Tehran University of Medical Sciences Publication
http:// tums.ac.ir

Iran J Parasitol

Open access Journal at
http:// ijpa.tums.ac.ir

Iranian Society of Parasitology
http:// isp.tums.ac.ir

Short Communication

Experimental Life Cycle of *Hypoderaeum conoideum* (Block, 1872) Diez, 1909(Trematoda: Echinostomatidae) Parasite from the North of Iran

Hekmat AZIZI, *Ali FARAHNAK, Iraj MOBEDI, MohamadBagher MOLAEI RAD

Dept. of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Received 20 Jun 2014
Accepted 12 Oct 2014

Keywords:
Echinostomatidae, *Hypoderaeum conoideum*, life cycle, *Lymnaea palustris*, Cercaria

***Correspondence**
Email:
farahnak@tums.ac.ir

Abstract

Background: Human *Echinostomiasis* is an intestinal disease caused by the members of family *Echinostomatidae* parasites. The aim of present research was to identify echinostomatidae cercariae emitted by *Lymnaea palustris* snails from Mazandaran province in the north of Iran based on the morphological and morphometrical characteristics of the different stages of experimental parasite life cycle.

Methods: Echinostomatidae cercariae were collected from *L. palustris* (Gastropoda: Lymnaeidae) of the north of Iran. To collect metacercaria, 50 healthy snails were infected with cercariae experimentally (50 cercariae for each). To obtain the adult stage, 9 laboratory animals (3 ducks, 2 rats, 2 mice and 2 quails) were fed with 60 metacercaria for each. To identify parasite, the different stages of worm were examined using light microscope and then the figures were draw under camera Lucida microscope and measures were determined.

Results: Averagely, 15metacercaria were obtained from each snail that had been previously exposed with cercariae. Ducks presented worm eggs in feces after 10-15 days post-infection. Intestinal worms were collected and identified as *Hypoderaeum conoideum* on the bases of figures and measures of cephalic collar, the number of collar spine, suckers diameter ratio, testes arrangement, etc.

Conclusion: *H. conoideum* cercariae and adult worm are described. This is the first report of the different stages of the experimental life cycle of this parasite in Iran.

Introduction

Hypoderaeum conoideum is the member of family *Echinostomatidae* that occurs in the posterior part of small intestine of the ducks, geese, swans, wild aquatic birds and human (1-3). Miracidia of *H. conoideum* develops almost in three weeks in the egg in environment and release in water. The first intermediate hosts are *Lymnaea. pere-*

gra, L. stagnalis, L. palustris and *L. ovate*. After development of Miracidia to sporocyst and redia, cercaria escapes from snail. Cercaria enters either same snail or others snails as second intermediate host (4-12). Definitive host is infected by feeding infected snails. Although *H. conoideum* is a common parasite that has been reported in many studies in the world, but no research has been found that surveyed the development of cercaria to adult stages in Iran (13-16).

The purpose of present paper was to identify echinostomatidae cercariae emitted by *L. palustris* snails that were collected from Mazandaran Province in the north of Iran based on morphological and morphometrical features of the different stages of experimental parasite life cycle.

Materials and Methods

Isolation of the infected snail and collecting cercariae

L. palustris (1-2 cm) was collected from the north of Iran (Mazandaran Province) and were transferred to the Helminthology Laboratory of the School of Public Health, Tehran University of Medical Sciences. Snails were plated for isolating infected snails. Five snails per petri containing 10 ml dechlorated water were kept for 24 h under standard laboratory condition. After this duration, petries were controlled under loop to observe cercariae. Snails of positive petries isolated individually to identify which snail excretes cercaria. The cercariae were treated by neutral red 0.1%, examined using light microscope, figures were drawn under camera Lucida microscope, and measures were determined as alive.

