



Experimental studies on physiological and morphological aspects of *Cysticercus cellulosae* in pigs

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Received: January 10, 2001 Revised: March 12, 2001 Accepted: August 16, 2001

Three Small-Ear-Miniature, 3 Landrace-Small-Ear-Miniature, and one Douc-Yorkshire-Landrace pigs were inoculated orally with 100 000 eggs of Zhengzhou strain or 10 000 eggs of Harbin strain of *Taenia solium*. A total of 3739 cysticerci were recovered from 3 Small-Ear-Miniature and 3 Landrace-Small-Ear-Miniature pigs, giving an infection rate of 85.7% and a cysticercus recovery rate of 1.1%. The predilection sites of *Cysticercus cellulosae* in descending order were leg muscles, abdominal muscles, thoracic muscles, liver, head muscles, diaphragm, tongue, heart, trachea, and omentum/testes. Except 2 calcified cysticerci in the tongue, 2 in the heart, and 176 in the liver, the remaining cysticerci were all alive. The greatest number of cysticerci per 100 g of muscles or viscera was found in the head muscles, followed by the leg, diaphragm, heart, tongue, thoracic, abdominal, omentum, testes, and trachea. All cysticerci were evaginated in pig's bile after fluid was drawn out from cysticerci, whereas evagination occurred in only 83.2% of those without fluid drawing. In 364 evaginated cysticerci, the mean length and width of scolex, proglottid, and bladder, and diameter of rostellum and sucker were 826 x 747 μ m, 5370 x 1734 μ m, 2885 x 3002 μ m, 155 μ m, and 253 μ m, respectively. In the protoscolex, the mean number of segments was 33. Each cysticercus had 2 rows of rostellar hooks on the scolex, and the mean length and width of inner and outer hooks were 151 x 18 μ m and 117 x 14 μ m, respectively. The number of paired hooks ranged from 10 to 18.

Key words: *Cysticercus cellulosae*, evagination, rostellar hooks, sites, *Taenia solium*

Pigs, wild boars, dogs, and monkeys can harbor the cysticerci of *Taenia solium*, with pigs being the most important intermediate host for this tapeworm [1]. To determine the predilection sites of cysticerci, surveys have been carried out in slaughter houses [2,3]. However, the examinations of pigs in these studies were rough, because a large number of pigs were examined at the slaughter houses over a very short period of time. In a previous study [4], we recovered 162 cysticerci of *T. solium* from 2 pigs and 21 cysticerci from 2 dogs after experimental inoculation of 20 000 eggs from a strain of this tapeworm from the Hainan Province in Mainland China. Four cats, 2 goats, and a calf were not infected. The cysticerci, all alive, were recovered mainly from muscles of the pigs. The rest was recovered in the liver. In dogs, all cysticerci were recovered from the muscles and all were alive. Two technicians carefully examined these animals in 11 days. Three adult worms

of *T. solium* were recently obtained from 3 patients with cysticercosis and taeniasis solium in Henan Province and an adult worm from a patient with taeniasis in Heilongjing Province. The mean number of 54 316 (12 160-187 825) eggs was determined in each of last 10 segments of 8 adult worms of *T. solium*. This number is 5 times more than those previously reported in experimental infection studies, which estimated 10 000 eggs in one gravid segment [5-7]. In this study, we infected different strains of 7 pigs with a known number of *T. solium* eggs to determine the susceptibility, cysticercus recovery, density, predilection sites, size, rostellar hooks, and evagination related to the fluid of the cysticerci. To understand the physiological and morphological aspects of *Cysticercus cellulosae*, measurements of the evaginated cysticerci in different organs were conducted.

Materials and Methods

Collection of worms

An adult worm of *T. solium* was collected from a patient who has received chemotherapy at the Harbin

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Table 1. Susceptibility and cysticercus recovery of pigs infected with *Taenia solium* eggs (Zhengzhou and Harbin strains)

Pig		Age at inoculation, days	No. of eggs/pig	Duration of infection, days	Cysticercus recovery	
Strain	No. of pigs				No. of cysticerci ^c	% of recovery
SEM	1	46	100 000 ^a	89	451 (176)	0.45
L-SEM	1	182	100 000 ^a	130	984	0.98
L-SEM	1	182	100 000 ^a	196	2240 (4)	2.24
L-SEM	1	52	10 000 ^b	62	8	0.08
SEM	1	56	10 000 ^b	218	24 (24)	0.24
SEM	1	51	10 000 ^b	89	32	0.32
DYL	1	52	10 000 ^b	46	0	
Total	7	46-182		46-218	3739 (204)	1.10

Abbreviations: L-SEM = Landrace-Small-Ear-Miniature; SEM = Small-Ear-Miniature; DYL = Douc-Yorkshire-Landrace

^aZhengzhou strain.

