# Studies on Intestinal Trematodes in Korea

XVII. Development and Egg Laying Capacity of *Echinostoma hortense* in Albino Rats and Human Experimental Infection

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# INTRODUCTION

# The fluke family Echinostomatidae, a group of intestinal trematodes of birds and mammals, is morphologically characterized by the presence of head crown with collar spines. Among them 14 species belonging to 5 genera are known to infect man and cause gastrointestinal troubles (Yamashita, 1964; Tani et al., 1974).

Echinostoma hortense Asada, 1926 is one of the human infecting echinostomes, for which more than 20 cases have been reported in Japan and in Korea (Makino et al., 1982; Seo et al., 1983). Various kinds of amphibia and freshwater fishes are known to serve as the second intermediate hosts (Asada, 1926; Ono, 1930; Mori, 1935; Tani, 1976 a&b) and the rats, the dogs and the weasles are the important reservoir hosts (Asada, 1927; Yamaguti, 1933 & 1939; Park, 1938; Seo et al., 1964 & 1981; Cho et al., 1981).

The present study was undertaken to estimate the susceptibility of albino rats to experimental *E. hortense* infection, by observing the development, maturation and egg-laying capacity of the worms within the rat host. In addition, by experimental infection of two human volunteers, the brief clinical course was observed.

# MATERIALS AND METHODS

In the experimental infection of the albino rats and the human volunteers, the metacercariae of E. hortense collected from the loaches (Chai et al., 1985) were used. A total of 21 albino rats (Wistar strain) were fed each with  $20\sim69$  metacercariae through a polyethylene tube inserted to their stomach. Each man swallowed 7 and 27 metacercariae in number respectively.

In order to obtain the worms of various ages to be studied, the rats were killed by cervical dislocation after 6, 10, 14, 21, 25, 28, 35, 42, 49 and 150 days from the experimental infection. The abdominal wall of the rats was opened and the whole length of small intestine, from duodenum to caecum, was resected. Then the intestinal wall was opened so as to search for the worms among the intestinal content. The flukes were collected and fixed in 10% formalin under slight pressure. They were washed with tap water, stained with acetocarmine, dehydrated in graded alcohols, cleared in xylol and mounted in canada balsam. The worms of various ages of infection were measured.

The stools of the albino rats as well as the human volunteers were collected from the 7th day after infection until the termination of infection. They were examined qualitatively by formalin-ether technique and quantitatively by Stoll's egg counting technique.

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In human volunteers, the clinical course was observed without laboratory tests up to  $27{\sim}28$  days after infection. The human parasitism was terminated by giving 15 mg/kg in single dose of praziquantel (Distocide®) followed by purgation with  $30{\sim}40$  g magnesium sulfate. The whole diarrheal stools discharged for 6 hours were collected and the echinostomatid flukes were searched for.

## RESULTS

# The Recovery Rate and Development of E. hortense

The recovery rate of *E. hortense* from the rats, according to the duration of infection, was in the range from 9.1% (6 days after infection) to 50.0% (49 days after infection), with an overall average value of 31.1% (Table 1). There was no decreasing tendency of the worm recovery rate according to the age of infection. Even on the 150th day the rate was not significantly low, compared with the average value, which suggests that the life span of this fluke may be fairly long in the rats. The recovery rate of the worms from two human volunteers was nearly the same as that from the rats (Table 2).

Based on the measurements of 98 specimens

**Table 1.** The recovery of *E. hortense* from the experimental rats

Duration of infection (days)	No. rats used	Total No. metacer. given	Total No. worms recovered*(%)		
6	2	22	2	(9.1)	
10	2	40	11	(27.5)	
14	3	109	25	(22.9)	
21	2	120	36	(30.0)	
25	3	36	11	(30.6)	
28	2	60	25	(41.7)	
35	2	40	7	(17.5)	
42	2	40	18	(45.0)	
49	2	60	30	(50.0)	
150	1	20	5	(25.0)	
Total	21	547	170	(31.1)	

<sup>\*</sup> The worms were recovered from the proximal and middle portions of the small intestine.

