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LYMNAEA CAILLIAUDI AS A NEW SECOND INTERMEDIATE HOST OF ECHINOSTOMA CAPRONI, IN EGYPT

(With 2 Tables, 3 Plates and One Figure)

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قوقع ليمنيا كايويدى كعائل جديد للطور اليرقى المعدى (السركاريا المتحوصلة) لطفيل اكينوستوما كابرونى فى مصر

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تم فحص ثلاثة مائه وخمسين قوقع ليمنيا كايويدى وهو من قواقع المياه العذبة ذو الحجم المتوسط لمعرفة نسبة اصابته بالطور اليرقى المعدى لطفيل اكينوستوما كابرونى والذى قد تم تجميعه من المجارى المائية الضحلة بمنطقة أبورواش بالجيزة. ووجد أن نسبة اصابتها حقليا بالطور اليرقى المعدى (السركاريا المتحوصلة) داخل أنسجتها كانت ١٣،٧ % وقد تم أيضا تجميع هذا الطور المعدى. ثم تم عدوى مجموعتين (كل مجموعة تتكون من ثلاثة من صغار الحمام البلدى) بالسركاريا المتحوصلة المجموعة الأولى تم عدواها بعدد ستة فى حين المجموعة الثانية تم عدواها بخمسة عشرة سركاريا متحوصلة. وقد تركت مجموعة ثالثة تتكون من ثلاثة من صغار الحمام البلدى كضوابط. وقد أمكن الحصول على الديدان البالغة والغير بالغة لطفيل اكينوستوما كابورينى من أمعاء صغار الحمام البلدى من المجموعتين الأولى والثانية فقط. وكذلك تم وصف التغيرات المرضية التى حدثت نتيجة الأصابة المعملية بهذا الطفيل. وقد أسفرت هذه الدراسة أن قوقع ليمنيا كايويدى يعمل كعائل جديد للسركاريا المتحوصلة لطفيل اكينوستوما كابرونى فى مصر.

SUMMARY

Thirty hundred and fifty *Lymnaea cailliaudi* snails were collected from shallow irrigation canals at Abou-Rawash, Giza, Egypt, during the period from September to December, 1997. Encysted metacercariae of the *Echinostoma caproni* were collected from the internal organ and tissues of field infected *Lymnaea cailliaudi* with prevalence (13.7 %). Two groups (3 squabs each) of one week old (*Columba livia domestica*) were inoculated with 6 and 15 encysted metacercariae, respectively. The

third group (3 squabs) was kept as non inoculated controls. *E. caproni* adults were recovered from the ileum of both experimentally inoculated groups after prepatent period of two weeks. The histopathological lesions induced by *E. caproni* were studied in these experimentally inoculated squabs and compared with the non inoculated controls. The inoculated jujenum (2 WPI) showed dispersion of connective tissue of submucosa by oedematus fluid. Mainly, histopathological lesions were observed in inoculated ileum (4 WPI); necrosis and slaughing of epithelial lining and haemorrhagic areas in submucosa were seen. Inflammatory reactions with lymphocytic and heterophic infiltration in lamina propria were also noticed. Necrosis and disquamation of epithelium at the tip of villi were observed. Increase numbers and activation of goblet cells with vaculation of epithelial lining villi in the mucosa of the inoculated ileum was observed.

Key Words: *Lymnaea Cailliaudi, Echinostoma Caproni*

INTRODUCTION

Man is a subsidiary host for some species of Echinostomes in South and Far east Asia acquiring infection by eating raw snails containing encysted metacercariae (Huffman and Fried, 1990; Lee et al., 1990 and Chao et al., 1993). Although, Echinostomiasis is apparently unknown among human in Africa, there is a potential danger for infection due to wide distribution of the snails (Brown, 1994).