^bHarbin strain.

^cNumber of calcified cysticerci in parenthesis.

University in Heilungkiang Province in Mainland China. Another 8 adult worms were collected from patients with cysticercosis and taeniasis solium after receiving chemotherapy with a mixture of areca and pumpkin seeds at the King-Shui Hospital in Henan Province in Mainland China. These worms were then sent to a laboratory in Taipei.

Experimental infection

Eggs were collected from the last 10 gravid proglottids of these adult worms. A total of 100 000 *T. solium* eggs of Zhengzhou strain were administered orally to one Small-Ear-Miniature (SEM) and 2 Landrace Small-Ear-Miniature (L-SEM) pigs, and 10 000 *T. solium* eggs of Harbin strain were inoculated orally to one Douc-Yorkshire-Landrace (DYL), L-SEM, and 2 SEM pigs. The animals were then kept in an animal center and fed with a regular diet.

Collection of cysticerci

Six pigs were sacrificed 62 to 218 days after experimental infection; examination of cysticerci was described in a previous study for the experimental

infection of Korean *Taenia* [8]. After the animals were sacrificed, cysticerci were collected from 15 parts of 6 carcasses in 4 to 16 days by 2 senior research technicians.

Viability of cysticerci

Viability was determined and compared by observing evagination and active movement of the cysticerci under a fluorescent light (15 foot-candles) after cysticerci from in 3 pigs have undergone fluid drawing or undrawing and were digested in pig bile for 5 to 15 min at room temperature.

Results

Susceptibility and cysticercus recovery

Of the 7 pigs inoculated with 10 000 or 100 000 eggs of *T. solium*, 6 (85.7%) were infected. The 3 SEM and 3 L-SEM pigs were susceptible, whereas the DYL pig was not susceptible to this strain of *T. solium*. A total of 3723 cysticerci were recovered from these pigs. The cysticercus recovery rate was 1.1%, ranged from 0.08% to 2.24% (Table 1).

Table 2. Survival of cysticerci of *Taenia solium* with drawing and undrawing fluid from cysticerci in 3 pigs

Pig		Undrawing out fluid		Drawing out fluid	
Strain	No. of pigs	No. of cysts	No. of survival (%)	No. of cysts	No. of survival (%)
SEM ^a	1	275	225 (81.8)		
L-SEM ^b	1	984	613 (62.3)		
L-SEM ^c	1	1651	1584 (95.9)	585	585 (100)
Total	3	2910	2422 (83.2)	585	585 (100)

Abbreviations: SEM = Small-Ear-Miniature; L-SEM=Landrace-Small-Ear-Miniature

^aCysticerci examined and collected from different parts of muscles and viscera from Day 1 to 4, kept at 4°C (body weight, 20 kg; liver weight, 490 g).

^bCysticerci examined and collected from Day 1 to 13, kept at 4°C (body weight, 39 kg; liver weight, 740 g).

^cCysticerci examined and collected from Day 1 to 16, kept at 4°C (body weight, 42 kg; liver weight, 780 g).