Infecting of the second intermediate host with cercariae and recovery metasercariae

To obtain the metacercaria stage, each of 50 healthy snails (*L. palustris* from the north of Iran/ *L. peregra* from Tehran University Campus), which was cercaria free after 1-2 months keeping in laboratory, was exposed with 50 cercariae (that had been previously shed from first intermediate host), in a Petri containing 10 ml water and was kept in room temperature for 48 h. In the some cases, snails were crushed and metacercariae were investigated and counted in the smooth tissues of them under the loop. Crushed materials were searched to observe sporocyst and redial stages of parasite however, due to non-specificity; the results were not published here.

Infecting of the laboratory reared animals with metacercariae

To recovery the adult stage, the following laboratory animals were fed with 60 metacercariae, 2-7 days, for each:

a) 3 one-week-old ducks, b) 2 twelve-weeks-old mice(*Balb/c*), c) 2 twelve-weeks-old rats(*Ratusnorvegicus)*, d) 2 six-weeks-old quails (*Coturnixypsilophora*).

To recognize parasite, the figures of staining adult worms were draw under camera Lucida microscope and measures estimated.

Results

Morphology and morphometry of cercaria

The cercariae discharged intermittently throughout the day. They typically swim near the bottom of the vessel. They swim actively by vigorous movements of the tail and during swimming; the body is bent ventrally and contracted. The cercariae were survived for 24 hours. The cercaria has a weak-developed head collar bearing 52 inconspicuous spines that Dorsal-spines seem arranged in a single row. Remaining arranged in double row (Fig.1, B). Body surface armed with small spines arranged in transverse rows. Cuticle is bearing spines on anterior and lateral margins as far as level of acetabular and on posterior margin of body. Oral sucker circular, smaller than acetabulum and located terminally or subterminally. Acetabulum is highly prominent, situated posterior to middle of body. Digestive system consists of mouth, short prepharynx, an elongate pharynx and a large slender esopha-

gus. Esophagus is divided just anterior to acetabulum into intestinal ceaca extending to posterior end of body. The totals of coarse excretory granules are 2x220 (Fig.1, c). At least 2x15 flame cells present but capillaries are very difficult to observe. Anterior margin of body with apertures of 6 narrow ducts which extend to posterior margin of oral sucker before becoming too difficult to trace. Paraoesophageal gland cells (n=16-17) are located around esophagus. Excretory bladder consists of two short, wide chambers that are connected by a narrow canal. Large primary excretory ducts arising from anterior chamber; ducts initially narrow but widening at level of acetabulum, connecting as far as pharynx and forming anterior loop by lateral to posterior margin of oral sucker before passing posteriorly as secondary ducts. Following a slightly sinuous course almost to posterior end of body, forming posterior loops at this level, and then passing interiorly to posterior level of acetabulum where bifurcation into anterior and posterior collected ducts located (Fig.1, A). Table 1 shows the measures of cercaiae obtained from *L.palustris*.

Morphology of metacercaria stage

Averagely 15 spherical metacercaria (150×150 micron) were obtained from each snail that had been previously exposed with 50 cercariae. Metacercaria were nearly spherical. The cyst wall is smooth, transparent and comprising two layers; outer layer is tough and inner layer is narrow. Often of cercariae encysted in digestive glands (Fig. 2).