Kanev (1985) redisgnateted the species in the 37- collar spined *E. revolutum* complex into *E. caproni* and *E. trivolvis* instead to African echinostome; *E. liei* (Jeyarasasingam et al. 1972) and American echinostome; *E. revolutum* (Froelich, 1802). In Egypt, Jeyarasasingam et al. 1972 mention that field collected *Biomphalaria alexandrina* could act as first and second intermediate host of African echinostome; *E. liei* (*E. caproni*, Kanev, 1985). In USA, Fried et al. 1988 obtained *E. caproni* after experimental infection of chicks with encysted metacercariae obtained from *B. glabrata*. Recently, El-Bahy 1998, obtained *Echinoparyphium recurvatum* after experimental infection of ducks with encysted metacercariae collected from *Lanistes carinatus*.

The purpose of this study, was to investigate the possible role of *Lymnaea caillaudi* to act as second intermediate host of field infected snails for echinostomes metacercariae at Abou-Rawash, Egypt. More accurate identification of parasitic stage (EMC) which were collected

from infected snails was through developing them to mature worms in suitable experimental avian host. Furthermore, the histopathological effects of *E. caproni* in experimentally inoculated squabs was also studied.

MATERIAL and METHODS

1- Collection of snails:

A total number of 350 *Lymnaea cailliaudi* snail samples were collected from shallow irrigation canals from Abou-Rawash Giza, Egypt, during the period from September to December, 1997. Identification of the collected snails was done according to Brown (1994).

2- Examination of snails:

All the internal organs and tissues of *L.cailliaudi* were examined microscopically to collect the encysted metacercariae (EMC). Snail tissues containing EMC were collected and counted, until they were used for experimental infection. The dimensions of the EMC were measured.

3- Experimental infection of squabs:

Nine apparently healthy, one week old domestic squabs (*Columba livia domestica*) were reared conventionally in special cages. They were fed on maize and water. Their feces were examined daily for 2 successive days befor inoculation to ensure that they were parasite free. They were divided into three groups each formed of 3 squabs. The 1st and 2nd groups were experimentally inoculated, while the 3rd group was left as non-inoculated control. The 1st and 2nd groups were inoculated with 6 and 15 EMC, respectively. One squab was necropsied from each group at 2, 4 and 6 (WPI). The intestinal content and intestinal wall were carefully examined. The obtained worms were counted and fixed, stained and mounted according to Pritchard and Kruse, 1982. The permenant specimens were measured and identified according to Jeyarasasingam et al. (1972) and Mc-Donald (1981).

4-Histopathological examination:

Parts of small intestine of experimentally inoculated squabs (especially the parts showing macroscopic lesions) were separated, trimmed, fixed in 10 % neutral buffered formalin and prepared for histopathological examination according to Carleton (1967).

RESULTS

This study revealed that out of the examined 350 snails (*L. cailliaudi*) 48 (13.7 %) were infected with encysted metacercariae (EMC). The number of EMC infecting each snail varied from 3-9. Each encysted metacercaria was ovoid to rounded in shape (Fig. 1C & Plate 1A) measuring 140-159 by 128-143 (average 149 by 132) μ . It was surrounded by a double wall 12-15 (average 14) μ. in thickness. Outer transparent cyst wall about 8μ., inner opaque layer about 2μ . The cysts contained fully mature metacercariae. Excretory granules, acetabulum, oral sucker and collar spines were usually visible through cyst wall (Plate 1A).

Experimental oral inoculation of one week old squabs with EMC resulted in its development in their ileum (Table 1). Severing of squab from each inoculated group at 2 (WPI) revealed immature and mature worms of *E. caproni*. While, severing at 4 (WPI) revealed that mature worms only (Table 1). The remaining squabs from each group were free from *E. caproni* at 6 weeks post-infection. The worms were obtained from four inoculated squabs (2,4 WPI) with total incidence (42.85 %). The control squabs were free from *E. caproni* at 2, 4, 6, weeks at the same time. The obtained worms (Fig. 1A & Plate 1B) have the same characteristic features of *E. caproni* (Jeyarasasingam et al., 1972 and Mc-Donald, 1981). The worms were recovered from ileum of experimental inoculated squabs. The dimensions of 10 recovered specimens were illusterated in (Table 2). Anterior collar (Fig. 1B) bearing 37 spines, 5 corner spines on ventral lobes of cephalic collar. The dimensions of the collar and corner spines were 54 ~ 83 (average 73) μ. and 72 ~ 85 (average 80 μ. long, respectively.

