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Vaccination trials against *Taenia solium* eggs in pigs injected with frozen oncospheres of *T. solium* or *Taenia saginata asiatica*

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In this study, 12 Small-Ear-Miniature pigs aged 142 to 185 days were used to determine whether pigs injected with nonviable oncospheres of *Taenia solium* or *Taenia saginata asiatica* can become resistant to the challenge of viable eggs of *T. solium*. The 12 pigs were equally divided into 4 groups: 3 experimental groups in which each pig was injected subcutaneously with a mixture of 0.2 mL complete Freund's adjuvant and 10^4/0.2 mL nonviable Taiwan/Asian *Taenia*, Indonesia *Taenia*, or *T. solium* oncospheres, and 1 control group in which each pig was injected subcutaneously with 0.2 mL phosphate buffer solution and 0.2 mL complete Freund's adjuvant. Each pig was orally inoculated with 10 000 viable *T. solium* eggs 1 month later. The infection rates were 100% (2/2), 100% (3/3), 33% (1/3), and 100% (3/3) and cysticerci recovery rates were 1.3% (254/20 000), 1.2% (371/30 000), 0.01% (4/30 000), and 8.6% (2,577/30 000), respectively. Except for the location of 72 cysticerci located in the viscera, 3134 cysticerci were recovered from the muscles. In the experimental groups, 4 cysticerci recovered were viable and the remaining 625 were either calcified or degenerated. However, 2567 cysticerci recovered from the control group remained viable and only 10 were calcified or degenerated. The results indicate that in addition to the vaccine of *T. solium*, those of Taiwan *Taenia* and Indonesia *Taenia* can also induce high-crossing immunologic reactions against *T. solium* infection.

Key words: Oncospheres, *Taenia saginata asiatica*, *Taenia solium*, vaccination

It is well known that cattle can develop strong immunity against reinfection with *Taenia saginata* and a high proportion of cysticerci which establish within them [1,2]. Immunization of cattle has been carried out using oral infection with irradiated eggs [3,4], and intramuscular or subcutaneous injection of activated oncospheres of *T. saginata* and *Taenia hydatigena* [5, 6]. Moreover, calves vaccinated with *T. saginata* were highly resistant to the challenge infection and those vaccinated with *Taenia ovis* and *T. hydatigena* were also significantly resistant [7].

In East Asia, *T. saginata asiatica* employs a pig as an intermediate host. The oncospheres almost always migrate to the liver of its swine host and develop into cysticerci [8-14]. Ito *et al* [15] determined that injection with nonviable oncospheres of Asian *Taenia* showed significant resistance to challenge infection with eggs of *T. taeniaeformis* in rats. However, those injected with nonviable oncospheres of *T. saginata* did not show any resistance. Since all developmental stages of cyclophyllidean cestodes are immunogenic and have some stage-specific immunogen [16-20], cross protection against infections of homologous and heterologous species may be induced between *T. solium* and *T. saginata asiatica*.

In our recent vaccination trials on frozen oncospheres of Taiwan *Taenia*, Korean *Taenia*, *T. saginata*, or *T. solium* emulsified with Freund's adjuvant were challenged orally with viable Taiwan *Taenia* eggs, The infection of Taiwan *Taenia* in pigs can be prevent by subcutaneous injection of nonviable homologous oncospheres as well as those of Korean *Taenia* and *T. saginata*. Although all pigs vaccinated with nonviable oncospheres of *T. solium* became infected, all cysticerci recovered were degenerated or calcified. These findings suggest that oncospheres of Taiwan *Taenia* and Korean *Taenia* are very similar to *T. saginata* in their immunogenicity in pigs [21].

In mainland China, taeniasis and swine cysticercosis are endemic in 20 provinces. A mixture of the homogenate of *Cysticercus cellulosae* (antigen Q83) and complete Freund's adjuvant has been found to be effective in the prevention of cysticercosis in pigs [22, 23]. Since the information on the vaccination against

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T. solium infection in pigs is not abundant, this study was designed to determine whether pigs injected with nonviable oncospheres of *T. solium* or *T. saginata asiatica* (Taiwan and Indonesian strains) can become resistant to the challenge of viable eggs of *T. solium* after vaccination.