**Table 2.** The recovery of *E. hortense* after praziquantel treatment from two human volunteers

Duration of infection (days)	Volunteer	No. metacer. given	No. worms recovered(%)
27	А	27	10 (37.0)
28	В	7	1 (14.3)
To	tal	34	11 (32.4)

of *E. hortense* recovered from the rats (Table 3), the growth of worms especially in body length was very rapid during the first 14 days after infection, but seemed to become slower thereafter until the 150th infection day. The average length of worms was 1.76mm at the age of 6 days, 3.49mm at 10 days, 7.59mm at 14 days, 9.04 mm at 28 days and 9.56mm at 42 days (Table 3). Even afterwards the worms grew continuously to become 12.62 mm in length at 150 days. In comparison, the growth of body width was negligible after 35 days.

There was some difference between the early growth pattern of the genital and non-genital organs of E. hortense (Fig. 1 & 2). In case of non-genital organs such as the oral sucker, head crown, pharvnx and ventral sucker, the growth pattern was expressed as nearly straight lines up to 14 days after infection (Fig. 1). On the other hand, in case of genital organs such as the cirrus sac, ovary, Mehlis' gland, and anterior and posterior testes, the growth pattern was of the initial part of a sigmoid curve (Fig. 2). The ovary no more enlarged later than the 28th day, and the Mehlis' gland attained its full size in 35~49 days and afterwards slightly regressed. But the male genital organs grew steadily up to 150 days after infection.

When the morphology of the worms of various ages was observed, it was considered that the growth of worm length was mainly due to the enlargement of the posterior portion (Fig.  $3\sim8$ ). The equatorial portion of the worms appears to be a good indicator to assure more remarkable growth of the posterior body than the anterior one. At the stage of metacercaria, the equatorial portion was preacetabular level, however, after

Table 3.	The measurements	of $E$ .	hortense	recovered	from	the rats	according	to t	he age of	worms
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Age of worm* (days)	No. specimens measured		Measurements in average value (μm)								
			Oral sucker	Head crown	Pharynx	Ventral sucker	Cirrus sac	Ovary	Mehlis' gland	Ant. testis	Post. testis
6	2	$1761.4 \times 359.9$	$98.8 \\ \times 102.7$	104. 0	$102.7 \times 63.2$	$\substack{226.1\\ \times 226.1}$	$142.4 \\ \times 57.0$	56. 9 ×51. 4	$83.0 \\ \times 59.3$	$78.9 \\ \times 114.6$	$98.8 \\ \times 101.0$
10	5	$3494.3 \times 799.3$	$125.1 \times 135.7$	196. 4	$^{165.2}_{ imes 105.6}$	$356.5 \times 397.7$	$\substack{228.8\\ \times 121.0}$	166. 3 ×154. 3	$208.8 \times 126.4$	$315.2 \times 292.6$	$395.0 \times 364.5$
14	9	$7586.5 \\ \times 1174.3$	$149.7 \\ \times 182.6$	258. 4	$^{168.1}_{\times 151.9}$	$524.1 \\ \times 563.0$	$^{410.8}_{ imes 226.9}$	$304.4 \times 284.5$	$399.0 \times 268.2$	$741.9 \\ \times 710.1$	$936.9 \\ \times 567.5$
21	20	$7948.0 \\ \times 1231.1$	$149.6 \\ \times 177.5$	273.6	$176.6 \\ \times 147.5$	$552.6 \\ \times 532.0$	$479.4 \\ \times 186.2$	$307.2 \times 288.6$	$467.5 \\ \times 307.9$	$776.1 \\ \times 688.3$	$951.0 \\ \times 588.5$
28	18	$9044.9 \\ \times 1295.0$	$^{160.5}_{\times 168.2}$	264. 3	$202.5 \times 164.7$	$589.7 \\ \times 551.2$	$569.7 \\ \times 225.0$	$336.0 \\ \times 327.9$	$553.5 \times 387.2$	$832.3 \times 698.7$	$1017.5 \\ \times 585.2$
35	4	$8437.8 \\ \times 1401.6$	$\begin{array}{c} 171.2 \\ \times 202.8 \end{array}$	292.6	$205.4 \times 163.3$	$^{631.8}_{ imes 665.0}$	$548.7 \\ \times 271.0$	$377.4 \times 334.2$	$631.8 \\ \times 360.8$	$939.3 \\ \times 811.3$	$1017.5 \times 690.0$
42	17	$9564.7 \times 1505.7$	$182.7 \times 199.6$	286. 5	$205.4 \times 170.5$	$629.4 \\ \times 579.0$	$521.8 \\ \times 280.9$	$351.3 \times 328.2$	$679.5 \\ \times 391.6$	$\begin{array}{c} 777.7 \\ \times 781.8 \end{array}$	$1051.5 \\ \times 667.8$
49	19	$10210.7 \times 1358.7$	$202.4 \times 222.5$	297. 9	$224.7 \times 194.3$	$639.6 \times 654.0$	$559.7 \times 255.9$	355. 3 ×335. 3	690.9 ×408.5	$868.6 \times 734.3$	$1049.7 \times 619.5$
150	4	$12618. 8 \\ \times 1453. 8$	$250.8 \times 265.0$	412. 2	$260.3 \times 246.0$	$753.0 \times 767.3$	$757.5 \times 303.0$	376. 5 ×357. 5	$558.8 \times 431.0$	$1084.3 \\ \times 861.8$	1231. 0 ×753. 0

