Short Communication

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

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**Abstract**

**Background:** Human Echinostomiasis is an intestinal disease caused by the members of family Echinostomatidae parasites. The aim of present research was to identify echinosomatidae 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 drawn under camera Lucida microscope and measures were determined.

**Results:** Averagely, 15 metacercaria 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. per...
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.

Infesting of the second intermediate host with cercariae and recovery metacercariae

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.

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. Paraeosophageal 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 cercariae 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).

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Fig. 1: A, Drawing picture of *Hypoderaeum conoidenum* sucker; B, Drawing picture of *Hypoderaeum conoidenum* spined collar arrangement; C, Photo picture of *Hypoderaeum conoidenum* 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 sucker length    | 46         | 35         | 40.5  |
| Oral sucker width     | 39         | 51         | 45    |
| Sucker distance       | 129        | 144        | 136.5 |
| Pharynx length        | 25         | 29         | 27    |
| Pharynx width         | 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 contiguous 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 Coturnixypsilophora) fed with metacercariae were parasite free in autopsy after 1-2 months post infection.

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

| Abbreviation | Name                  |
|--------------|-----------------------|
| F.C          | Flame cell            |
| O.S          | Oral sucker           |
| H.C          | Head collar           |
| V.S          | Ventral sucker        |
| L.C          | Intestinal cecae      |
| O.S          | Oesophagus            |
| E.G          | Excretory glands      |
| P            | Paraoesophageal gland |
| V.F          | Vitellari follicular  |
| T            | Testis                |
| V.S          | Ventral sucker        |
| I.C          | Intestinal cecae      |
| U            | Uterus                |
| O            | Oesophagus            |
| E.G          | Excretory glands      |
| P.G          | Paraoesophageal gland |

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

| Measurement                           | 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 sucker length                     | 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 testis length                 | 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

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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.

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