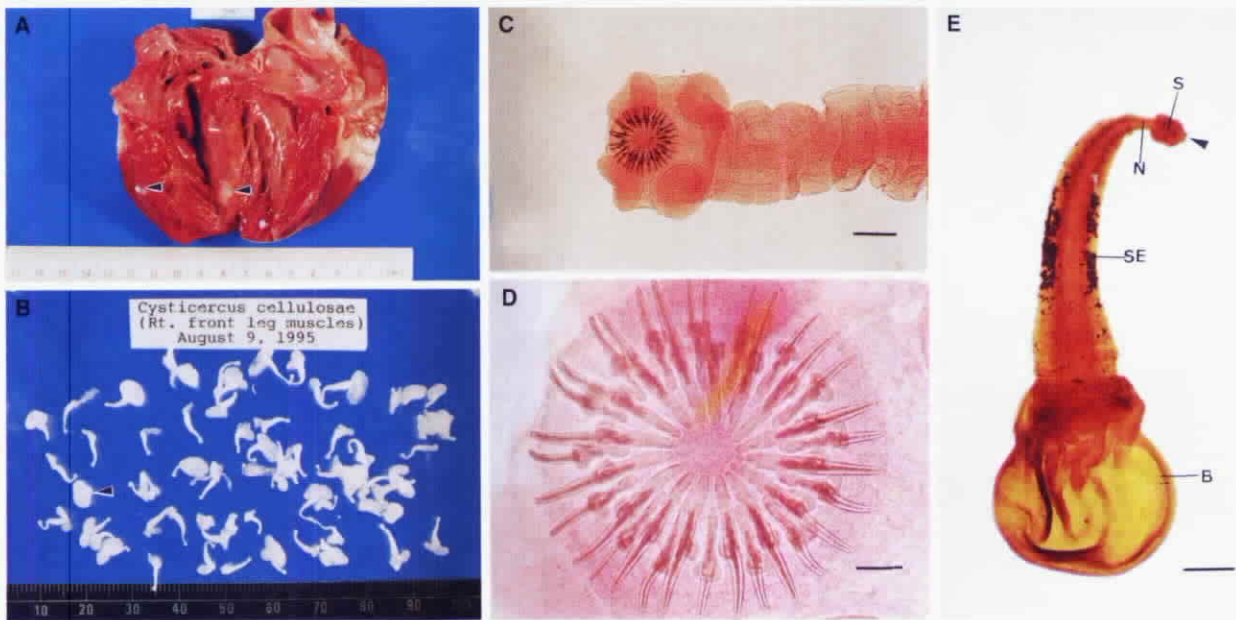


Fig. 1. The evaginated cysticerci of *Taenia solium* in a Landrace-Small-Ear-Miniature pig. **(A)** Two cysticerci in the heart (arrowhead). **(B)** Sixty-two cysticerci including 56 evaginated, 6 invaginated (arrowhead). Each evaginated cysticercus had a long proglottid with more segments and different sizes of ovoid bladder. **(C)** An enlarged evaginated *Cysticercus cellulosa* with 10 segments and a roseleaf-like rostellum (16 large and 16 small hooks) and 4 suckers on the scolex (Bar = 250 μ m). **(D)** Enlarged from Fig. 1C (Bar = 50 μ m). **(E)** An enlarged-evaginated cysticercus had 82 segments and a big bladder with a long-square form of scolex, short neck, and 2 rows of rostellum hooklets (Bar = 1000 μ m). Abbreviations: S = scolex; N = neck; SE = segment; B = bladder

Predilection sites of cysticerci

Of the 3723 cysticerci recovered from the 6 infected pigs, 46.9% were found in the leg muscles (Fig. 1B), 18% in the abdominal muscles, 17.5% in thoracic muscles, 4.7% in liver, 4.5% in head muscles, 2.8% in diaphragm, 1.3% in tongue, 1% in heart (Fig. 1A), 0.2% in trachea muscles, 0.05% in omentum, and 0.05% in testes. A total of 3425 cysticerci were alive. Only 2 calcified cysticerci were found each in the tongue and the heart. A total of 176 cysticerci in the liver were found to be calcified; 24 cysticerci, including 3 each in the thoracic muscles and the abdominal muscles and 18 in the leg muscles, were found to be calcified in a SEM pig. In addition, 110 cysticerci were dead as a result of being dropped down from unknown part of muscles into tap-water about 4 h. No cysticerci were found in the brain, kidney, pancreas, spleen, and lung (Fig. 2).

Density of cysticerci per 100 g of muscles and viscera

After locating the predilection sites, the number of cysticerci per 100 g of muscles and viscera in 2 heavily infected L-SEM pigs was determined. A mean number of 14.4 cysticerci were found in head muscles, 13.2 in leg muscles, 12.5 in the diaphragm, 11.9 in the heart, 11.3 in the tongue, 11 in thoracic muscles, 6.5 in

abdominal muscles, 1.4 in omentum, 0.7 in testes, and 0.3 in trachea muscles (Fig. 3).