Macroscopic lesions of the experimentally inoculated squabs were evident in the form of dilated ileum and haemorrhagic areas. Microscopically, a marked increase in numbers and activity of goblet cells in the jujenum (2 WPI), (Plate 2B) together with hyperactivity of mucous secreting glands (Plate 2B). Dispersion of connective tissue of submucosa by oedematus fluid was also observed (Plate 2C). The normal architecture in the jujenum of the control squabs is shown in Plate 2A.

Mainly histopathological lesions were observed in the inoculated ileum (4 WPI) of experimentally inoculated squabs. Necrosis and slaughing of the epithelial lining and haemorrhagic areas in submucosa

were observed (Plate 3A&B). Inflammatory reactions were also noticed with lymphocytic and heterophilic infiltration in lamina propria (Plate 3C). Increase numbers and activation of goblet cells with vaculation of epithelial lining the villi. A small area of necrosis and disquamation of epithelium at the tip of villi were observed (Plate 3E). The normal architecture of the ileum of the control squabs is shown in Plate 3D.

DISCUSSION

The present findings revealed that *L. cailliaudi* is a new second intermediate host of *E. caproni* in Egypt. Several species of the fresh water snails, such as *Vivipara vivipara, Vivipara malleatus* and *Lymnaea japonica* are known to serve as the second intermediate host of echinostome parasites (Soulsby, 1978 and Lee et al., 1990).

Biomphalaria alexanderina, is naturally known as first and second intermediate host of *E. caproni* in Egypt (Jeyarasasingam et al. 1972). In addition, *Biomphalaria glabrata* have been proved experimentally to act as second intermediate host (Jeyarasasingam et al. 1972 and Ursone & Fried 1995). Various fresh water snails; such as *Lymnaea stagnalis, L. peregra* and *Lanistes carinatus* were commonly reported as first and second intermediate host of other echinostomes (*Echinoparyphium spp.*) Drageneva and Kanev (1983), Mc Carthy (1990) and El-Bahy (1998), respectively.

Echinostome cercariae were stimulated to encyst either by chemosensory function or attracted by chemical substance produced by second intermediate host snails (Fried & Fujino, 1987 and Fried & King, 1989). However, Lee et al., (1990) recorded that the second intermediate host provides only the space for encystment of the cercariae. This indicates that spectrum of the snail to act as second intermediate hosts is wider than the first. This study, demonstrated that, the ecological conditions prevailing inside *L. cailliaudi* were favourable for encystement of *E. caproni* cercariae in its tissues. This finding agreed with the same observation of Jeyarasasingam et al. (1972); Lee et al., (1990) and El-Bahy (1998). They proved that *B. alexandrina* act as first and second intermediate host for *E. caproni* and *E. hortense*. The latter author recorded that *Lanistes carinatus* act as first and second intermediate host for *Echinoparyphium recurvatum*. Furthermore, this study added the most important snail, *L. cailliaudi* for the first record as a second intermediate host for African echinostome; *E. caproni*.

The obtained flukes from the intestine of experimentally inoculated squabs with EMC were morphologically identical to *E. caproni* described by Jeyarasasingam et al. (1972); Mc donald (1981); Kanew (1985) and Christensen et al. (1989). The recovered number of flukes was relatively small in comparison with the number of inoculated EMC. This result could be due to some losses in the number of EMC during inoculation, reduced susceptibility of the bird species or aging of some EMC. Similar observation was reported by El-Bahy (1998).

Histopathological observations in the present study, showed certain similarities to those previously described for *E. caproni* in the mouse intestine (Bindseil and Christensen, 1984) specially gut dilutation, cellular infiltration and dispersion of connective tissue of submucosa. Extensive haemorrhagic zones in the lamina propria and decrease numbers of goblet cells previously described by Fried & Nelson (1978) and Kim and Fried (1989) were not reported in this study. The differences in this observations in the histopathological lesions may be due to dose of inoculation or the experimental host. In this study, 6 and 15 encysted metacercariae were inoculated to squabs, but Kim and Fried (1989) inoculated 400 (EMC) for chicken.