Materials and Methods

Parasites

Adult worms of *T. solium* were collected from patients with cysticercosis and taeniasis solium in King-Shui Hospital, Zhengzhou City, Henan Province, mainland China, after chemotherapy with a mixture of areca and pumpkin seeds. These worms were intact but without scolex. They were sent immediately to National Yang-Ming University (NYMU) in Taipei by courier.

Adult worms of *T. saginata asiatica* (Taiwan strain) were recovered from aboriginal patients at a mountainous area (Wufeng District, Hsienchu County) in Taiwan after chemotherapy with Atabrine. Gravid segments of *T. saginata asiatica* (Indonesian strain) were collected from patients on Samosir Island, Sumatra, Indonesia. These segments were sent immediately to NYMU for further study.

Experimental animals

Twelve Small-Ear-Miniature (SEM) pigs (age, 145-185 days) were obtained from an animal farm with very good sanitary conditions in Taitung County, Taiwan. They were kept in the Animal Centre of NYMU with a regular diet and drinking water daily day in separate cleaned accommodation without exposure to Taenia infections (Fig. 1A).

Preparation of oncospheres

Eggs of Taiwan *Taenia*, Indonesian *Taenia*, and *T. solium* were collected from the last 10 gravid segments. *In vitro* hatching (without activation) of the oncospheres was carried out using 0.5% sodium hypochlorite in phosphate buffer solution (PBS) [24,25]. The hatched oncospheres were then suspended in sterile PBS and adjusted to 5×10^4 /mL in Eppendorf tubes and kept immediately in deep frozen (–80°C) for 2 months or more before vaccination. Thawed oncospheres were emulsified with complete Freund's adjuvant (CFA).

Vaccination

The 12 SEM pigs were equally divided into 4 groups –3 experimental groups and 1 control group. Each SEM pigs in the 3 experimental groups (Group A, *T. saginata asiatica* of Taiwan strain; Group B, *T. saginata asiatica* of Indonesian strain; Group C, *T. solium*) were injected subcutaneously with a mixture (0.4 mL) of 10 000 nonviable oncospheres (0.2 mL) of one of the 3 strain/species of *Taenia* with 0.2 mL CFA. In the control group (Group D), each of the 3 SEM pigs was injected subcutaneously with a mixture (0.4 mL) of PBS and CFA.

Experimental infection, sacrifice, and examination

One month after vaccination, 10 000 viable eggs of *T. solium* were orally inoculated to each pig. These pigs were then sacrificed separately by the electric shock technique 41 to 55 days after inoculation of eggs of *T. solium*. The methods employed for experimental infection, necropsy of infected animals, examination of cysticerci, and classification of cysticercus development were as described by Fan *et al* [9].

Results

Results are summarized in Table 1. No adverse effects or local reactions were observed after vaccination. However, 1 SEM pig in Group A died naturally on day 21 after subcutaneous injection of the vaccine. The remaining ones were found to harbor cysticerci on days 41 and 43 after oral inoculation of viable *T. solium* eggs. However, the cysticercus recovery rates were very low (0.89% and 1.65%) and all cysticerci were degenerated or calcified. Most of the cysticerci were recovered from the muscles and a few from the viscera. Although the cysticercus recovery rates were extremely low (0.72%-1.98%), all 3 SEM pigs in Group B were found to have cysticerci in the muscles and viscera. However, only 4 of 371 cysticerci were found to be mature and the remaining ones were degenerated or calcified. In Group C, no cysticerci were found in 2 SEM pigs and only 4 degenerated or calcified cysticerci were recovered from the muscles of the remaining one (Table 1) (Fig. 1E).

