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

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SUMMARY

When Small-Ear-Miniature pigs subcutaneously injected once with frozen oncospheres of Taiwan *Taenia*, Korea *Taenia*, *T. saginata* or *T. solium* emulsified with Freund's complete adjuvant ($1{\cdot}6 \times 10^4$/0·4 ml) were challenged orally with $1{\cdot}6 \times 10^4$ viable eggs of Taiwan *Taenia* 41 days later, they all showed strong resistance compared with pigs vaccinated with *T. solium*. Most pigs (5/8) of the former 3 groups harboured no cysticerci. The number of cysticerci was $5{\cdot}5 \pm 9{\cdot}1$ (mean $\pm$ S.D.), whereas pigs of the *T. solium* group and control group harboured $160 \pm 30{\cdot}1$ and $661 \pm 637{\cdot}2$ cysticerci, respectively. All cysticerci recovered from vaccinated pigs and most cysticerci in control pigs were degenerated or calcified at 36–55 days after oral egg challenge. These results strongly suggest that oncospheres of Taiwan *Taenia* and Korea *Taenia* are very similar to *T. saginata* in their immunogenicity in pigs.

Key words: vaccination, SEM pig, oncosphere, Taiwan *Taenia*, Korea *Taenia*, *Taenia saginata*, *Taenia solium*.

INTRODUCTION

Taenia saginata and *T. solium* are well-known cestodes which infect humans as definitive hosts. Their life-cycles also require an intermediate host (domestic animals). The disease in the intermediate host caused by these taeniid cestodes is called cysticercosis, whereas that in the definitive host is called taeniasis. In these taeniid cestode infections, it is well established that the intermediate mammalian host becomes completely immune to reinfection with eggs. There are some reports on the cross-immunity evoked by the heterologous species but the induced immunity is less effective than that induced by the homologous species. Cross-immunity is insufficient for practical vaccination trials in domestic animals (reviewed by Lightowlers, Mitchell & Rickard, 1993). In the experimental animal model system for cysticercosis, *T. taeniaeformis*/rat system, it has been reported that a single dose of frozen oncospheres with or without adjuvant is sufficient to evoke almost complete immunity to challenge infection with the homologous eggs (Ito & Hashimoto, 1993; Ito *et al.* 1994).

Recently, Fan and his colleagues reported a newly described human *Taenia* mainly from Asia (reviewed by Fan, 1988). Asian *Taenia* has a unique life-cycle intermediate between *T. saginata* (beef tapeworm) and *T. solium* (pork tapeworm). The definitive hosts for *T. saginata*, *T. solium* and Asian *Taenia* are only humans, while their intermediate hosts are pigs, except for cattle in *T. saginata* (Fan, 1992). The morphology of the adult tapeworm of Asian *Taenia* is very similar to *T. saginata* but the larval cysticercus is similar to *T. solium*. Although cysticerci of *T. saginata* or *T. solium* develop mainly in the muscle, those of Asian *Taenia* develop mainly in the liver (for more details, see Fan (1988)). Control or prevention of human taeniasis due to *T. saginata*, *T. solium* or Asian *Taenia* is expected to be effective only by (1) vaccination of cattle or pigs and/or (2) treatment and education of humans.

This study was designed to investigate whether pigs vaccinated with frozen oncospheres of Taiwan *Taenia*, Korea *Taenia*, *T. saginata* and *T. solium* become resistant to the challenge infection with viable eggs of Taiwan *Taenia* 41 days after vaccination. This work will provide additional information on the immunological relationships between these parasites.

MATERIALS AND METHODS

Parasites

Eggs of Taiwan *Taenia* and *T. solium*, collected in Taiwan and China respectively, were prepared by Fan and Chung in Taiwan (Ito *et al.* 1997*a*). Eggs of Korea *Taenia* were prepared by Eom in Korea (Eom & Rim, 1992). Eggs of *T. saginata* were prepared by

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Ito in Japan (Ito *et al*. 1994). Eggs were prepared from gravid segments of these 4 specimens according to Takemoto *et al*. (1995). *In vitro* hatching of oncospheres was carried out according to the method of Rajasekariah, Mitchell & Rickard (1980) and Lightowlers, Mitchell & Rickard (1984) with some modification (Negita & Ito 1994). Viabilities of Taiwan *Taenia* and *T. solium* eggs were checked by our novel method using *scid* (severe combined immunodeficient) mice (Ito *et al*. 1997*a*, *b*). All oncospheres were kept frozen at −80 °C for at least 2 months (for Taiwan *Taenia* and *T. solium*), 6 months (for Korea *Taenia*), or 2 years (for *T. saginata*).