REFERENCES

Bindseil, E. and Christensen, N.O. (1984): Thymus independent crypt hyperplasia and villous atrophy in the small intestine of mice infected with the trematode *Echinostoma revolutum*. Parasitol. 88: 431-138.

Brown, D.S. (1994): Fresh water snails of Africa and their medical importance. 2nd Ed. Taylor and Francis, 57-67.

Carleton, H.N. (1967): Carleton's histological technique fourth Ed., Oxford, Univ. Press, New York, Toronto.

Chao, D. Wang, L.C. and Huang, T.C. (1993): Prevalence of larval helminths in fresh water snails of Kinmen Island. J. Helminthol. 67: 259-264.

Christensen, N.; Fried, B. and Kanev, I. (1989): Taxonomy of the 37 collar-spined *Echinostoma* (Trematoda: Echinostomatidae) in studies on population regulation in experimental rodent hosts. Angewandte Parasitologie.

Drageneva, N. and Kanev, I. (1983): Antigen similarity between rediae and cercariae of *Echinoparyphium aconiatum* Dietz, 1909 (Trematoda: Echinostomatidae) and their intermediate hosts. Khelmintologiya, 16: 29-36.

El-Bahy, N. M. (1998): Parasitological aspects of *Lanistes carinatus* (Olivier, 1804, Cyclostoma) snails and their role in transmission of parasitic diseases to animal and man. Assiut Vet. Med. J., 38 (76): 282-306.

Fried, B. and Fujino, T. (1987): Argentophilic and scanning electron microscopic observations of the tegumentary papillae of *Echinostoma revolutum* (Trematoda) cercariae. J. Parasitol. 73: 1169-1174.

Fried,B. and King, B.W. (1989): Attraction of *Echinostoma revolutum* cercariae to *Biomphalaria glabrata dialysate*. J. Parasitol. 75: 55-57.

Fried, B. and Nelson, P.D. (1978): Host parasite relationships of *Zygocotyle lunata* (Trematoda) in the domestic chick. Parasitol. 77: 49-55.

Fried, B. Donovick, R.A. and Emili, S. (1988): Infectivity, growth and development of *Echinostoma liei* (Trematoda) in the domestic chick. Inter. J. for Parasitol. 18: 413-414.

Froelich (1802): Cited by Mc Donald (1981).

Huffman, J.E. and Fried, B. (1990): Echinostoma and Echinostomiasis. Advances in Parasitology. 29: 215-269.

Jeyarasasingam, U.; Heyneman, D.; Lim. H.K. and Mansour, N. (1972): Life cycle of a new Echinostome from Egypt, *Echinostoma liei* sp. n. (Trematoda: Echinostomatidae). Parasitol. 65: 203-222.

Kanev, L. (1985): On the morphology, biology, ecology and taxonomy of the *Echinostoma revolutum* group (Trematoda: Echinostomatidae). Ph. D. Thesis, Bulgarian Acad. Scien., Sofia, Bulgaria.

Kim, S. and Fried, B. (1989): Pathological effects of *Echinostoma caproni* (Trematoda) in the domestic chick. J. Helminthol. 63: 227-230.

Lee, S.H.; Chai, J.Y.; Hong, S.T. and Sohn, W.M. (1990): Experimental life history of *Echinostoma cinetorchis*, Korean J. Parasitol. 28: 39-44.

Mc Carthy, A.M. (1990): The influence of second intermediate host dispersion pattern upon the transmission of cercariae of *Echinoparyphium recurvatum*. Parasitol. 101: 43-47.

Mc Donald, M.E. (1981): Key to trematodes reported in water fowl. Resource publication 142. Washington, D.C. United states.

