INFECTIVITY AND DEVELOPMENT OF THE HUMAN STRAIN OF *HYMENOLEPIS NANA* IN ICR MICE

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Abstract. In order to study the infectivity and development of the human strain of *Hymenolepis nana* in mice, a human strain of *H. nana* was inoculated into ICR mice. *H. nana* eggs were concentrated by the sedimentation method and inoculated by a disposable syringe (1 ml) connected to a long needle (8 cm) into the stomach of mice. Mouse feces were examined daily beginning day 5 after inoculation and the mice were sacrificed from days 19 to 65 post-infection (PI). The infection rate and worm recovery rate were 69% and 17%, respectively. The prepatent period ranged from 7 to 23 days. Autoinfection was found to occur in an ICR mouse infected with 60 eggs; 102 worms were recovered from its small intestinal lumen on day 19 PI. One row of hooklets was found on the scolex and the mean number of hooks was 19. The average length, width, and number of segments were 51 mm, 0.6 mm, and 1,099, respectively. The mean length and number of immature segments were 9 mm and 414 segments, mature segments 20 mm and 390 segments, and gravid segments 22 mm and 295 segments. The average length, width, and number of segments in 26 autoinfected worms were 11.5 mm, 0.3 mm, and 189 segments. The mean length and number of immature segments were 3.9 mm and 41 segments, mature segments 4.4 mm and 65 segments, and gravid segments 3.2 mm and 83 segments, respectively.

INTRODUCTION

Hymenolepis nana infection has a cosmopolitan distribution and is more common in warm than cold climates. It is prevalent in South Asia and the Far East (Hsieh, 1970; Cross and Basaca-Sevilla, 1981; Fan *et al,* 1982), southern Europe, the region of the former Soviet Union, India, South America, the southeastern United States, Hawaii, and most of the islands of the South and Southwest Pacific (Beaver *et al,* 1984).

Although the mouse strain of *H. nana* is morphologically similar to the human strain (Belding, 1965), there is much confusion about the relationship between these strains. Grassi (1887) suggested both strains to be varieties of the parasite. However, Al-Baldawi *et al* (1989) failed to infect mice by feeding them eggs of the human strain of *H. nana*, while Ferretti *et al* (1981) succeeded in infecting mice with the human strain. In order to shed light on this topic, we infect ICR mice with the human strain of *H. nana*. The purposes of our study were to: (1) determine the susceptibility of ICR mice to the human strain of *H. nana*, (2) make measurements of *H. nana* (human strain) in ICR mice, (3) make measurements of *H. nana* in autoinfected ICR mice, and (4) evaluate for the presence of *H. nana* eggs in the feces of those ICR mice.

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MATERIALS AND METHODS

Egg collection

A survey of intestinal helminthic infections was conducted in the Sanming district, Kaohsiung County, southern Taiwan. A four-year old aboriginal girl was found to be infected with *H. nana*. A positive sample of feces was obtained from the infected girl and brought to our laboratory in Taipei.

Mice

ICR mice, bred in the Animal Center, National Yangming University, were used. These mice were 25 days old. They were kept in the

animal room of our department and were allowed to feed *ad lib* on a standard diet and water. Plastic cages with iron wire covers and sawdust bedding were used, and cleaned once every other day. Before experimental infection, a single stool specimen from each mouse was examined. Only the ones with negative results were included in the present study.

Experimental infection and feces examination

The eggs of *H. nana* were collected by the sedimentation method. Each mouse was inoculated orally with 20, 30, or 60 eggs using a disposable 1 ml syringe connected to a special long needle (8 cm). After infection, the feces were examined by the sedimentation method daily starting on day 5 after inoculation in order to find the eggs of *H. nana*. The first appearance of the prepatent period and the intermittent finding pattern of eggs were determined.

Sacrifice and examination

At various intervals 19-65 days after inoculation, the mice were sacrificed by cervical dislocation. The abdominal cavity was opened, the gastrointestinal canal from the anterior end of stomach to the posterior end of rectum was removed and placed in a large Petri dish (15 cm) filled with normal saline, and then placed in a refrigerator (5ºC) overnight. The next morning, the gastrointestinal canal was taken out and dissected carefully and the worms were collected. The number was recorded and fixed with a 10% formalin solution. Before measuring, the worms were dehydrated directly with absolute ethyl alcohol for 10 minutes twice, cleared in m-kresol (Merck-Schuchardt) then placed on a microscope slide (25 x 75 mm), covered with a coverslip (24 x 50 mm) and examined under an optical microscope. The scolex, length and width of the worms, and number and length of immature, mature and gravid segments, and number of hooks on the scolex in each of the ICR mice were measured and counted. In the meantime, the above figures were obtained for each mouse, including those with autoinfection. Natural autoinfection was considered to occur in the mice with abundant cysticercoids and immature worms in the intestinal canal (Sano *et al*, 1975).