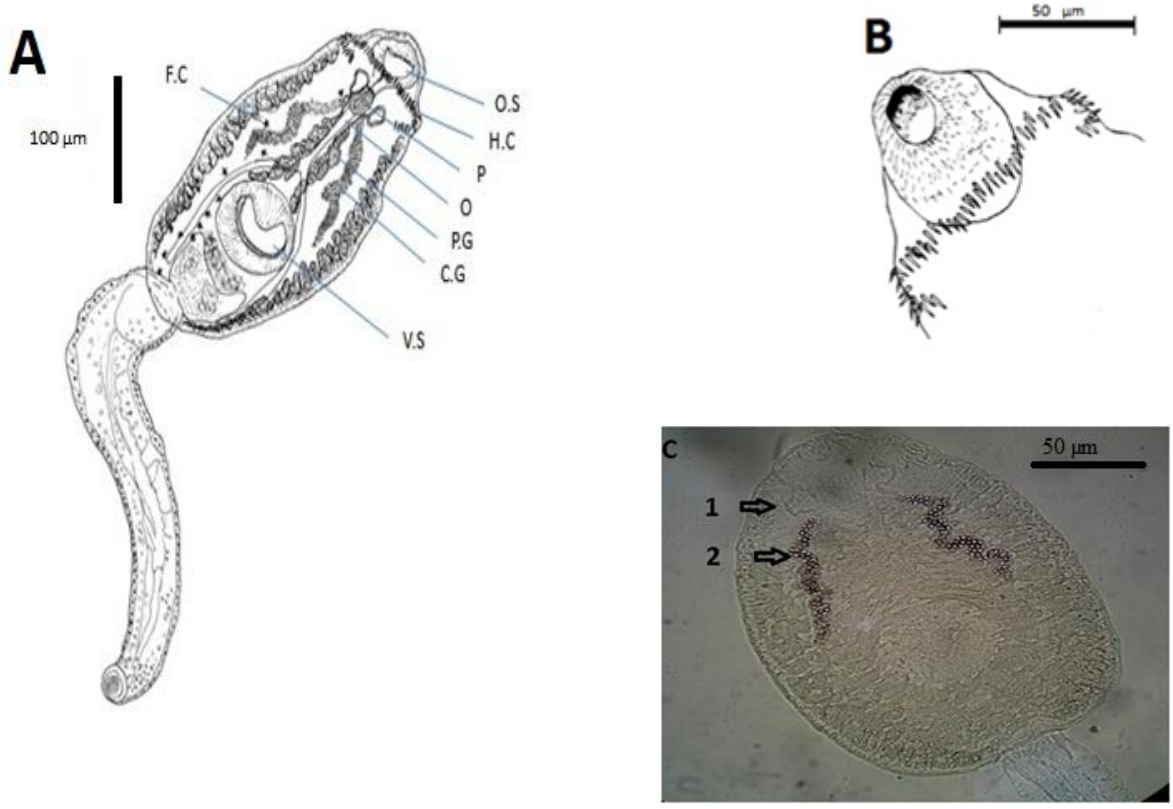

Fig. 1: A, Drawing picture of *Hypoderaeum conoideum* sucker; B, Drawing picture of *Hypoderaeum conoideum* spined collar arrangement; C, Photo picture of *Hypoderaeum conoideum* cercaria with simple loop 1) and coarse granules 2)

Table 1: Measurements (in micron) of the Cercariae (Larval stage) of *Hypoderaeum conoideum* Obtained from *L. palustris*

	Specimen 1	Specimen 2	Mean
Body length	297	322	310
Body width at acetabular level	127	169	145
Body width at cephalic level	68	108	88
Ventral sucker length	59	72	69
Ventral sucker width	57	81	69
Oral suckerlength	46	35	40.5
Oral sucker width	39	51	45
Sucker distance	129	144	136.5
Pharynxlength	25	29	27
Pharynxwidth	13	18	15.5
Oesophagus length	73	64	68.5
Cecae length	135	110	72.5
Tail length	322	381	351.5
Tail width at anterior end	51	64	57.5
Tail width at posterior end	33	35	34
Excretory granules	220×2	220×2	220×2
Flame cell	15	15	15
Paraoesophageal gland cells	18	16	17

