

Classification of Anomalies in Protoscoleces of *Echinococcus granulosus* and *E. multilocularis*

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Introduction

Reports on morphological anomalies of cyclophyllidean cestodes are not so rare, particularly in larval and adult tapeworms of family Taeniidae. Concerning anomalies of genus *Echinococcus*, however, only a few reports have been published up to the present. The first record of abnormality of *Echinococcus* has been described by Siebold⁸⁾ who found a protoscolex with 6 suckers. Gashen³⁾ found a pair of duplication of protoscoleces from hydatid cyst in the liver of pig. Lubinsky^{4,5)} described abnormal numbers of hooks in oncospheres of *E. granulosus* and *E. multilocularis*. He⁵⁾ also reported anomalies of hooks in adult and larval scoleces of *E. multilocularis* and *E. granulosus* and protoscoleces jointed by their bases from hydatid cyst in a human case.

The present paper deals with morphological classification of anomalies found among protoscoleces of *E. granulosus* from sheep and *E. multilocularis* from cotton rats during *in vitro* culture.

Materials and Methods

Protoscoleces of *Echinococcus granulosus* were obtained from hydatid cysts in the liver and lung of naturally infected sheep in New Zealand, and those of *E. multilocularis* were collected from the liver of experimentally infected cotton rats. In each case, echinococcal cysts were separated aseptically from connective tissue of the host. The protoscoleces were severed mechanically from the brood capsule following trypsinization, and these were washed several times with Hanks' solution for the purpose of removing degenerated protoscoleces and debris of echinococcal tissue. About 15,000 protoscoleces were incubated at 37°C in each culture flask (30 ml in capacity) with 5 ml of medium consisting of a 1 : 1 mixture of Medium 199 and Hanks' solution containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract and 20% inactivated calf serum.

The observations were carried out using normal and phase contrast microscopes and supravital staining with 0.2% neutral red and 0.2% Janus green during a 10-day culture period.

Results

1. Abnormalities of *Echinococcus granulosus*

Of 24,574,501 protoscoleces examined, 129 (5.25 protoscoleces per million of population sampled) showed the morphological abnormalities. The abnormalities observed were classified as follows: protoscoleces with aberrant number of suckers ranging 4 to 8, protoscoleces with double rostella and aberrant number of suckers ranging 4 to 6, protoscoleces with 4 rostella each with 4 suckers, protoscoleces with bifurcate trunk, and duplications of protoscoleces (duplicitas). The duplications were classified by the part of diffusion into three types of parabiogenic or lateral duplication (duplicitas parabolica) and telobiotic duplication at the anterior part (duplicitas anterior) and at the posterior part (duplicitas posterior). The frequencies of the abnormalities for *E. granulosus* are shown in Table 1. Besides, aberrant hooks in number, arrangement, size and shape were observed occasionally on the rostella of protoscoleces. The anomalies of hooks observed were classified as follows: complete absence of hook, increase and decrease of number of hooks, accessory (third) row of hooks, irregular distribution of hooks, abnormally large and small hooks, minute rudiment of hooks, complete and partial deletions of handle and garde in hooks, and duplication of hooks. The frequencies of protoscoleces having aberrant hooks were not shown in the table, because standard range of variability in number, arrangement, size and shape of hooks has not been established for the present.

Of the protoscoleces with atypical number of suckers, those with 6 suckers were most prevalent, 36.43% per all anomalies. Frequency of protoscoleces with even-numbered suckers was higher than that of protoscoleces with odd-numbered ones. The fre-

Table 1. Frequency of anomalies in protoscoleces of *Echinococcus granulosus*

Number of suckers and rostella on protoscoleces, or kinds of duplication	Number of anomalies	Number of anomalies per million	Percentage for all anomalies
Suckerless and 1 rostellum	4	0.16	3.10%
1 sucker and 1 rostellum	1	0.04	0.78%
2 suckers and 1 rostellum	5	0.20	3.88%
3 suckers and 1 rostellum	2	0.08	1.55%
5 suckers and 1 rostellum	10	0.41	7.75%
6 suckers and 1 rostellum	47	1.91	36.43%
7 suckers and 1 rostellum	2	0.08	1.55%
8 suckers and 1 rostellum	5	0.20	3.88%
4 suckers and 2 rostella	1	0.04	0.78%
6 suckers and 2 rostella	9	0.37	6.98%
16 suckers and 4 rostella	1	0.04	0.78%
Parabiogenic duplication	21	0.85	16.28%
Anterior telobiotic duplication	2	0.08	1.55%
Posterior telobiotic duplication	18	0.74	13.95%
Protoscoleces with bifurcate trunk	1	0.04	0.78%
Total	129	5.25	

Table 2. Frequency of anomalies in protoscoleces of *Echinococcus multilocularis*

Number of suckers and rostellae on protoscolex, or kinds of duplication	Number of anomalies	Number of anomalies per million	Percentage for all anomalies
6 suckers and 1 rostellum	1	4.08	16.67%
Parabiotic duplication	2	8.16	33.33%
Posterior telobiotic duplication	2	8.16	33.33%
6 suckers and bifurcate trunk	1	4.08	16.67%
Total	6	24.49	

quency of protoscoleces with double rostellae and 6 suckers was higher than that of protoscoleces with double rostellae and 4 suckers also. In the protoscolex with bifurcate trunk, the minute remnant of the peduncle was found on each end of the trunk.