In the control group (Group D), all 3 SEM pigs were infected and the cysticercus recovery rate ranged from 4.1% to 12.1%. Most of the cysticerci were recovered from the muscles (Fig. 1B and C) and the majority was mature (Fig. 1D, F-H). Only 10 cysticerci recovered were found to be degenerated or calcified (Table 1).

Discussion

Although intramuscular injection of hatched oncospheres of *T. saginata* into calves has reduced expected challenge burdens substantially, this procedure may result in the development of local focus of viable cysticerci at the site of injection [6]. Moreover,

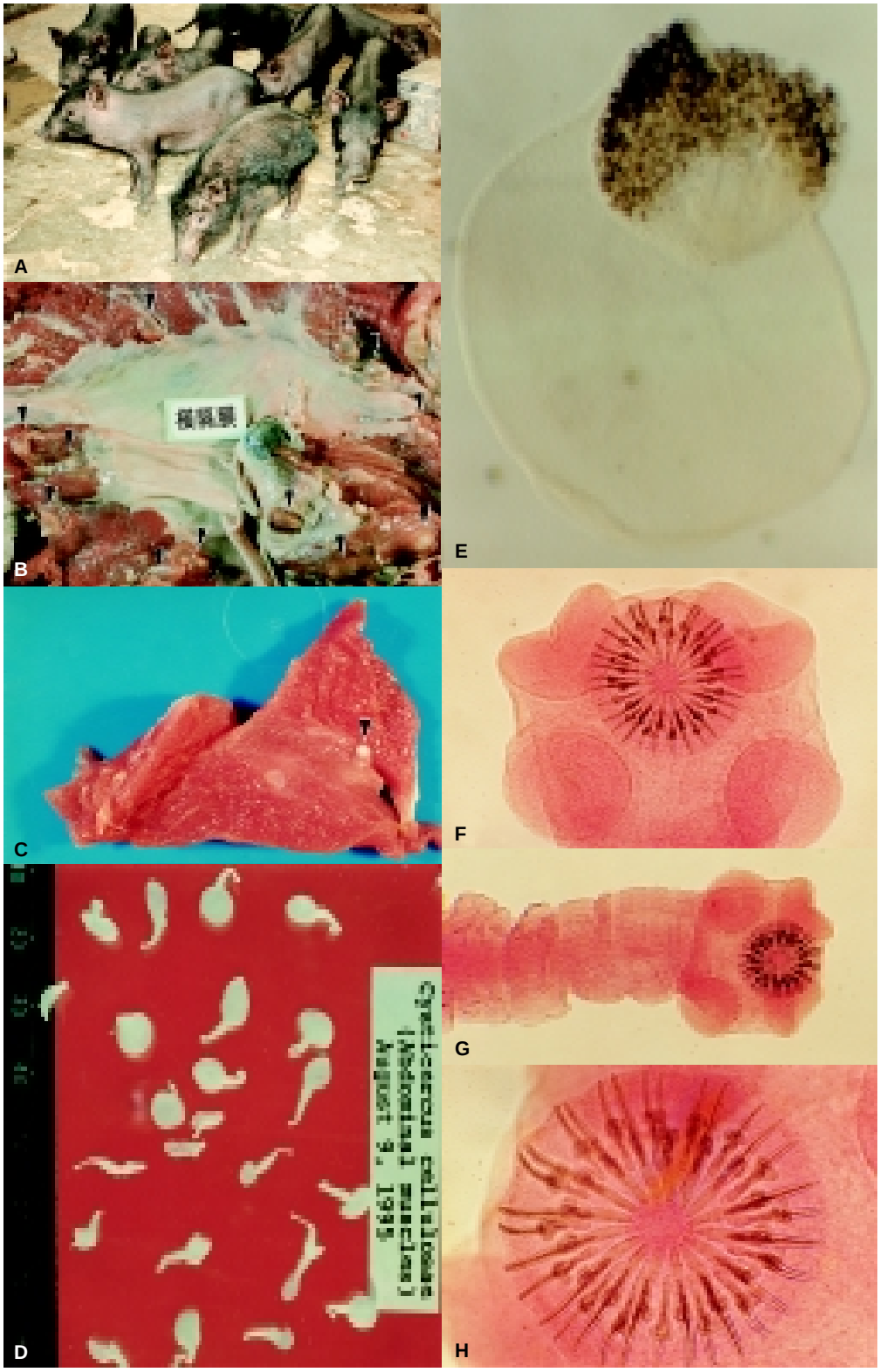

Fig. 1. Development, distribution, and morphological characteristics of *Cysticercus cellulosae* in the Small-Ear-Miniature pigs. **(A)** Eight young Small-Ear-Miniature pigs. **(B)** Twelve cysticerci observed in the diaphragm muscles. **(C)** A cysticercus located in the right hind leg's muscles. **(D)** Twenty-four evaginated cysticerci. **(E)** A 41-day-old calcified cysticercus (16x). **(F)** Sixteen pairs of rostellar hooks (100x); **(G)** (250x); **(H)** (500x).

Table 1. Cross protection against *Taenia solium* infection in SEM pigs vaccinated with nonviable oncospheres of homologous and heterologous species of the family *Taeniidae*

Group	Age at immunization,	Days after inoculation		Cysticercus	Distribution in			Development
	days	Feeding	Killed	recovery, n (%)	Viscera	Muscles	Mature	Degenerated/calcified
A	185	30	41	89 (0.89)	1	88	0	89
	185	21[a]						
	185	30	43	165 (1.65)	5	160	0	165
B	150	33	44	72 (0.72)	3	69	1	71
	150	33	45	198 (1.98)	18	180	0	198
	150	33	46	101 (1.01)	10	91	3	98
C	142	30	48	0				
	142	30	49	4 (0.04)		4	0	4
	142	30	50	0				
D	145	30	51	414 (4.14)	9	405	412	2
	145	30	53	950 (9.50)	15	935	946	4
	145	30	55	1213 (12.13)	11	1202	1209	4

Note: Group A = Taiwan/Asian *Taenia*, Group B = Indonesia *Taenia*, Group C = *Taenia solium*, Group D = Control. Subcutaneous injection of 10 000 nonviable oncospheres/pig with complete Freund's adjuvant and challenge with 10 000 viable *T. solium* eggs/pig.