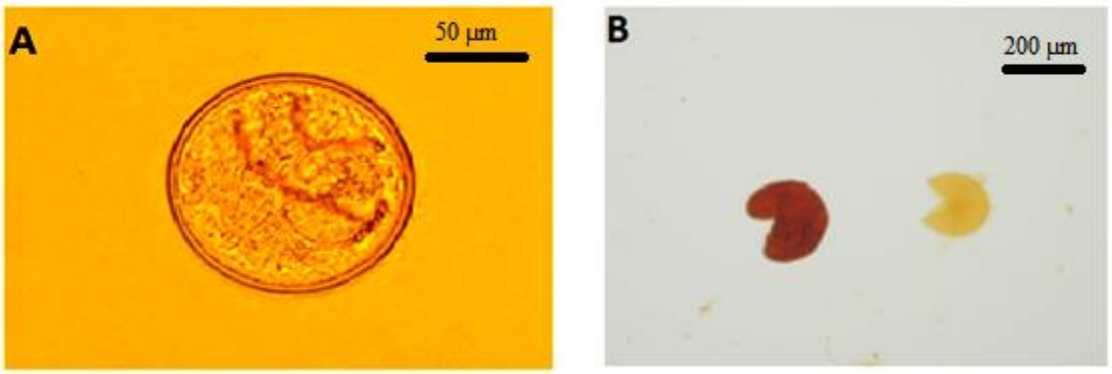

Fig. 2: A, Spherical *Hypoderaeum conoideum* metacercaria picture; B, Excysted metacercaria of *H. conoideum* with Red-brown juvenile larva and yellow ruptured cyst

Morphology and morphometric of adult-parasite

Stool examination revealed parasite egg (100×50 micron) in ducks on the 10th post infection day. From three infected ducks (one duck dead during 3rd day infectivity), after 10-15 days, 15 *hypoderaeum* were collected from small intestine. The body of *H. conoideum* is elongated. The anterior part of body covered with minute spines extending to half-point of ventral sucker on ventral side and to half-point to the mid-posterior on the dorsal side. The collar is poorly developed with 52 spines in two rows (on lateral side two rows exist is more sensitive). Ventral sucker is placed in the first sixth of the body and is bigger than oral sucker and sucker diameter ratio is 1:4. Digestive system includes short and muscular pharynx. Esophagus bifurcating is in front of the ventral sucker. The two intestinal cecae extend

to near the posterior end of the body. The cirrus and genital sac are well developed. Testes are arranged in tandem and are slightly lobulated that contiguously located in the posterior half of the body. The cirrus-sac is club-shaped reaching back almost to the posterior margin of the ventral sucker. Ovary is located in front of the testes. Linear uterus is located between oviduct and ventral sucker and containing many eggs. Vitellarium follicular is laterally extending from about just behind the posterior end of ventral sucker to near the posterior extremity (Fig.3). Table 2 shows the measures of adult worm were obtained from ducks and Table 3 shows the Figures abbreviations. Other animals (Balb/c, *Rattus norvegicus* and *Coturnix ypsilophora*) fed with metacercariae were parasite free in autopsy after 1-2 months post infection.