Viability between drawing and undrawing fluid from cysticerci

Survival between drawing and undrawing fluid from cysticerci collected from 1 SEM and 2 L-SEM pigs was compared. All 585 cysticerci evaginated in pig's bile after drawing fluid from cysticerci were alive, and 2422 (83.2%) of 2910 cysticerci were found to evaginate in those with undrawing fluid (Table 2).

Measurement of evaginated cysticerci

Fig. 4 shows the measurements of 364 evaginated cysticerci in 2 L-SEM pigs. The mean length and width of scolex, segment, and a big bladder, as well as the diameter of rostellum and sucker were 826 \times 747 μ m, 5370 \times 1734 μ m, 2885 \times 3002 μ m, 155 μ m, and 253 μ m, respectively. In the proglottids, the mean number of cysticercus was 33 (range, 12-82) (Table 3, Fig. 1C and 1D).

An enlarged cysticercus was collected and measured from front leg muscles. The mean size of scolex, neck, 82 segments, and a big bladder, as well as the diameter of rostellum and sucker were 938 \times 750 μ m,

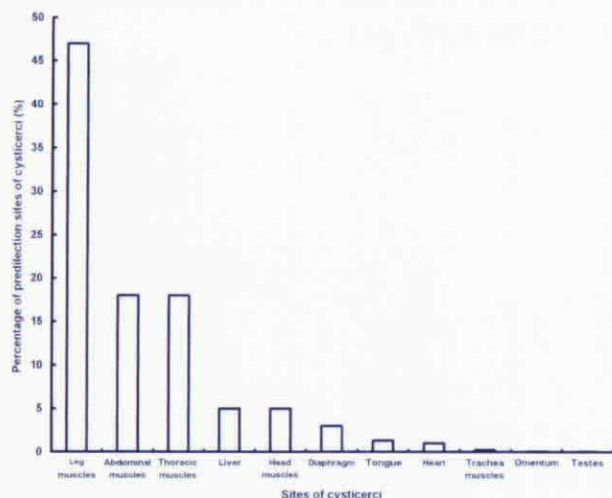


Fig. 2. Predilection sites of cysticerci of *Taenia solium* in 6 pigs.

850 x 245 μm , 6275 x 1500 μm , 3663 x 3575 μm , 250 μm , and 288 μm , respectively (Fig. 1E).

Measurement of rostellar hooks

There were 2 rows of rostellar hooks on the scolex of each cysticercus. The number of paired hooks ranged from 10 to 18. The mean length and width of inner (large) and outer (small) hooks in 364 cysticerci were 151 x 18 μm and 117 x 14 μm , respectively (Table 3).

Discussion

In a survey of natural infection of *C. cellulosae* in pigs on Cheju Island, Han and Loh [2] reported that 7.4% of 979 pigs at a slaughter house were infected. A total of 78.2% of the infected pigs had cysticerci recovered in the thigh and gluteal muscles, 65.2% in the neck and masticatory muscles, 39.1% in the heart and tongue, 26% in shoulder muscles, 19.3% in axial muscles, 8.7% in the diaphragm, and 4.3% in eye and liver. The mean number of cysts in central thigh and gluteal muscles among 23 pigs was 70 per 300 cm^3 (range, 1-352/300 cm^3). Kuo *et al* [3] noted that in Kansu Province of Mainland China, the infection rate of *C. cellulosae* in frozen pigs decreased from 10.64% in 1977 to 5.52% in 1985. The distribution of cysticerci in 161 naturally infected pigs was also determined. Loin muscles (13.42%) had the highest positive rate of 91.3%, and brain (85.7%) had the highest density of 27.91 per 40 cm^3 (range, 1-420/300 cm^3). The positive rate and density muscles were 90.1% and 11.5 per 40 cm^3 in shoulder, 87.6% and 10.9 per 40 cm^3 in high muscles, 80.6% and 12.3 per 40 cm^3 in heart, 78.9% and 8.27 per 40 cm^3 in diaphragm, and 64.6% and 8.13 per 40 cm^3 in abdominal muscles [3].