<sup>\*</sup> From metacercarial infection to worm recovery

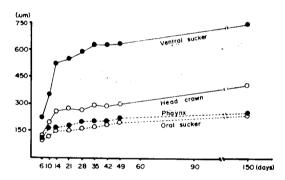


Fig. 1. The growth curve of non-genital organs of E. hortense up to 150 days after infection.

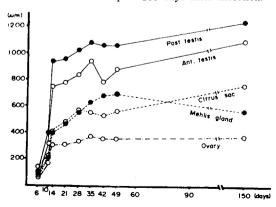


Fig. 2. The growth curve of the genital organs, male or female, of *E. hortense* up to 150 days after infection.

6 days of infection, it was changed to the ovarian level (Fig. 3). At 10 days it was at the level of anterior testis or at the junctional area between two testes (Fig. 4) and at  $14\sim35$  days, in the majority of worms, it was at the posterior testis level (Fig. 5 & 6). Later than 42 days of infection it was observed at the post-testicular level (Fig. 7 & 8).

# 2. The Preparent Period and Egg Laying Capacity of *E. hortense*

The eggs of *E. hortense* observed in the feces of the rats(Fig. 9) and humans(Fig. 10) were not containing mature miracidia, golden yellow in colour, and ellipsoid to elliptical in shape, with very thin egg shells and opercula. The size of 10 measured eggs from the rats and from humans was  $115\sim122\times68\sim74~\mu m$  and  $116\sim130\times69\sim80\mu m$  respectively.

The preparent period of *E. hortense* was different in the two kinds of definitive hosts. Out of 18 rats of which their stools were examined, 4 revealed the eggs on the 10th day, 12 on the 11th day, and 2 on the  $12\sim13$ th day after infection. Therefore, the preparent period of this fluke seems to be  $10\sim12$  days in the rat host. On the other hand, from human volunteers, the eggs firstly appeared in the feces after  $16\sim17$  days,



Figs. 3-8. Developmental stages of E. hortense.

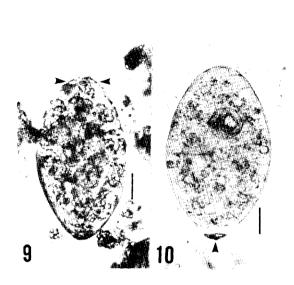
- 3. A 6-day old worm recovered from albino rat (scale: 250µm). Dorsal view.
- 4. Ventral view of a 10-day old worm. The genital organs already developed and several eggs are seen in uterus (scale:  $400\mu$ m).
- 5. A 14-day old worm. The body is greatly elongated and the genitalia fully matured. Numerous eggs are seen in uterus (scale:  $750\mu$ m).
- 6. A 28-day worm. The body is more elongated and the equatorial portion is at the posterior testis level (scale:  $750\mu m$ ).
- A 42-day worm. This specimen is a little wider than the others and the posterior body elongated more (scale: 750µm).
- A 150-day worm. The body did not enlarge significantly but the equatorial portion is behind the testes (scale: 750μm).

which appears to be the prepatent period in human host.