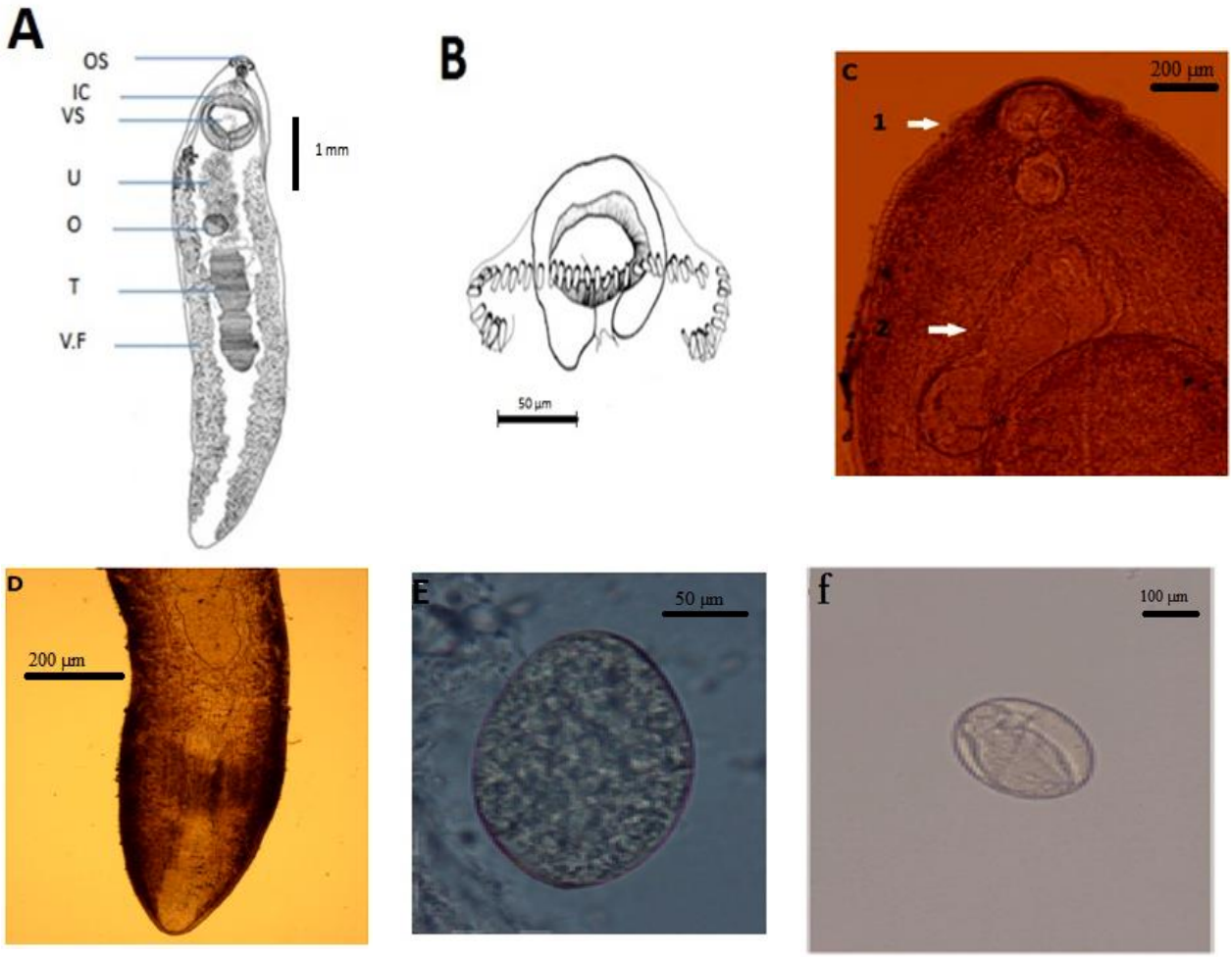

Fig. 3: A, Drawing picture of *Hypoderaeum conoideum* adult parasite in ventral view sucker; B, Drawing picture of *Hypoderaeum conoideum* adult parasite with spined collar arrangement in anterior portion; C, Photo Picture of Anterior portion of *Hypoderaeum conoideum* adult parasite with (1- collar and 2- cirrus sac) ; D, Photo Picture of Posterior portion of *Hypoderaeum conoideum* adult parasite with testis and excretory duct;E, Photo Picture of *Hypoderaeum conoideum* egg; F, Photo Picture ofMiracidium in *Hypoderaeum conoideum* egg

Abbreviations of figures

F.C	Flame cell	O.S	Oral sucker	H.C	Head collar	P	Pharynx
O	Oseophagus	E.G	Excretory glands	V.S	Ventral sucker	I.C	Intestinal cecae
U	Uterus	T	Testis	V.F	Vitellarium follicular	P.G	Paraoseophageal gland cells

Table 2: Measurements (in micron) of the *Hypoderaeum conoideum* (Adult stage) Obtained from Experimentally Infected Ducks

	Specimen 1	Specimen 2	Mean
Body length	7340	8670	8005
Body width at acetabular level	1000	1160	1080
Body width at testis level	1320	1410	1365
Pharynx length	146	154	150
Ventral sucker length	970	910	940
Ventral sucker width	730	730	730
Oral suckerlength	240	122	181
Oral sucker width	233	326	279.5
Ventral sucker- oral sucker distance	414	422	418
Anterior end- ventral sucker distance	693	816	754.5
Ventral sucker- anterior testis distance	1730	2100	1915
Posterior testis- Posterior end distance	2700	3340	3020
Anterior testislength	693	1060	876.5
Anterior testis width	530	548	539
Posterior testis length	877	1000	938.5
Posterior testis width	306	448	377
Cirrus sac length	500	506	503
Ovary length	380	387	383.5
Ovary width	280	285	284.5