After examining the cysticerci of *T. solium* in pigs

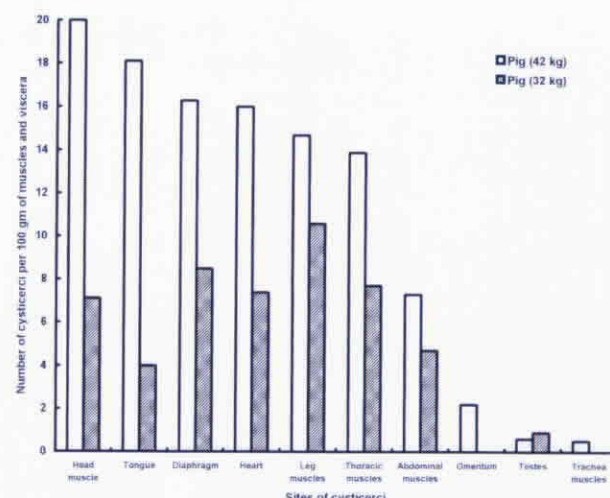


Fig. 3. Predilection sites and mean number of *Taenia solium* cysticerci per 100 g of muscles and/or viscera of 2 Landrace-Small-Ear-Miniature pigs.

in the Chin-Feng City of Inner-Mun in Mainland China, Huang [5] reported that in 40 naturally infected pigs, 60% of cysticerci were found in the brain, 37.5% in lymph glands, 30% in stomach and rectum, and 15% in pancreas and surrounding fat. Liu *et al* [6] recovered cysticerci of *T. solium* (Henan strain) from 19 pigs after inoculating 5 to 25 gravid segments, with an estimated 50 000 to 250 000 eggs per pig. The distribution and density per 40 cm^3 of cysticerci were as follows: masseter muscles (50%, 93), hind leg muscles (47%, 70), fore leg muscles (63%, 64), thoracic muscles (64%, 57), tongue (47%, 47), diaphragm (36%, 44), heart (54%, 37), brain (40%, 21), liver (25%, 6), kidney (13%, 3), and lung (8%, 2). In a previous study, we recovered cysticerci of *T. solium* (Hainan strain) from 2 pigs each inoculated

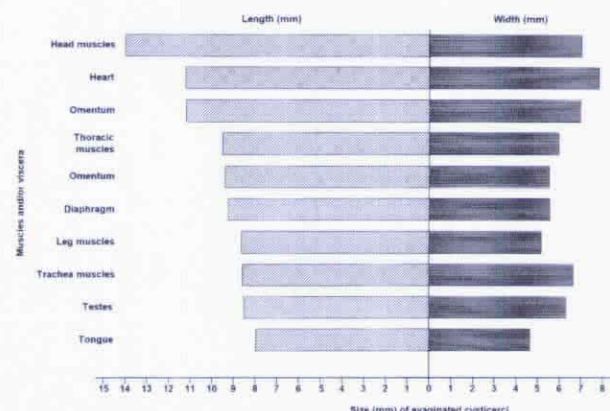


Fig. 4. The size (mm) of 364 evaginated cysticerci of *Taenia solium* in muscles and/or viscera of 2 Landrace-Small-Ear-Miniature pigs.

Table 3. Measurement of 364 evaginated cysticerci of *Taenia solium* in 2 Landrace-Small-Ear-Miniature pigs^a