Pritchard, M.H. and Kruse, G.O.W. (1982): The collection and preservation of animal parasites. Univ. of Nebraska Press, Nebraska, USA.

Soulsby, E.J.L. (1978): Helminths, arthropods and protozoa of domesticated animals 6th Ed. Tindall and Cassell Ltd, London.

Ursone, R.L. and Fried, B. (1995): Light microscopic observations of *Echinostoma caporoni* metacercariae during in vitro excystation. J. Helminthol. 69: 253-257.

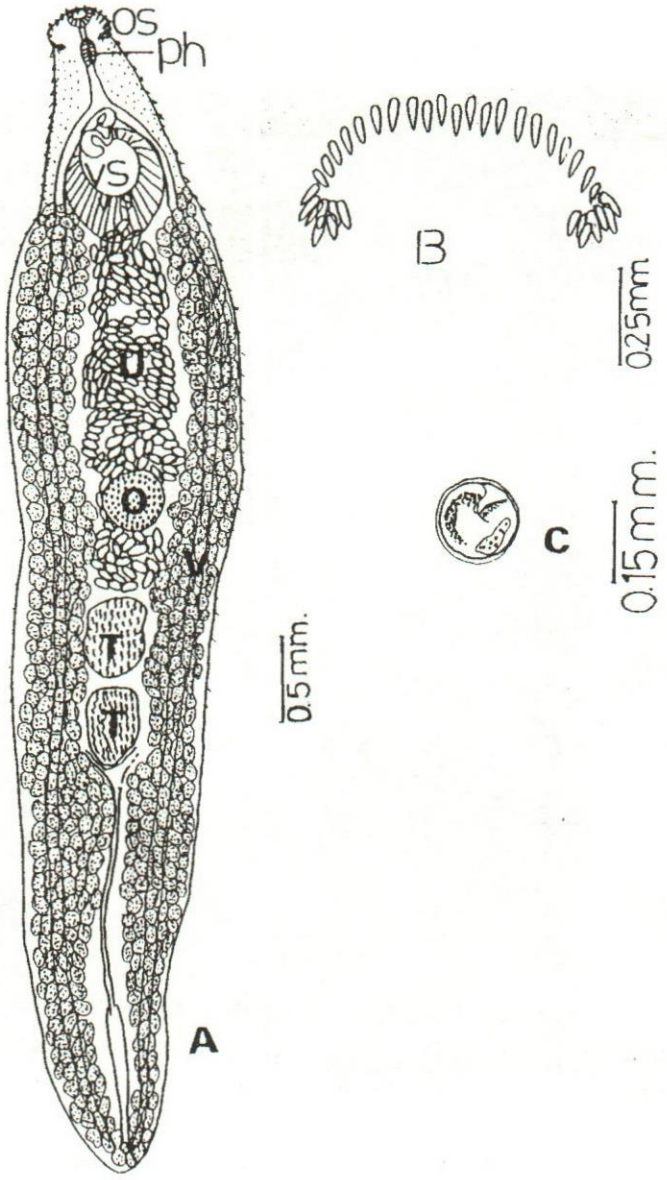
Table 1: Experimental oral inoculation of one week old squabs with EMC.

Squabs groups (3 each)	Source of EMC and inoculation dose	Number of the recovered worms		
		2 WPI	4 WPI	6 WPI
First group (group A)	Field collected *L. cailliaudi* snails 6 / squab	4 W / S 2 immature and 2 mature	3 W / S mature	Negative
Second group (group B)	Field collected *L. cailliaudi* snails 15 / squab	9 W / S 3 immature and 6 mature	11 W / S mature	Negative
Non- inoculated Control group (group C)	Non- inoculated	--------	-------	-------

EMC : Encysted metacercariae
WPI : Week post-inoculation
W/S : Worms / squab

Table 2: Dimensions of *Echinostoma caproni* in mm. (obtained from 10 specimens).