Discussion

Larval stage (Cercaria)

Our cercaria specimens are *H. conoideum* based on published key (17). The main diagnostic feature is the number and arrangement of collar spine. The range of collar spine is 47-54 and arranged in a double row (9, 18). Diaz et al. reported 47-52 collar spines that are in a single row (19). Faltynkova et al. recorded 46-55 collar spines that the dorsal spines were in a single row (17). The number of collar spine in our observation is 52 that dorsal spines are in a single row and remaining spines arranged in double row. The inconsistency about spines row is not clear but it probably is visual problem. The reported data about number of flame cells are also different. Diaz et al. described at least 20 flame cells for each side of the body (19). Jurlova presented 19 pairs (20) and Wesenberg-Lund reported 13 flame cells (21). In the present study, at least 15 flame cell per hemi-body were observed and the location of them was shown.

Adult stage

Our adult specimens, which obtained from duck, are *H. conoideum* on the bases of following characteristics: Cephalic collar is weakly developed. The mean number of collar spine is 52. Suckers are close together and their diameter ratio is 1:4, Testes are arranged in tandem and are located in the posterior half of the body. These findings of the current study

are consistent with those of Toledo et al. who described the morphology of the obtained adult flukes from naturally infected ducks. He recorded 47-53 collar spines and a sucker diameter ratio 1:4 (22). Hosseini et al. recorded *H. conoideum* from Gray lag goose in the north of Iran (15) and Albert et al. reported *H. conoideum* from waterfowl in southwest Texas (13). Donald et al and Farias et al. reported this species from Florida and Mexico respectively (14, 16). These authors did not describe the morphology of the obtained adult flukes (13-16). Khan et al. explained the adult of *H. conoideum* but one major criticism of his study is that he did not show the number and arrangement of cephalic collar spine (23). Toledo et al. in a detailed study described reproductive system of *H. conoideum*and presented several characteristics such as body dimensions and sucker ratio to distinguish *H. conoideum* from other species with similar number of collar spine (22).Former studies are shown rather similar results in relative to range of collar spine, 47-54, for *H. conoideum*, but some authors for instance: Rees and Biverley-Burton respectively reported 43-45 and 47-49 (24, 25). Collar spines have been arranged in a double row (22, 24) but in this experiment, we observed dorsal spines were in a single row and remaining spines were in a double row. The reason of this difference is not clear but it may refer to illusion.

Conclusion

We identified the cercaria and adult worm specimens as *H.conoideum* and this is the first report of the different stages of the experimental life cycle of this fluke in Iran.

Acknowledgments

This work was supported by the Research grant # 18186 from the Tehran University of Medical Sciences. We are deeply grateful to Dr. A.B. Mansourian for review of snail samples. The authors declare that there is no conflict of interests.

References

1. Toledo R, Esteban JG, Fried B. Current status of food-borne trematode infections. Eur Clin Microbiol Infect Dis. 2012; 31:1705-18.
2. Chai JY, Shin EH, Lee SH, Rim HJ. Food-borne Intestinal Flukes in Southeast Asia. Korean J Parasitol. 2009; 47:69-102.
3. Yousuf MA, Das PM, Anisuzzaman F, Banowary B. Gastro-intestinal helminths of ducks: Some epidemiologic and pathologic aspects. J Bangladesh Agril Univ. 2009; 7(1):91-7.
4. Fried B. Biology of Echinostomes Except Echinostoma. Adv Parasit. 2001; 49:65-308.
5. Vayrynen T, Siddall R, Valtonen T, Taskinen J. Patterns of trematode parasitism in Lymnaeid snails from northern and centeral finland. Ann Zool Fennici. 2000; 37:189-99.
6. Haas W, Körner M, Hutterer E, Wegner M, Haberl B. Finding and recognition of the snail intermediate hosts by 3 species of Echinostome cercariae. Parasitology. 1995; 110(2):133-42.
7. Toledo R, Muñoz-Antolí C, Perez M, Esteban JG. Encystment sites of Metacercarae of *Hypoderaeum conoideum* (Trematoda: Echinostomatidae) in freshwater gastropods. Folia Parasit. 1999; 46:157-58.
8. Toledo R, Muñoz-Antolí C, Pérez M, Esteban JG. Miracidial infectivity of *Hypoderaeum conoideum* (Trematoda: Echinostomatidae) differential susceptibility of two lymnaeid species. Parasitol Res. 1999; 85(3):212-15.
9. Toledo R, Muñoz-Antolí C, Pérez M, Esteban JG. Larval trematode infections in freshwater gastropods from the Albufera Natural Park in Spain. J Helminthol. 1998; 72(1):79-82.
10. Niewiadomska K, Siddall R, Valtonen E. Cercariae from *Lymnaea stagnalis* in lake Kuuhankavesi (central Finland). Acta Parasitol. 1997; 42:132-7.
11. Faltynkova A, Nasincova V, Kablaskova L. Larval trematodes (Digenea) of planorbid snails (Gastropoda: Pulmonata) in Central Europe: a survey of species and key to their identification. Syst Parasitol. 2008; 69:155-78.