[a]Naturally died 21 days after subcutaneous injection.

cysticerci derived from this immunizing injection may be dispersed throughout the body occasionally [26]. Immunization by the use of X-ray irradiated eggs of *T. saginata* given orally or intraluminally has reduced expected challenge burdens up to 90% [4]. Antigens derived from *in vitro* cultures of cysticerci of *T. saginata* combined with complete Freund's adjuvant have induced highly significant levels of immunity to *T. saginata* infection in calves [7].

Hatched oncosphere of *T. hydatigena* may induce some immunity to *T. saginata* in cattle [5]. Moreover, cross protection against *Taenia* and *Hymenolepis* species may be induced by nonviable intact oncospheres of heterologous species [27-29]. In our previous study, we demonstrated that Taiwan *Taenia* in pigs may be prevented by subcutaneous injection of nonviable homologous oncospheres, and those of Korean *Taenia* and *T. saginata*. Although all pigs vaccinated with nonviable heterologous oncospheres of *T. solium* were infected, all cysticerci recovered were degenerated or calcified. This finding suggests that Taiwan *Taenia* cysticerci can only be viable in the liver of the swine host for a very short period [13].

In this study, we conducted the vaccination trials against *T. solium* eggs in pigs injected with frozen oncospheres of *T. solium* or *T. saginata asiatica*. The results were similar to our previous study that the homologous vaccine produces nearly complete immunity whereas heterologous vaccines induce only partial acquired immunity against *T. solium*. These findings indicate that the cross-immunity evoked by the heterologous *Taenia* species is less effective than that induced by the homologous species.

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