2. Abnormalities of *Echinococcus multilocularis*

Of 245,007 protoscoleces of *E. multilocularis* examined, 5 anomalies (5.25 protoscoleces per million) were found. The abnormalities were classified into 4: parabiotic and posterior telobiotic duplications, protoscolex with 6 suckers and protoscolex with 6 suckers and bifurcate trunk. In addition, the same abnormalities of hooks as those observed in *E. granulosus* were recognized on rostellae of protoscoleces of *E. multilocularis* also.

Discussion

The abnormalities of protoscoleces observed in the present experiment are essentially composed of aberrant number of suckers (0 to 16 suckers), aberrant number of rostellae (double to tetradic rostellae), bifurcate trunk, duplications of protoscoleces (parabiotic duplication and telobiotic duplications at anterior or posterior part) and aberrance of hooks in number, arrangement, size and shape. Of those anomalies, the frequency of protoscoleces with even-numbered suckers was higher than that of protoscoleces with odd-numbered suckers corresponding to each of the even numbers, particularly the frequency of protoscoleces with 6 suckers was most prevalent. An anomaly of echinococcal protoscolex found at first by Siebold⁸⁾ was the one having 6 suckers. The similar tendency also has been reported by the workers who observed anomalies in other cestodes. Namely, Foster²⁾ and Clapham¹⁾ stated that the most frequent type of the abnormalities was scolex with 6 suckers, and the one with 8 suckers came next in order. Schiller⁷⁾ reported the occurrence of scolexes having 2 to 7 suckers in larval *Taenia crassiceps*, stating that the frequency of scolexes with 6 suckers was most prevalent.

As far as the author is informed of, there is no report dealing with protoscolex having double rostellae in genus *Echinococcus* for the present. However, Railliet⁶⁾, Clapham¹⁾ and Schiller⁷⁾ described the occurrence of double rostellae on scolexes with various numbers of suckers in *Coenurus serialis*, adult *T. pisiformis* and larval *T. crassiceps*, respectively. The first record of duplication of protoscoleces of *E. granulosus* was reported by Gaschen³⁾. Lubinsky⁵⁾ described that protoscoleces jointed in pairs were

found in less than 1% of protoscoleces from hydatid cysts of human liver in Canada. This frequency is very high in comparison with that in the present examination.

Various deformations of hooks in number, arrangement, size and shape were observed on the rostellum of protoscoleces of *E. granulosus* and *E. multilocularis* in this experiment. Lubinsky⁵⁾ detailed on anomalies of hooks of adult and larval *E. granulosus* and *E. multilocularis*, and he classified those according to transformation and aberrant number of hooks. Schiller⁷⁾ reported that the aberrant hooks were observed on the rostellum of scoleces of larval *T. crassiceps*. The anomalies of hooks observed in the present experiment are similar to those reported by them.

Summary

Of 24,574,501 protoscoleces of *Echinococcus granulosus* collected from naturally infected sheep, 129 showed morphological abnormalities including atypical numbers of suckers, double and tetradic rostellum, bifurcate trunk and duplications of protoscoleces. The number of suckers ranged from 0 to 8 in the anomalous protoscoleces with a rostellum, 4 and 6 in those with double rostellum, and 16 in the one with tetradic rostellum. Of the anomalies, the frequency of protoscoleces with 6 suckers was highest. Of 245,007 protoscoleces of *E. multilocularis* from experimentally infected cotton rats, 5 anomalies consisting of 4 pairs of duplications of protoscoleces, a protoscolex with 6 suckers and the one with 6 suckers and bifurcate trunk were observed. Besides the anomalies as above, aberrance of hooks in number, arrangement, size and shape were recognized on the rostellum of protoscoleces.

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References

- 1) Clapham, P.A.: *J. Helminth.*, **17**, 163-176 (1939)
- 2) Foster, W.D.: *J. Parasit.*, **2**, 7-12 (1915)
- 3) Gaschen, H.: *Acta Trop.*, **3**, 247-248 (1948)
- 4) Lubinsky, G.: *Can. J. Zool.*, **36**, 883-887 (1958)
- 5) Lubinsky, G.: *Ibid.*, **37**, 793-801 (1959)
- 6) Railliet, A.: *C. R. Soc. Biol.*, Paris, 2nd Ser., **1**, 18-21 (1899)
- 7) Schiller, E.L.: *J. Parasit.*, **59**, 122-129 (1973)
- 8) Siebold, C.T.E. von: *Z. Wiss. Zool.*, **4**, 409-424 (1853)

Explanation of plate

Figures 1~6 and 8 are anomalous protoscoleces of *E. granulosus*, and figure 7 is that of *E. multilocularis*.

Fig. 1 Protoscolex with 5 suckers × 170

Fig. 2 Protoscolex with 6 suckers × 100

Fig. 3 Protoscolex with 4 suckers and 2 rostellum × 100

Fig. 4 and 5 Protoscolex with 6 suckers and 2 rostellum × 100

Fig. 6 Lateral duplication of protoscoleces × 100

Fig. 7 Protoscolex with 6 suckers, bifurcate trunk and 1 rostellum having aberrant hooks × 100

Fig. 8 Anterior telobiotic duplication of protoscoleces with aberrant hooks × 130