Location	No. of cysticerci	Scolex			Diameter of Rostellum, μm	Diameter of Sucker, μm	Diameete Sucker, μm	Proglottid			Bladder			Rostellar hook ^b			
		Length, μm	Width, μm	No. of segment				Length, μm	Width, μm	Length, μm	Width, μm	No. of rows	Inner hook		Outer hook		
													Length, μm	Width, μm	Length, μm	Width, μm	Length, μm
Leg muscles	149	768 (400-1650)	763 (425-1575)	140 (100-175)	251 (135-300)	2185 (1413-6388)	2794 (1188-8188)	35 (15-82)	5702 (2503-9450)	1605 (688-2625)	2185 (1413-6388)	2794 (1188-8188)	2	157 (140-175)	18 (15-20)	122 (110-140)	14 (10-15)
Thoracic muscles	75	955 (400-1850)	869 (600-1225)	266 (225-325)	260 (150-335)	3413 (1256-7963)	3137 (1375-5450)	32	5134 (1813-7000)	1940 (1000-2563)	3413 (1256-7963)	3137 (1375-5450)	2	154 (90-175)	20 (15-25)	113 (100-130)	14 (10-18)
Abdominal muscle	52	1003 (550-1250)	792 (488-1275)	102 (75-125)	258 (190-300)	3288 (1800-8600)	3109 (750-7815)	32	5092 (1563-8000)	1609 (625-2500)	3288 (1800-8600)	3109 (750-7815)	2	144 (133-155)	17 (15-25)	118 (60-130)	13 (10-15)
Diaphragm	67	677 (400-1075)	598 (475-938)	ND	240 (175-300)	3483 (1775-8075)	3113 (1875-5625)	33	5076 (2938-7188)	1833 (1000-3125)	3483 (1775-8075)	3113 (1875-5625)	2	13 (100-1557)	18 (15-25)	110 (65-125)	15 (10-20)
Tongue	9	791 (625-975)	735 (600-900)	ND	238 (200-300)	2454 (2275-7563)	2299 (3200-4913)	25	4699 (2938-6025)	1614 (1225-1950)	2454 (2275-7563)	2299 (3200-4913)	2	137 (120-160)	19 (15-20)	100 (85-125)	14 (10-15)
Heart	5	809 (688-1025)	720 (558-938)	ND	253 (200-325)	4298 (3500-5438)	4493 (3650-5825)	28	6418 (4313-8625)	2585 (1450-2075)	4298 (3500-5438)	4493 (3650-5825)	2	135 (120-150)	18 (15-20)	102 (85-125)	13 (10-15)
Trachea muscle	5	700 (625-813)	846 (825-888)	ND	285 (270-300)	2733 (2450-3150)	3899 (3638-4250)	14	5151 (4750-5588)	1883 (1700-2050)	2733 (2450-3150)	3899 (3638-4250)	2	163 (160-165)	18 (15-20)	123 (120-130)	12 (10-15)
Omentum	1	1688	775	ND	275	3825	4688	14	5688	1500	3825	4688	2	150	18	120	10
Testis	1	750	800	ND	300	3275	3813	15	4500	1725	3275	3813	2	150	18	120	10
Total	364	826 (400-1850)	747 (425-1575)	155 (75-335)	253 (135-335)	2885 (1256-8600)	3002 (750-8188)	33	5370 (1563-9450)	1734 (625-3125)	2885 (1256-8600)	3002 (750-8188)	2	151 (90-175)	18 (15-25)	117 (60-140)	14 (10-20)

Abbreviations: L-SEM = Landrace-Small-Ear-Miniature; ND = not done

^aData are expressed as mean (range).

^bThe number of paired rostellar hooks ranged from 10 to 18.

Note: The mean length of 364 cysticerci of *T. solium* = scolex + segment + bladder = 826 + 5370 + 2885 = 9081 μm .

with 10 000 eggs. The parasitic sites were mainly found in muscles (75% hind leg, 25%; fore leg, 22%; thoracic, 21%; abdominal, 7%) and liver (25%) of pigs [4]. In this study, we determined predilection sites of *C. cellulosae* in 6 pigs as follows: 46.9% in leg muscles, 18% in abdominal muscles, 17.5% in thoracic muscles, 4.7% in liver, 4.5% in head muscles, 2.8% in the diaphragm, 1.3% in the tongue, 1% in the heart, 0.2% in trachea muscles, 0.1% in the omentum, and 0.1% in testes. Complete and careful examination revealed no cysticerci in the kidney, spleen, brain, pancreas, and lung. These findings were not consistent with those obtained by Huang *et al* [5] and Liu *et al* [6], which may have caused by the differences in the isolates of *T. solium* from different strains of pigs. The number of cysticerci per 100 g of muscles or viscera was 14.4 in head muscles, 11.3 in the tongue, 12.5 in the diaphragm, 11.9 in the heart, 13.2 in leg muscles, 11 in thoracic muscles, 6.5 in abdominal muscles, 1.4 in the omentum, 0.7 in testes, and 0.3 in trachea muscles. The calculation using the number of cysticerci per 100 g of muscles or viscera is preferred over cysticerci per 40 cm³ (volume of muscles) because the former is clearer, and the volume of 40 cm³ muscles cannot be measured in the hind leg, fore leg, tongue, heart, liver, diaphragm, and lung because each part of muscles or viscera is much smaller than 40 cm³ [3,5,6]. Results of previous studies [2-6] as well as this study indicated that the muscles seem to be more suitable for the development of the cysticerci of *T. solium* than the viscera as there are more blood vessels and prosperous nutrition in the muscles.

