increase while lymphocytes showed significant decrease. Non significant changes occurred in basophils (Table 1). The total protein of the infected fish showed significant decrease along the days post infection. The albumin showed non-significant differences while the globulin showed significant increase in 3, 5, 20 25, and 30 days post infection (Table 2).

DISCUSSION

The result of the present study revealed that the nematode species *Procamallanus laeviconchus* was collected from stomach of *Clarias gariepinus* and this was in agreement with those reported by Negm El-Din (1987), Imam and El-Askalany (1990), Ramadan (1994), Abd El-Al (1996) and Shagar (1999). Others as Abu El-Ezz (1988), Imam *et al.*, (1991) and El-Nagger (1995) detected it from Egyptian *Synodonits shall, Barges bayad, Tilapia militia, Tilapia area* and *Clarias gariepinus* respectively.

The morphological criteria of collected species was similar as described by Bayils (1923), Imam (1971), Sahlab (1982), Negm El-Din (1987), Abu El-Ezz (1988), Imam and El- Askalany (1990), Ramadan (1994), Abd El-Al (1996) and Shagar (1999), with some differences which may be due to the methods of preservation and fixation used.

Regarding the experimental development of *Procamallanus laeviconchus,* only one copepod crustacean species(*Mesocyclops leukarti)* was infected and this agrees with Moravec (1974 and 1975) who used the

same copepod species for the life cycle of *Procamallanus cyathopharynx* and *Procamallanus laeviconchus*. Sinha (1988) used *Mesocyclops seocyclops leukarii* and *M. haylinus* as intermediate host of *Procamallanus spiculogubernaculus*. Thatcher *et al.* (1998) mentioned that copepod crustaceans representing an important source for transmitting several Camallaniid species. Moreover the specificity of fish nematode at the level of intermediate host is much broader than that of the definitive host which seems to be valid particularly for Camallanids Moravec (1994).

Concerning the morphology of the larval stage, the present investigation revealed that first and second stage larvae are similar to those of other *Procamallanus species*. The larvae are characterized by the presence of dorsal tooth, undivided oesophagus and the body is transparent and slender and these agreed with those reported by Moravec (1975), Moravec *et al.* (1995) and Moravec and Vargas-Vazquez (1996). The third stage larvae showed distinct differences other than *Procamallanus species* that the buccal capsule is continuous without a distinct separation into anterior and posterior portions and without spiral thickenings. These differences agree with Li (1935), De (1995) and Moravec *et al.* (1995) . At the same time these results disagree with Pereira *et al.* (1936) Fusco (1980), Moravec and Vargas-Vazquez (1996) .The conical processes or spines of the tail tip of the third stage larvae are typical of *Procamallanus species* which are similar to that described by Moravec *et al.* (1993) and Pereira *et al.* (1936) and disagree with that reported by Li (1935), Fusco (1980), De (1995), Moravec *et al.* (1995) Moravec and Vargas-Vazquez (1996).

The fourth stage larvae of *Procamallanus laeviconchus* recovered from the definitive host differ from conspecific third stage larvae principally in the buccal capsule which was enlarged, caudal processes became shorter, the body increase in size and the glandular part of oesophagus became longer than the muscular one. The structure of buccal capsule is rather similar to that reported by Moravec (1975) but differs from that of *P. rebecae* which having 10 spiral thickenings (Moravec *et al.* 1995) and the fourth stage larvae of *P. neocaballeroi* having 11 spiral thickening (Moravec and Vargas-Vazquez, 1996). The caudal processes of *Procamallanus laeviconchus* are shorter while the same larval stage of *P. pimelodus* has no caudal processes although these are present in the conspecific third stage larvvae (Moravec *et al.* 1993). In the present study the fourth stage larvae of *Procamallanus laeviconchus* recovered at 12-16 days post infection of *Clarias gariepinus* and this closely agrees with those recorded by Moravec

and Vargas-Vazquez (1996) who obtained the fourth stage larvae of *P. neocaballroi* at 12 days post infection of *Astyanax fasciatus.*

The present study revealed that *Procamallanus laeviconchus* adult female was obtained from *Clarias gariepinus* at 30 day post infection and male specimen 25 day post infection. These development seems to be faster than the development recorded by De (1995) who found that the last (fourth) moult of male and female larvae of *P. mystii* in the definitive host was at 37and 67 day post infection respectively.