The egg laying capacity of *E. hortense* was observed in 8 rats which were given with 20 metacercariae in each number. For convenience, they were divided into 4 groups according to the day of sacrifice after infection (Fig. 11 & Table 4); 28-day group (Rat A, B), 35-day (Rat C, D), 42-day(Rat E, F) and 49-day ones (Rat G, H). After the start of oviposition in the rats, the value of E.P.G. (eggs per gram of feces) rapidly and remarkably increased up to 35 days (Fig. 11). Thereafter, however, the value began to decrease (42-day and 49-day

groups). Especially in the latter group, from which 30 adult flukes were recovered, the decreasing tendency of E.P.G. was more conspicuous during the 35~49th day after infection.

When the E.P.G./worm value was calculated in each rat, it was 14~17 (mean; 16) at 10~11th day, 50~86 (62) at 12~13th day, 96~200 (146) at 20~21th day, and 224~389 (321) at 28~29th day. It became a maximum value of 317~443 (390) at 32~33th day (Table 4). Thereafter, it gradually decreased. From this result, it can be said that the maximum egg production per worm of *E. hortense* occurs during the 30~40th day after infection (Fig. 11).



Figs. 9-10. The eggs of E. hortense in the feces

- 9. From an albino rat. Operculum (arrows) is seen(scale: 20μm).
- From a human volunteer. A germ cell and a wrinkling at abopercular end(arrow) are seen(scale: 20µm).

**Table 4.** The egg laying capacity of *E. hortense* in the experimental rats by post-infection days

Days after	EPG* per worm								
infection	Rat A, B	Rat C, D	Rat E, F	Rat G, H	Mean				
10-11**	16	14	17	17	16				
12-13	60	86	50	53	62				
20-21	96	200	128	160	146				
22-23	124	200	194	210	182				
24-25	148	186	256	193	196				
26-27	232	171	228	203	209				
28-29	224	343	389	327	321				
30-31		257	378	377	337				
32-33		443	317	410	390				
34-36		414	278	417	370				
37-38			333	403	368				
39-43			300	387	344				
44-45	_	_	_	267	267				
46-47			_	247	247				
48-49		_		210	210				

<sup>\*</sup> Eggs per gram of feces

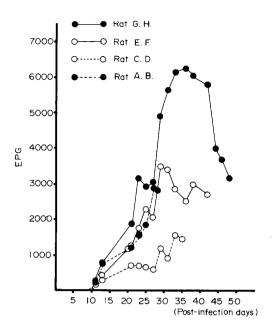


Fig. 11. The fluctuation pattern of EPG in the stool of the rats experimentally infected with E. hortense. The number of the recovered worms was 25 from Rat A & B after 28 days of infection, 7 from Rat C & D after 35 days, 18 from Rat E & F after 42 days, and 30 from Rat G & H after 49 days.

# 3. The Brief Clinical Course in Human Volunteers

Volunteer A: JLK, 42-year old male to whom 27 metacercariae in number were orally given. He experienced slight degree of vague abdominal pain on the 7th day after infection. This symptom did not persist thereafter, and instead, developed abruptly a gastric ulcer-like pain and general weakness on the 16th day. The gastric pain persisted for over 10 days until treatment on the 27th day. Change of bowel habit and episodes of diarrhea occurred on the 19th day, which continued intermittently until treatment. Insomnia due to the gastric pain was sometimes complained. All these symptoms were subsided after giving 15mg/kg praziquantel and recovery of 10 adult flukes of *E. hortense*.

Volunteer B: JYC, 34-year old male to whom 7 metacercariae were given. He also experienced ulcer-like pain after 5 days. General weakness developed on the 7th day. Both of these symptoms intermittently continued until deworming on the

<sup>\*\*</sup> The prepatent period of E. hortense in the rats

28th day. However, the symptoms were milder than in volunteer A. Diarrhea never occurred in this case. One adult fluke of *E. hortense* was recovered from the stool after treatment with 15mg/kg praziquantel.

## DISCUSSION

Many studies have revealed that the experimental final hosts of E. hortense could be the rat, mouse, dog and hamster (Ono, 1930; Asada, 1939; Arizono et al., 1976; Tani, 1976 a & b, 1978; Saito, 1984). However, the host susceptibility to infection and the development of worms in the hosts were not described in detail except in two papers (Tani, 1978; Saito, 1984). Tani (1978) reported that the most suitable laboratory host was the rats, followed by hamsters and mice. According to him, the worm recovery rates from hamsters and mice significantly decreased after 6 weeks of infection. Such decreasing tendency was, however, less marked up to 21 weeks in the rats. In the present study, the worm recovery rate from the rats was not significantly affected by the duration of infection and it was variable by host individual;  $9.1\sim$ 50.0%. The rate by Tani (1978) was  $16.7 \sim$ 40.0% in the rats, which is approximately the same as in this study.