Aspect	Minimum	Maximum	Average
Total length	5.5	7.8	6.6
Max. width	1.1	1.5	1.3
Head collar	0.72 by 0.36	0.77 by 0.38	0.73 by 0.36
Collar spines	0.0 54	0.083	0.073
Corner spines	0.072	0.085	0.080
Pharynx	0.18 by 0.16	0.19 by 0.16	0.18 by 0.16
Oesophagus	0.54 by 0.24	0.58 by 0.26	0.55 by 0.25
Oral sucker	0.32 by 0.12	0.38 by 0.13	0.36 by 0.12
Ventral sucker	0.60 by 0.56	0.77 by 1.0	0.73 by 0.96
Ovary	0.36 by 0.38	0.48 by 0.51	0.43 by 0.47
Testes			
Ant. Testis	0.42 by 0.36	0.48 by 0.51	0.47 by 0.47
Post. Testis	0.54 by 0.33	0.57 by 0.51	0.55 by 0.49
Eggs	0.120 by 0.060	0.128 by 0.064	0.126 by 0.063

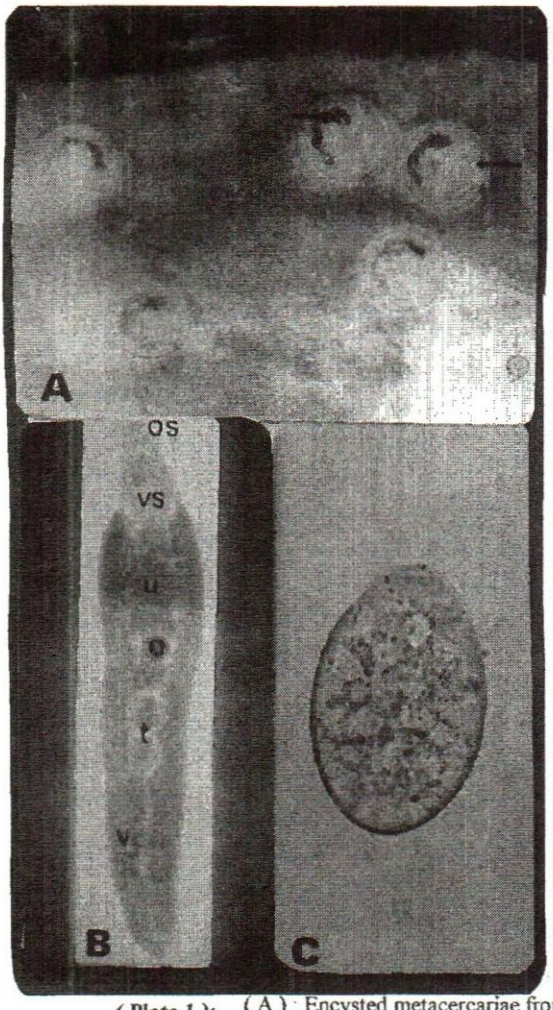


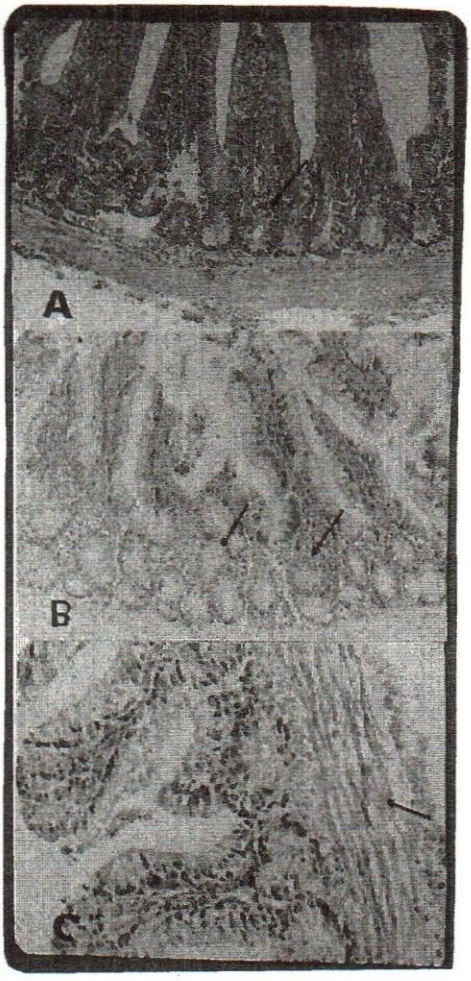
(**Fig. 1**) : An adult *Echinostoma caproni* recovered from ileum of squabs experimentally inoculated with metacercarial cysts.