Animals

Small-Ear-Miniature (SEM) pigs are known to be the most susceptible for Taiwan *Taenia* egg infection (Fan, 1988). Fourteen SEM pigs (103–379 days old) and 1 Landrace-Small-Ear-Miniature (L-SEM) pig (181 days old) kept under very good sanitary conditions were bought from an animal farm in Taitung County, Taiwan. After purchase, they were kept in the animal centre at Yangming University, Taipei, Taiwan, where they were given a regular diet and drinking water every day in separate accommodation which was cleaned daily. Therefore, they were not exposed to *Taenia* infections.

Preparation of oncospheres

In vitro hatching (without activation) of oncospheres of Taiwan *Taenia*, Korea *Taenia*, *T. saginata* or *T. solium* was carried out using 0·5 % sodium hypochlorite (NaClO in PBS instead of distilled water) (Rajasekariah *et al*. 1980; Lightowlers *et al*. 1984). Hatched oncospheres were suspended in sterile PBS and adjusted to approximately 5×10^4/ml in Eppendorf tubes and kept immediately deep frozen (−80 °C) for 2 or more months before vaccination. Thawed oncospheres were emulsified with complete Freund's adjuvant (CFA).

Vaccination

Three SEM pigs each in the 4 experimental groups (Group A, Taiwan *Taenia*; Group B, Korea *Taenia*; Group C, *T. saginata*; Group D, *T. solium*) were injected subcutaneously with a mixture (0·4 ml) of $1{\cdot}6 \times 10^4$ non-viable oncospheres (0·2 ml) each of the 4 samples of *Taenia* and 0·2 ml of CFA. In the control group (Group E), 2 SEM pigs and 1 L-SEM pig were injected subcutaneously with a mixture (0·4 ml) of PBS and CFA.

Experimental infection, sacrifice and examination

All pigs were orally inoculated with viable eggs of Taiwan *Taenia* ($1{\cdot}6 \times 10^4$/0·5 ml PBS) each 41 days after vaccination. These pigs were killed by the electric shock technique 39–55 days after inoculation under the guideline for animal care. 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*. (1995).

RESULTS

The results are summarized in Table 1. Three pigs were used for each group, but 1 pig in Group A died before challenge. In Group A (Taiwan *Taenia*), 1 of the 2 SEM pigs was found to be infected with 20 cysticerci. All cysticerci were found only in the liver and were degenerated or calcified. In group B (Korea *Taenia*) and group C (*T. saginata*), only 1 of the 3 SEM pigs from each group was found to be infected with, respectively, 20 and 4 degenerated or calcified cysticerci. In group D (*T. solium*), all 3 SEM pigs were found to be infected with $160{\cdot}3 \pm 30{\cdot}1$ cysticerci (mean ± S.D.). All 481 cysticerci, recovered from group D as well as a total of 44 cysticerci from group A, B and C were degenerated or calcified and found exclusively in the liver. In group E (control), all 3 pigs were infected with $695{\cdot}3 \pm 637{\cdot}8$. One L-SEM pig harboured only 76 degenerated or calcified cysticerci, whereas the 2 SEM pigs harboured 668 and 1342 cysticerci. Of the 2010 cysticerci from the 2 SEM pigs 102 were mature but all others were degenerated or calcified. All cysticerci were found exclusively in the liver.