Regarding the effect of the experimental infection of third, fourth stage larvae and adults of *Procamallanus laeviconchus* on the immune status of *Clarias gariepinus,* it was found that, the total leukocytic counts of the infected fish significant increased especially neutrrophils, eosinophils and monocytes while lymphocytes showed significant decrease. Non significant changes occurred in basophils. The results agree with Imam (1971) and Sopinska (1985) who reported these observations in Clarias and Carp infected with Cestode species. On the other hand Mackinnon (1993) and Ranzan- Paiva *et al.* (1997) reported non-significant differences in infected fish compared with the non infected one.

The total protein of the infected fish showed significant decrease along the days post infection. Albumin showed non-significant differences while the globulin showed significant increase in 3, 5, 20, 25, and 30 days post infection. These results agree with those reported by Boon *et al.* (1990), Hoglund *et al.* (1992) and Nielsen (1999) who recorded the same results in fish infected with different parasites. Moreover Awad (1992) mentioned that the total protein did not show any significant changes between the infected and apparently healthy fish

From the present study, it was concluded that, the experimental infection of *Clarias gariepinus* with the third stage larvae of *Procamallanus laeviconchus* causing immune suppression indicated by increasing of globulin and total leukocytic count.

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Table (1): Effect of *Procamallanus laeviconchus* larval stages and adults on total and differential leucocytic count of *Clarias gariepinus* fish specimens

Day P.i●	*TWCs* (x10mm3)*		*Neutrophils %*		*Eosinophils %*		*Basophils %*		*Lymphocytes %*		*Monocytes %*	
	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control
3 day P.i	21.75±0.25	19.75±1.06	29.5±2.12	28.5±0.71	10.5±2.12	9.5±0.71	5.5±0.71	5.5±0.71	48.5±0.71	51±0	6±0	5.5±0.71
5 day P.i	22.33±1.88	20.6±2.26	29.5±0.71	28.5 ±0.71	10.5± 2.12	9.5±2.12	5.5±212	5.5±0.71	48±1.41	51± 1.41	6.5±0.71	5.5±0.71
8 day P.i	32.9±1.27	19.5± 0.71	30.5±0.71	28± 0	11.5±0.71	9.5±0.71	6.5±0.71	5.5±2.12	45±1.41	51.5±2	6.5±0.71	5.5±0.71
12 day P.i	33.75±1.06	20.5± 2.12	32±0	28.5 ±2.12	12±0	9±0	6.5±0.71	5 ± 0	43±0	52±1.41	6.5±0.71	5.5±0.71
16 day P.i	31.25±2.47	19.9± 0.14	33.5±2.12	28±1.41	12.5±0.71	9.5±0.71	6.5±0.71	5.5±0.71	41±2.83	52±1.41	6.5±2.12	5±0
20 day P.i	26.9±0.14	20.7± 1.84	32.5±0.71	28.5±0.71	10.5±2.12	9.5±0.71	6±1.41	5±0	44.5±2.12	51.5±0.71	6.5±2.12	5.5±0.71
25 day P.i	26.8±1.69	20.4± 0.85	31.5±0.71	28±1.41	10.5±0.71	9.5±0.71	5.5±0.71	5.5±0.71	46.5±0.71	51.5±0.71	6 ± 0.71	5.5±0.71
30 day P.i	24.3±0.98	20.1± 1.27	30.5±0.71	28.5±0.71	10±1.41	9±0	5.5±0.71	5.5±0.70	48± 1.41	51.5±0.5	6 1±.41	5.5±0.71

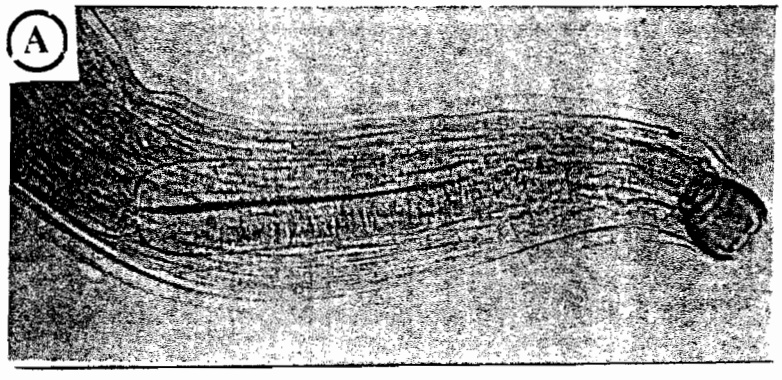
* Significant at P< 0.05 ●Post-infection

Table (2): Effect of *Procamallanus laeviconchus* larval stages and adults on total protein, albumin and globulin of *Clarias gariepinus* fish specimens.