Liu *et al* [6] reported that all cysticerci recovered from mildly infected pigs (5 gravid segments/50 000 eggs/pig) 90 to 360 days after infection were all mature. However, in 18% to 20% of worms from the moderately (13 gravid segments/130 000 eggs/pig) and heavily (25 gravid segments/250 000 eggs/pig) infected pigs, some cysticerci remained premature 90 days after infection. In a previous study, all cysticerci were alive in the muscular tissues of pigs, whereas only one was alive in the liver [4]. In this study, we found that most of the cysticerci were alive, except 2 cysticerci each in the tongue and the heart, and 176 in the liver. These findings were similar to those obtained in our previous study [4].

Masuda [9] and Ono [10] reported that the size of cysticerci of *T. solium* in pigs were 10 to 10.9 mm long and 5 to 5.9 mm wide. However, CH Kim (personal communication, 1986) noted much smaller cysticerci, ranging from 1.5 to 3.5 mm in length and 1 to 2.5 mm in width. In a previous study, we demonstrated a mean

length and width of cysticerci of *T. solium* of 3.6 mm and 2.8 mm in pigs and 3.2 mm and 2.8 mm in dogs, respectively [4]. In this study, we determined the corresponding figures of 9.1 x 5.5 mm in 2 L-SEM pigs. The difference in the measurements may be related to the differences in the strains of *T. solium*, the experimentally infected pig strain, and age of infection (days). In addition to the size of the evaginated cysticerci, we also determined the scolex, proglottid, bladder and sucker, and rostellum in diameter. These figures were similar to those obtained by Masuda [9] and Ono [10], but were much larger than those reported by Kim.

The reported number of rostellar hooks of cysticerci in pigs by Eshinu [11], Masuda [9], Slais [12], and Soulsby [1] were 28, 26, 26, and 22 to 32, respectively. In a previous study, we found a mean number of 27 (range, 20-36) rostellar hooks of cysticerci in pigs [4]. In this study, we found that the pig infected over a period of 130 to 169 days had 10 to 18 pairs of hooks. These findings agree with the previous figures. We also found that the growth and development of small (outer row) hooks precedes the large (inner row) hooks in the formation of the 2-row pattern of rostellar hooks in *C. cellulosae*.

The measurements of rostellar hooks of cysticerci in pigs have also been investigated by Okabe [13], Verster [14], and Soulsby [1]. The length of large and small hooks have been reported to be 128 to 162 µm and 100 to 130 µm by Okabe [13], 160 to 170 µm and 110 to 140 µm by Verster [14], and 140 to 180 µm and 110 to 140 µm by Soulsby [1], respectively. Kim reported that large and small hooks measured 140 to 161 µm and 103 to 131 µm, respectively, in our previous study, we found that the mean length of large and small hooks were 147 µm (range, 90-210 µm) and 94 µm (range, 50-125 µm), respectively, in 2 infected pigs [4]. In this study, we measured the evaginated cysticerci and found that the length and width of large and small rostellar hooks were 151 µm (range, 90-175 µm) x 18 µm (range, 15-25 µm) and 117 µm (range, 60-140 µm) x 14 µm (range, 10-20 µm), respectively, as shown in Table 3.

In determining the viability of the cysticerci, we found that all cysticerci were evaginated in pig's bile after drawing out fluid from cysticerci, whereas only 83.2% were found to evaginate in those with undrawing out fluid (Table 2). These findings indicated that there may be some factors in the fluid of cysticerci that inhibit the evagination of cysticerci. However, the functions of these factors in the evagination require further investigations.

Acknowledgments

The authors wish to express their thanks to the National Science Council, ROC for the research grant NSC89-2320-B010-039, and to the Department of Health, Executive Yuan, ROC for the research grant DOH89-DT-1022. Thanks were also given to Mr. CY Lin, Miss PHuang, and Miss CW Yen for their technical assistance.

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