DISCUSSION

This study is the first demonstration of highly effective immunization of pigs against Taiwan *Taenia* infection using homologous or heterologous non-viable oncospheres of human *Taenia*. SEM pigs vaccinated with Taiwan *Taenia*, Korea *Taenia*, or *T. saginata* showed very strong resistance to challenge infection with viable eggs of Taiwan *Taenia*. Infection with Taiwan *Taenia* in pigs may be prevented by a single subcutaneous injection of non-viable homologous, Korean *Taenia* and *T. saginata* oncospheres, but not by those of *T. solium*, although the viability of *in vitro*-hatched oncospheres of *T. solium* and Taiwan *Taenia* used for this study was confirmed using our new method for evaluation using *scid* mice (Ito *et al*. 1997*a*). All cysticerci recovered from SEM pigs vaccinated with these *Taenia* were found to be degenerated or calcified within approximately 2 months of oral viable egg challenge, whereas some of them (5·1 %, 102/2010) recovered from the 2 SEM pigs in the control group were mature but not degenerated or calcified. Therefore, judged from degeneration or calcification of Taiwan *Taenia* cysticerci, all 4 group SEM pigs vaccinated with taeniid oncospheres showed some resistance. However, much more clear evidence of induced resistance

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Table 1. Protection against challenge infection with viable eggs of Taiwan *Taenia* in SEM pigs vaccinated with frozen oncospheres of Taiwan *Taenia* (group A), Korea *Taenia* (group B), *T. saginata* (group C), *T. solium* (group D), or none (group E)

(Vaccination and challenge infection for all individuals were carried out on the same days (day 0 and day 41). All pigs for vaccination trials (groups A, B, C, D) were injected subcutaneously with $1{\cdot}6 \times 10^4$ frozen oncospheres/0·2 ml of sterile PBS emulsified with the same volume of complete Freund's adjuvant (CFA). Pigs of control Group E were injected with PBS without any oncospheres emulsified with CFA. All pigs, challenged orally with $1{\cdot}6 \times 10^4$ viable eggs of Taiwan *Taenia* on day 41, were killed between 36 and 55 days after egg challenge (between day 77 and day 96).)

Group	$1{\cdot}6 \times 10^4$ frozen oncospheres of	Age of pig* (days after birth)	No. of pigs infected/No. of pigs challenged	No. of cysticerci
A	Taiwan *Taenia*	366, 379	1/2	0, 20
B	Korea *Taenia*	151, 271, 271	1/3	0, 0, 20
C	*T. saginata*	151, 151, 183	1/3	0, 0, 4
D	*T. solium*	182, 182, 183	3/3	151, 136, 194
E	None	103, 103, 181†	3/3	1342 (1)‡, 668 (101)‡, 76†

* At immunization (day 0).
† L-SEM pig. All others were SEM pigs.
‡ Number of mature cysticerci in parentheses.

was the recovery rates between the former 3 groups (A, B, C) and group D. In the majority of SEM pigs of groups A (1/2), B (1/3), and C(1/3), there was no recovery of cysticerci. In contrast, all SEM pigs of group D (3/3) were found to be infected. Furthermore, Taiwan *Taenia* cysticerci could only be viable in the liver of the swine host for a very short period (Fan *et al*. 1995), since most of the 36 to 55-day-old cysticerci from the 2 SEM pigs (1908/2010) in control group E were already degenerated or calcified.

Ito *et al*. (1994) reported that frozen oncospheres of Taiwan *Taenia* and *T. saginata* showed some minor difference in their immunogenicity as assessed in rats by the resistance to challenge infection with *T. taeniaeformis* eggs. Based on this report and the present results, it may be rational to conclude that immunogenicity of oncospheres of Asian *Taenia* assessed in vaccinated pigs by the resistance to challenge infection with Taiwan *Taenia* eggs is very similar to *T. saginata* but not to *T. solium*. This immunobiological information, however, does not exclude any difference but might rather suggest at least some minor difference between Asian *Taenia* and *T. saginata*, which may be supported by the previously reported DNA analysis (Zarlenga *et al*. 1991; Bowles & McManus 1994; Zarlenga & George 1995).

Vaccination by a single dose with more frozen oncospheres may be expected to show complete resistance but approximately $1{\cdot}6 \times 10^4$ oncospheres used in this study appeared to be sufficient to evoke immunity in pigs, similar to that in the *T. taeniaeformis*/rat system (see Ito & Hashimoto 1993). Vaccination with more oncospheres appears not to be feasible for practical vaccination trials as discussed by Johnson *et al*. (1989) (reviewed by Rickard 1989; Lightowlers *et al*. 1993). However, if we have a small local focus for taeniasis/cysticercosis like in Taiwan (Fan 1992), vaccination trials in pigs using native killed oncospheres and/or education of the local people may be fruitful.