RESULTS

Susceptibility

Each of the 67 ICR mice inoculated with 20, 30, or 60 *H. nana* eggs was sacrificed and examined 19 to 65 days after infection and 43

Table 1
Hymenolepis nana (human strain) infection in ICR mice.

No. of eggs fed/ mouse	No. of mice autopsied	Day of infection	No. of mice infected	Infection rate (%)	No. of worms recovered	Worm recovered (%)	Eggs found the 1st time (days)
30	13	54-65	5	50	60	20	ND[a]
30	8	23-33	7	88	15	6	ND
60	3	19	3[b]	100	116	64	7
20	3	52	2	67	8	13	18
20	5	22	5	100	15	15	19
20	3	23	3	100	6	10	19
20	5	22	5	100	22	22	19
20	5	23	5	100	11	11	19
20	5	24-30	5	100	17	17	19
20	1	23	1	100	1	5	20
20	5	30	1	40	3	3	20
20	5	19-30	1	33	1	2	23
20	4	30	0	0	0	0	0
20	5	19	0	0	0	0	0
20-60	70	19-65	38	39	275	61	7-23

[a]ND = not done; [b]102 worms were recovered from an infected mouse with 60 eggs caused by autoinfection.

Table 2
Measurements of *Hymenolepis nana* (human strain) infection in ICR mice.

Age of worms (days)	No. of worms exam	Scolex		No. of hooks/ scolex	Worm (mm)		Length (mm)			No. of segments			
		W	Wo		Length	Width	Immat segment	Mature segment	Gravid segment	Immat segment	Mature segment	Gravid segment	Total no.
19	2	2		18[a]	39	0.5	6	12	21	304	130	162	596
				18-18[b]	31-37	0.4-0.7	5-7	7-17	19-24	154-454	121-139	126-197	419-722
22	5	1	4	22	107	0.8	23	34	50	532	486	455	1,474
					94-123	0.7-0.9	18-28	28-39	46-56	457-579	447-522	378-532	1,400-1,552
23	10	5	5	11	123	0.7	28	52	43	564	647	288	1,499
				6-16	63-183	0.3-1.0	15-41	33-88	13-62	141-933	459-984	95-465	695-2,222
24	6	5	1	22	102	0.7	17	53	32	603	659	298	1,560
				18-24	84-119	0.6-0.9	10-31	36-65	23-49	491-712	568-711	212-575	1,400-1,879
30	6	2	4	19	100	0.7	15	39	45	714	590	519	1,823
				18-20	87-123	0.6-0.8	9-16	28-48	28-61	478-893	497-691	243-840	1,403-2,164
33	3	2	1	19	33	0.4	9	10	14	604	204	286	1,093
				18-20	26-35	0.3-0.5	6-14	9-11	2-21	477-728	167-225	78-399	1,024-1,230
53	2	2		18	31	1.0	5	5	21	306	249	351	906
				18-18	28-34	0.9-1.0	4-6	5-6	20-23	164-447	95-403	248-453	507-1,303
54	28	25	3	19	23	0.5	4	8	14	314	337	263	914
				16-24	13-38	0.2-0.8	2-9	4-18	2-19	132-728	161-702	102-532	604-1,398
60	1	1		22	25	0.7	4	12	9	879	757	162	1,739
63	5	3	2	20	21	0.4	5	7	8	144	94	97	335
				18-22	15-24	0.4-0.5	3-7	5-9	7-9	86-215	69-130	34-134	234-449
Total	61	43	18	19	51	0.6	9	20	22	414	390	295	1,099
				16-24	13-183	0.2-1.0	3-41	3-88	2-62	86-933	69-984	34-840	234-2,222

[a]Mean; [b]Range; W = With scolex; Wo = Without scolex.

Table 3
Comparative development of *Hymenolepis nana* in rodents.

Length (mm)	No. of segments	No. of hooklets	Reported by
15-25 (rare 45)	200	20-30	Yokogawa *et al*, 1960
20	200	ND	Watson, 1960
15-25	100-120	20-22	Swellengrebel and Sterman, 1941
10-45	100-200	20-30	Piekarski *et al*, 1962
5-45	Up to 200	20-30	Belding, 1965
20-60	ND	20-27	Itagaki and Itagaki, 1965
50-60	ND	20-27	Wardle and McLeod, 1968
20-40	200	20-30	Faust *et al*, 1974
20	200	ND	Brown, 1975
5-30 (rare 120)	100-200 (rare 1,000)	ND	Chao, 1983
Up to 40	ND	20-30	Beaver *et al*, 1984
25-40	200	ND	Zamen and Keong, 1994
Seldom exceeding 40	ND	20-30	Schmidt and Roberts, 1989
13-183	234-2,222	16-24	In the present study, 1991

ND=Not done.

were found to be infected. A total of 275 worms were recovered. The infection rate and worm recovery rates were 39% and 61%, respectively (Table 1).