12. Cichy A, Faltynkova A, Bikowskai E. Cercariae (Trematoda: Digenea) in European freshwater snails – A checklist of records from over one hundred years. Folia Malacol. 2011; 19(3):165-89.
13. Albert CG, Mena AC, Bristol JR. Parasits of waterfowl from Southwest Texas: The Green-winged teal, *Anas crecca*. J Wildl Dis. 1981; 17(1):57-64.
14. Donald JF, Oseph AC, John UB. Lead poisoning and parasitism of non-migratory Canada Geese in Florida. Fla Field Nat. 1987; 15:71-6.
15. Hosseini SH, Saifurl P, Eslami A, Nabieian S. Parasitic infection of Gray lag goos (*Anser anser*) in Gilan Province, Iran. Vet J. 2001; 56(1):57-60.
16. Farias JD, Canaris A. Gastro-intestinal helminths of the Mexican duck, Anas Platyrhynchos Diazi Ridgway, from north central Mexico and southwestern United States. J Wildl Dis. 1986; 22(1):51-4.
17. Faltynkova A, Nasincova V, Kablaskova L. Larval trematodes (Digenea) of the great pond snail, *Lymina stangalis*, (Gastropoda, Pulmonata) in centeral Europe: A survey of species and key to their identification. Parasite. 2007; 3:39-51.
18. Muñoz-Antolí C, Toledo R, Esteban JG. The life cycle and transmission dynamics of the larval stages of Hypoderaeum conoideum. J Helminthol. 2000; 74(2):165-72.
19. Diaz D. Studies on the Life Cycles of Digenetic Trematodes [PhD dissertation]. University of Leeds; 1976.
20. Jurlova NI. Morphology and biology of trematodes of *Hypoderaeum cubicanum* comb and *H. conoideum* (Echinostomatidae). Parazitologya. 1987; 21:32-5.
21. Wesenberg-Lund C. Contribution to the development trematode digenea. Mem Acad Roy Sci Lett. 1934; 9: 1–223
22. Toledo R, Muñoz-Antolí C, Perez M, Esteban JG. Redescription of the adult stage of *Hypoderaeum conoideum* (Bloch, 1782) (Trematoda: Echinostomatidae) and new record in Spain. Res Rev Parasitol. 1996; 56(4):195-201.
23. Khan R, Ahmao F. Redescription of *Hypoderaeum conoideum* (Bloch, 1782, Dietz, 1909) from Anas Platyrhynchos domesticus in Kashmir. Indian J Helminthol. 1991; 43:162-4.
24. Rees FG. On the anatomy of the trematode *Hypoderaeum conoideum* Bloch, 1782 together with attempts at elucidating the life cycle of two other digenetic trematodes. Proc Zool Soc Lond. 1933; 1:819-26.
25. Beverley-Burton M. Studies on the trematoda of British frehwater birds. Proc Zool Soc Lond.1961; 137:13-39.