There are two reports on the experimental human infection with *E. hortense*; 2 human volunteers each with 10 metacercariae (Arizono et al., 1976) and 5 volunteers each with 30 metacercariae (Tani, 1979). However, in their study, the clinical course and egg laying pattern were only observed, and it was not tried to recover the worms by treatment. In the present study, two volunteers revealed 14.3 and 37.0% (32.4% in average) of worm recovery rates after praziquantel treatment. This result suggests that the susceptibility of man to *E. hortense* infection may be as high as the rodent host.

The present observation on the developmental pattern of *E. hortense* in the rats was much similar to the report of Tani (1978) and Saito (1984). Saito (1984) reported that the develo-

pment of genital organs was quite different from that of nongenital organs, which was confirmed again in this study. Up to 10~14th day after the present experimental infection the development of genital organs was expressed as sigmoid curves whereas that of non-genital organs was as nearly straight lines. Later than the 49th day, however, the Mehlis' gland, of which the development was not described by other workers, nearly stopped growing and rather regressed in size. The growth of ovary in size was also negligible after 30~40 days. These findings seem to be related to the egg production pattern of worms; maximum E.P.G./worm during 30~40 days after infection and marked decrease thereafter. In comparison, however, the male genital organs continued to grow in size up to the 150th day of infection.

The prepatent period of *E. hortense* was studied in a variety of animals and man by many workers (Ono, 1930; Asada, 1939; Arizono et al., 1976; Tani, 1979). According to them the eggs firstly appeared on the  $9\sim16$ th day after infection in the stool of the rats,  $10\sim11$ th day in the hamsters,  $10\sim13$ th day in the mice,  $12\sim15$ th day in the rabbits and dogs, and  $16\sim17$ th day in men. The present data from the rats and men agreed well to the above reports.

The egg laying capacity of this fluke in human host, especially its fluctuation pattern by the age of infection, was studied only in two human volunteers by Tani (1979). The volunteers were infected with 30 metacercariae each and the eggs were first detected in their stools on the  $16\sim17$ th day after infection. The number of eggs produced reached maximum on the 20th day in one man and  $25\sim30$ th day in the other, but markedly decreased thereafter. In the present study, the egg counting in the feces of two volunteers was done only up to the 25th day after infection, so that not much information could be derived.

In the present study, the egg laying pattern of *E. hortense* was observed in the experimental rats. The value of E.P.G./worm was lower than 200 until the 25th day but rapidly increased to

390 on the 32~33th day followed by decrease thereafter. Based on Fig. 11, it is expected that the value would continuously decrease after the 50th infection day. In practice one rat sacrificed on the 150th day revealed no eggs in its stool, despite the presence of 5 worms in the intestine. There is no available literature to compare the egg laying pattern of this fluke in experimental animals.

The subjective symptoms in human volunteers experimentally infected with *E. hortense* were described in two papers (Arizono *et al.*, 1976; Tani, 1979). The major symptoms were, as in other intestinal helminthiases, abdominal pain and diarrhea. The symptom severity of human echinostomiasis in general is known to be related to the worm burdens (Yamashita, 1964). In the present study with *E. hortense*, the volunteer A experienced moderate to severe degrees of abdominal pain and diarrhea, from whom 10 worms were recovered by treatment. But the symptoms were milder in volunteer B from whom only 1 fluke was collected.

As to the change of blood picture due to this fluke infection, Arizono et al. (1976) described early monocytosis followed by eosinophilia (up to 19%). Also Tani (1979) observed the eosinophil count of 5 human volunteers and reported that they revealed 5~22% eosinophilia during the 20~80th day after infection. He also observed significant elevation of serum immunoglobulins such as Ig G, Ig M, Ig A and Ig E from 16~ 17th day, which showed peak values during the 20~40th day. Pathological aspects of human echinostomiasis in the intestinal tract has not been studied, which should be pursued in order to obtain more comprehensive understandings on the clinical significance and host-parasite interaction.