Prepatant period and autoinfection

The eggs of *H. nana* in the feces were first found on day 7 postinfection. Autoinfection was also found in an ICR mouse infected with 60 eggs, and 102 worms were recovered on day 19 postinfection (Table 1).

Measurements of *H. nana* (human strain) in ICR mice

Of the 61 worms examined, 43 had a scolex and 18 did not. The mean number of hooks on each scolex was 19 (range 16-24). The average length and width and the mean total number of segments were 51 mm (range 13-183 mm), 0.6 mm (0.2-1.0 mm), and 1,099 (range 234-2,222), respectively. The mean length and number of immature segments were 9 mm (range 3-41 mm) and 390 (range 86-933), mature segments 20 mm (range 3-88 mm) and 390 (range 69-984), and gravid segments 22 mm (range 2-62 mm) and 295 (range 34-840), respectively (Table 2).

Measurements of autoinfected worms

Twenty-six autoinfected worms from an ICR mouse 19 days after infection were measured. Of these worms, 13 had a scolex and 13 did not. The mean number of hooks on each scolex was 19 (range 17-22). The average length and width and the mean total number of segments were 11.5 mm (range 8.1-16.1 mm), 0.3 mm (range 0.3-0.4 mm), and 189 (125-303), respectively. The mean length and number of immature segments were 3.9 mm (range 1.6-6.0 mm) and 41 (range 22-79), mature segments 4.4 mm (range 3.0-7.0 mm) and 65 (range 42-119), and gravid segments 3.2 mm (range 1.3-5.2 mm) and 83 (23-149), respectively.

DISCUSSION

H. nana was described by von Siebold in 1852 as a parasite found in man, which he called *Taenia nana*. Later Stiles (1906) described *H. fraterna* as a parasite in several wild rodents (rats, mice, etc). These species are morphologically identical to each other and Grassi (1887) first suggested that the human and murine strains were identical. However, Brumpt (1949) regarded them as separate species. Yamaguti (1959) considered these two species distinct and indicated that man and various rodents were hosts for both.

Although many workers have studied the specificity of the human and murine strains of *H. nana*, they have not reached a satisfactory

conclusion. Tsuchiya and Rohlfing (1932), Roman (1939), and Ferretti *et al* (1981) succeeded in experimentally infecting mice and rats with human strains of *H. nana*, however, Woodland (1924) and Al-Baldawi *et al* (1989) did not. According to Ferretti *et al* (1981), the unconvincing results of Woodland (1924) may be due to the infective methods used, especially the selection of experimental animals. In the experimental infection study of Al-Baldawi *et al* (1989), no eggs were found in the mouse feces from six groups of male Swiss albino mice inoculated with human *H. nana* of the Basrah strain. In addition, no worms were recovered at the post-mortem examination. In attempts to induce cysticercoids to infect mice, beetles (*Tribolium confusum*) were either fed on infected human feces or given *H. nana* eggs on filter paper. Both methods were unsuccessful, as no cysticercoids were recovered six days after exposure to the beetles. In another study, artificially immunosuppressed mice were fed with the eggs of the human strain but again none appeared to become infected (Andreassen, 1981). Al-Baldawi *et al* (1989) concluded that the Basrah strain of *H. nana* is very host-specific.

In our study, we used ICR mice. We inoculated the mice with a tuberculine syringe connected to a long needle into the stomach. This technique ensured that the eggs of *H. nana* could reach the stomach of the mice. We succeeded in infecting the ICR mice with the human stain of *H. nana* and obtained an infection rate of 69% with a worm recovery rate of 17%. At the same time, we obtained the shortest prepatent period of 7 days. This period is much shorter than those reported by Belding (1965) (30 days) and Beaver *et al* (1984) (2 or more weeks).

According to Belding (1965), the adult worm of *H. nana* is 5-45 mm long and 0.5-0.9 mm wide and has as many as 200 segments and 20-30 hooklets on the scolex. Similar findings have also been reported by other parasitologists (Yokogawa *et al*, 1960; Watson, 1960; Swellengrebel and Sterman, 1961; Piekarski, 1962; Itagaki and Itagaki, 1965; Wardle and McLeod, 1968; Faust *et al*, 1974; Brown, 1975; Chao, 1983; Beaver *et al*, 1984; Zamen and Keong, 1994; Schmidt and Roberts, 1989) (Table 3). In our study, the average length and width and the mean total number of segments were found to be 51 mm (range 13-183 mm), 0.6 mm (range 0.2-1.0 mm) and 1,099 (range 234-2,222), respectively. The total number of segments (1,099) was about five times more than those previous reported (100-200). Although the width was similar to those previous reported, the length was also greater than those reported by Belding (1965) and Beaver *et al* (1984). We also measured the length and number of the immature, mature, and gravid segments. These figures are reported for the first time.