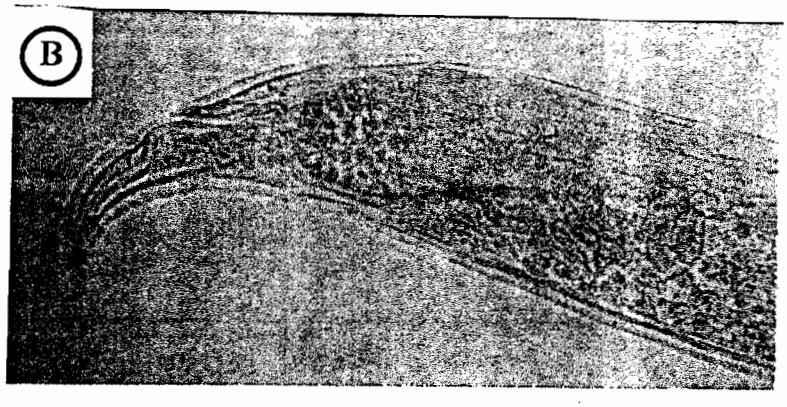
Days P.i •	*Total protein** *(gm / dl)*		*Albumin* *(gm/ dl)*		*Globulin**	
	Infected	**Control**	**Infected**	**Control**	**Infected**	**Control**
3rd day P.i.	3.45 ± 0.14	4.05 ± 0.01	1.16 ± 0.04	185 ± 0.04	2.29 ± 0.09	2.2 ± 0.06
5th day P.i.	3.36 ± 0.08	4.05 ± 0	1.05 ± 0.04	1.83 ± 0.01	2.31 ± 0.04	2.22 ± 0.01
8th day P.i.	3.2 ± 0.04	4.06 ± .01	1.06 ± 0.04	1.85 ± 0.03	2.14 ± 0	2.21 ±0.01
12th day P.i.	2.85 ± 0.08	4.06 ± 0.01	0.91 ± 0.06	1.84 ± 0.01	1.94 ± 0.03	2.22 ± 0
16th day P.i.	2.81 ± 0.14	4.04 ± 0	0.84 ±0.04	1.83 ± 0.01	1.97 ± 0.09	2.21 ± 0.01
20th day P.i.	3.66 ± 0.04	4.03 ± 0.01	1.12 ± 0.03	1.83± 0	2.45 ± 0.01	2.2 ± 0.01
25th day P.i.	4.07 ±0.03	4.03 ±0.03	1.35 ± 0.01	1.81 ±0.01	2.72 ± 0.01	2.22 ± 0.01
30th day P.i.	4.07 ± 0.01	4.06 ± 0	1.33 ± 0.01	1.85 ± 0.01	2.74 ± 0	2.21 ± 0.01

*Significant at P< 0.05

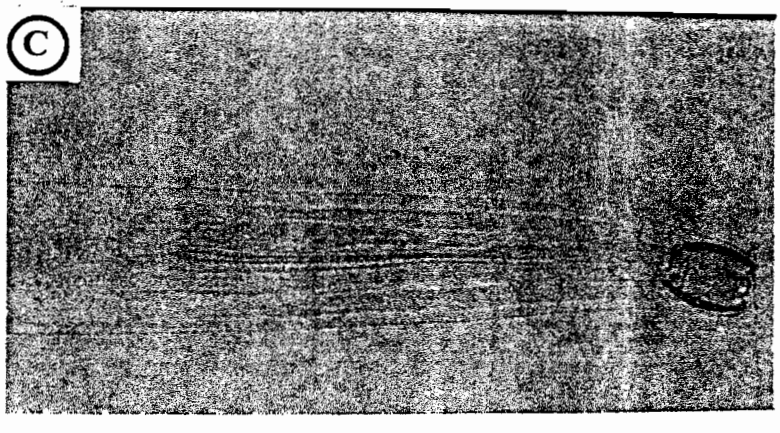
• Post-infection



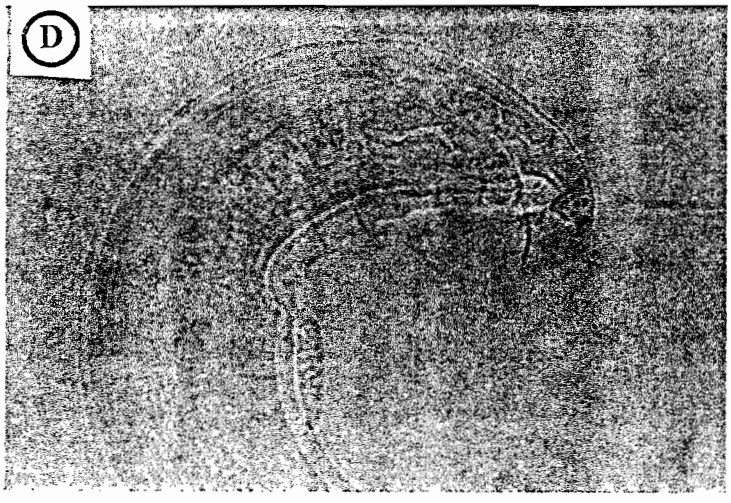
(Fig. 1A): Anterior end of *Procamallanus laeviconchus* female (x720).