## **SUMMARY**

The worm development and egg laying pattern of *Echinostoma hortense* (Trematoda; Echinosto-

matidae) were studied in albino rats and the brief clinical course was observed in human volunteers. A total of 21 rats were infected with 20~69 metacercariae each and two humans were with 7 and 27 metacercariae, which were collected from the loaches. For recovery of worms, the rats were sacrificed at irregular intervals from the 6th to 150th day after infection and the human volunteers were treated with praziquantel and purged with magnesium salt on the 26~27th day. The stools of the rats and humans were examined for the eggs.

The results were as follows:

- 1. The worm recovery rate from the rats was not affected by the increase of infection time but varied individually;  $9.1 \sim 50.0\%$  (31.1% in average). From humans, 14.3% and 37.0% (32.4% in average) of challenged were recovered.
- 2. In the rats, it was revealed that the worms rapidly grew for the first 14 days to become 7.59mm in average length and 1.17mm in average width but the growth became much slower thereafter until the 150th day; 7.95mm in length on the 21th day, 9.04mm on the 28th day, 10.21mm on the 49th day and 12.62mm on the 150th day. During the early stage of infection, the growth of genital organs (male or female) was expressed as sigmoid curves whereas non-genital organs (such as suckers) was simply as straight lines.
- 3. The preparent period of this fluke was  $10\sim12$  days in the rats and  $16\sim17$  days in men. After the start of oviposition, the egg production by the worms remarkably increased, reached maximum on the  $32\sim33$ th day, followed by decrease thereafter. The maximum value of E.P.G./worm was 390.
- 4. The major subjective symptoms in human volunteers were abdominal pain and diarrhea during the early stage of infection.

The results show that human is as susceptible as the rats to *E. hortense* infection and the amount of egg production in the rats is greatly affected by the age of worms.

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## =국문초록=

# 韓國의 腸吸蟲에 관한 硏究

# XVII. 호르텐스棘口吸蟲의 흰쥐내 發育, 蟲卵產出樣相 및 人體實驗感染

호르텐스棘口吸蟲(Echinostoma hortense)의 흰쥐내 發育樣相, 蟲卵產出樣相 및 實驗的 人體感染에 있어서의 간단한 臨床 경과를 관찰하였다. 被囊幼蟲은 미꾸리로부터 분리한 것을 사용하였고 흰쥐 21마리에 대하여 각각 20~69個, 人體感染 지원자 2名에 대하여 각각 7個 및 27個를 感染시켰다. 흰쥐는 感染 6日부터 150日사이에 희생시킨후 小腸으로부터 蟲體를 回收하였고, 지원자는 感染 26~27日後 praziquantel과 下劑를 사용하여 治療하고 泄寫便으로부터 蟲體를 回收하였다.

결과는 다음과 같다.

- 1. 흰쥐로부터의 蟲體回收率은 感染期間에 따라 거의 변동이 없었고 그대신 個體別로 9.1~50.0%(平均 31.1%)의 다양한 樣相을 보였다. 人體 지원자에서는 14.3% 및 37.0%(平均 32.4%)의 回收率을 보였다.
- 2. 흰쥐에 있어서, 蟲體는 첫 14日 동안 매우 급격히 成長하여 평균 길이 7.59mm, 폭 1.17mm에 달하였으나 그 후에는 150日까지 느린 成長을 보여 감염 21日後 길이 7.95mm, 28日後 9.04mm, 49日後 10.21mm 및 150日後 12.62mm로 成長하였다. 感染初期에 蟲體의 生殖器官(雄性 또는 雌性)은 S字狀 成長曲線을 보였으나 非生殖器官(吸盤 등)은 단순한 直線狀 曲線을 보였다.
- 3. 感染後 蟲體가 蟲卵을 產出할 때까지의 期間은 흰쥐에서 10~12日, 人體에서는 16~17日이 所要되었다. 흰쥐에 있어서 蟲卵產出量은 32~33日경에 最高値에 달하고(390 E.P.G.) 그 후 차차 減少하였다.
  - 4. 人體感染에 있어서 感染初期(26~27日)의 主要 自覺症狀은 腹痛 및 泄瀉이었다.
- 이 결과는 人體가 흰쥐와 거의 마찬가지로 호르텐스棘口吸蟲에 잘 感染될 수 있으며, 또 흰쥐에서의 蟲卵產出 온 蟲體의 感染연령에 따라 크게 좌우됨을 나타내는 것으로 해석되었다.