Of the experimentally infected mice, 102 worms were recovered from an infected mouse with 60 eggs caused by autoinfection. The mean size of the 26 worms in the mouse with autoinfection was 11.5 mm long, 0.3 mm wide and with 189 segments. In addition, the mean length, number of immature, mature and gravid proglottides were also demonstrated. In order to obtain more information on autoinfection, we conducted further experimental infections. Autoinfection will be the next topic in our study of the human strain of *H. nana* in ICR mice.

REFERENCES

Al-Baldawi FA, Mahdi NK, Abdul-Hafidh BA. Resistance of mice to infection with the human strain of *Hymenolepis nana*. *Ann Trop Med Parasitol* 1989; 83: 275-7.

Andreassen J. Immunity to adult cestodes. *Parasitolgy* 1981; 82: 153-9.

Beaver PC, Jung RC, Cupp EW. *Clinical parasitology.* 9th ed. Philadelphia: Lea & Febiger, 1984: 509-11.

Belding DL. Textbook of parasitology. 3rd ed. New York: Appleton-Century-Crofts, 1965: 419-23.

Brown HW. Basic clinical parasitology. 4th ed. 1975: 183-5.

Brumpt E. Precis de Parasitologie. Paris: Masson et Cie Editeurs, 1949.

Chao WS. Human parasitology. Peking: Health Publishing, 1983: 524-9.

Cross JH, Basaca-Sevilla V. Intestinal parasitic infections in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1981; 12: 262-74.

Fan PC, Chung WC, Chan CH, Lee KM, Wang CC, Wu CC. A pilot study of common intestinal parasites and head louse among aboriginal children in Tai-

wan, R.O.C. *Nat Sci Counc Monthly* 1982; 10: 773-98.

Faust EC, Russell PF, Jung RC. Craig and Faust's Clinical parasitology. 8th ed. 1974: 525-7.

Ferretti G, Gabriele F, Palmas SC. Development of human and mouse strain of *Hymenolepis nana* in mice. *Int J Parasitol* 1981; 11: 425-30.

Grassi B. Entwicklungscyclus der *Taenia nana*. *Cbl Bakt Parasitenk* 1887; 2: 305-12.

Hsieh HC. Parasites and parasitic diseases other than trematodiasis of the human gastrointestinal system in Taiwan. Taipei: Proceedings of Seminar on Infective Diseases of Gastrointestinal System in Southeast Asian and the Far East, September 28 - October 2, 1970.

Itagaki S, Itagaki H. Parasitology of domestic animals. Tokyo: 1965: 144-5.

Piekarski G. Medical Parasitology in Plates. Germany: Bayer, 1962.

Roman E. Sur l'infestation des rongeurs par l'*Hymenolepis nana* de l'Homme. *Ann Parasitol* 1939; 17: 167-9.

Sano M, Majumder S, Watarai S, Odaka S. Spontaneous infection of *Hymenolepis nana* in hamsters. *Jikken Dobutsu* 1975; 24: 41-4.

Schmidt GD, Roberts LS. Foundations of parasitology. St. Louis: CV Mosby. 1989.

Stiles CW. Illustrated key to the cestode parasites of man. US Public Health Service. *Hyg Lab Bull* 1906; 25: 1-104.

Swellengrebel NH, Sterman MM. Animal parasites in man. New York: D. Van Nostrand, 1941: 283-90.

Tsuchiya H, Rohlfing EH. *Hymenolepis nana*. Report of additional cases and an experimental transmission from man to rats. *Am J Dis Children* 1932; 43: 865-72.

von Siebold CT. Uber die Verwandlung der Echinococcus-Brut in Taenien. *Z Wiss Zool* 1852; 4: 409-24.

Wardle RA, McLeod JA. The zoology of tapeworms. New York: Noble Offset Printers, 1968: 460-2.

Watson JM. Medical helminthology. London: Bailliere, Tindall & Cox, 1960: 318-22.

Woodland WNF. On the development of the human *Hymenolepis nana* (Siebold 1852) in the white mouse: with remarks on *H. fraterna*, *H. longior* and *H. diminuta. Parasitology* 1924; 16: 425-35.

Yamaguti S. Systema Helminthum. Volume II. The Cestodes of Vetebrates. New York: Interscience Publishers, 1959.

Yokogawa S, Murishita K, Yokogawa M. Human parasitology. Tokyo: 1960: 323-5.

Zaman V, Keong LA. Handbook of medical parasitology, 3nd ed. Singapore: KC Ang Publishing. 1994: 119-21.