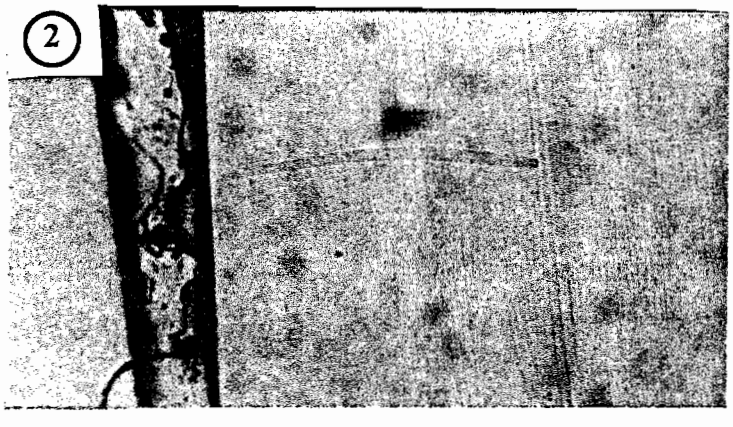
(Fig. 1B): Posterior end of *Procamallanus laeviconchus* female (x720).



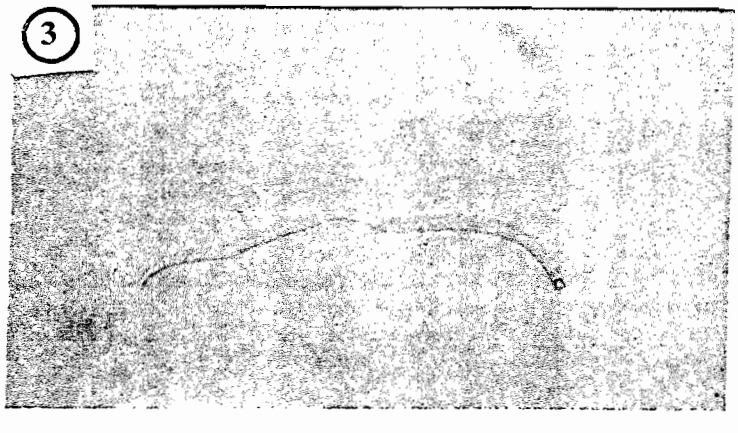
(Fig. 1C): Anterior end of *Procamallanus laeviconchus* male (x720).



(Fig. 1D): posterior end of *Procamallanus laeviconchus* male (x720).

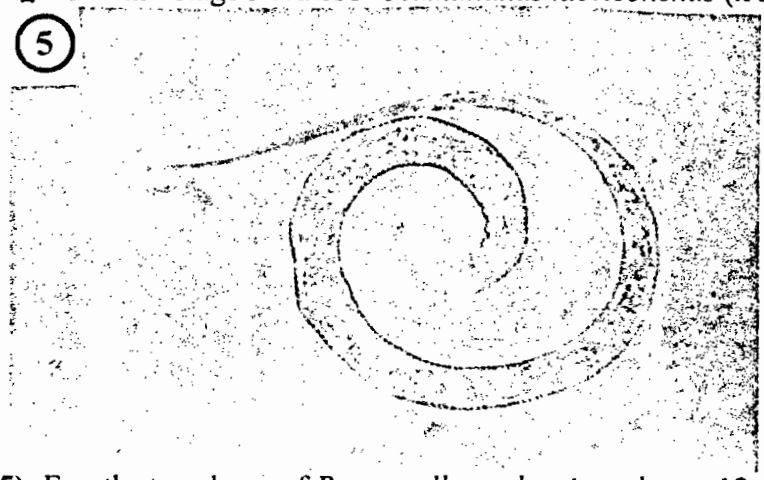


(Fig. 2): First stage larva of *Procamallanus laeviconchus* (x 720)



(Fig. 3): Second stage larva of *Procamallanus laeviconchus* (x 720)

(Fig. 4): Third stage larva of *Procamallanus laeviconchus* (x 360)



(Fig. 5): Fourth stage larva of *Procamallanus laeviconchus* at 12 day post infection of the definitive host (x 144).

(Fig. 6): Fourth stage larva of *Procamallanus laeviconchus* at 16 day post infection of the definitive host (x 144)