(A) Whole worm : os., oral sucker ; ph., pharynx ; o., ovary ; vs., ventral sucker ; u., uterus ; v., vitelline follicles ; t., testis.

(B) Arrangement of spines around the anterior collar.

(C) : Encysted metacercariae.





(Plate 1): (A) : Encysted metacercariae from field collected *Lymnaea caillaudi* snails (x 100).

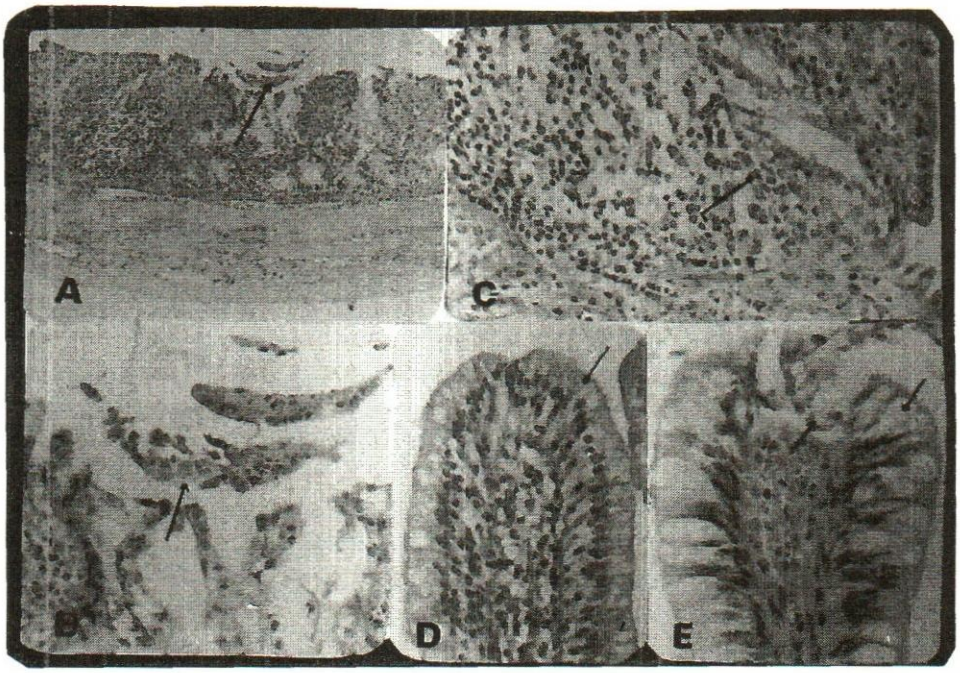
(B) : Adult worm of *Echinostoma caproni* after experimentally inoculated squabs (2 week p.i.).

(C) : Egg of *Echinostoma caproni* (x 400).

(Plate 2): (A) : Jujenum of control group squab (H. & E., x100).

(B) : Jujenum showing increase in number and activity of the goblet cells and mucous secreting glands. (H. & E., x100).

(C) : Jujenum showing oedema in the submucosal layer (H. &.E., x100).



(Plate 3):

(A) : Ileum showing necrosis and slaughing of the epithelium lining the villi (H. & E. x 100).

(B): Higher magnification of previous photo to show the slaughing of epithelium (H. & E., x 400).

(C): Ileum showing infilteration of lamina propria by inflammatory cells ; heterophyles and lymphocyte (H. & E., x 400).

(E): Ileum showing increase the number and activation of goblet cell with vaculation of epithelium, noticed the disquamation of epithelium of the tip of villi (H. & E. x 400).

(D): Ileum of control group squab. (H. & E., x 400).