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REFERENCES

BOWLES, J. & MCMANUS, D. P. (1994). Genetic characterization of the Asian *Taenia*, a newly described taeniid cestode of humans. *American Journal of Tropical Medicine and Hygiene* **50**, 33–44.

EOM, K. S. & RIM, H. J. (1992). Experimental human infection with Asian *Taenia saginata* metacestodes obtained from naturally infected Korean domestic pigs. *Korean Journal of Parasitology* **30**, 21–24.

FAN, P. C. (1988). Taiwan *Taenia* and taenaisis. *Parasitology Today* **4**, 86–88.

FAN, P. C. (1992). Taeniasis in Taiwan: a review. *Chinese Journal of Parasitology* **5**, 1–21.

FAN, P. C., LIN, C. Y., CHEN, C. C. & CHUNG, W. C. (1995). Morphological description of *Taenia saginata asiatica* (Cyclophylidea: Taeniidae) from man in Asia. *Journal of Helminthology* **69**, 299–303.

ITO, A. & HASHIMOTO, A. (1993). Vaccination with hatched but non-activated, non-viable oncospheres of *Taenia taeniaeformis* in rats. *Journal of Helminthology* **67**, 165–168.

ITO, A., FAN, P. C., CHUNG, W. C. & SUZUKI, M. (1994). Cross-protection against *Taenia taeniaeformis* in rats vaccinated with non-viable oncospheres of Asian *Taenia* or *T. saginata*. *Journal of Helminthology* **68**, 83–85.

ITO, A., CHUNG, W. C., CHEN, C. C., ITO, M., ENDO, S., OKAMOTO, M. & FAN, P. C. (1997*a*). Human *Taenia* eggs develop into cysticerci in *scid* mice. *Parasitology* **114**, 85–88.

ITO, A., EOM, K. S., CHUNG, W. C., CHEN, C. C., FAN, P. C., MA, L., ENDO, S. & ITO, M. (1997*b*). *In vitro* hatched oncospheres of Asian *Taenia* from Korea and Taiwan develop into cysticerci in the peritoneal cavity of female scid (severe combined immunodeficient) mice. *International Journal for Parasitology* **27**, (in the Press.)

LIGHTOWLERS, M. W., MITCHELL, G. F. & RICKARD, M. D. (1984). Immunisation against *Taenia taeniaformis* in mice: studies on the characterisation of antigen from oncospheres. *International Journal for Parasitology* **14**, 321–333.

LIGHTOWLERS, M. W., MITCHELL, G. F. & RICKARD, M. D. (1993). Cestodes. In *Immunology and Molecular Biology of Parasitic Infections* (ed. Warren, K. S.), pp. 438–472. Blackwell Scientific Publications, Oxford.

NEGITA, T. & ITO, A. (1994). *In vitro* hatching of oncospheres of *Taenia taeniaeformis* using eggs isolated from fresh, frozen, formalin-fixed and ethanol-fixed segments. *Journal of Helminthology* **68**, 271–272.

RAJASEKARIAH, G. R., MITCHELL, G. F. & RICKARD, M. D. (1980). Density-gradient separation of *Taenia pisiformis* oncospheres. *Journal of Parasitology* **66**, 355–356.

RICKARD, M. D. (1989). A success in veterinary parasitology: cestode vaccines. In *New Strategies in Parasitology* (ed. McAdam, K. P. W. J.), pp. 3–14. Churchill Livingstone, Edinburgh.

TAKEMOTO, Y., NEGITA, T., OHNISHI, K., SUZUKI, M. & ITO, A. (1995). A simple method for collecting eggs of taeniid cestodes from fresh, frozen or ethanol-fixed segments. *International Journal for Parasitology* **25**, 537–538.

ZARLENGA, D. S., MCMANUS, D. P., FAN, P. C. & CROSS, J. H. (1991). Characterization and detection of a newly described Asian taeniid using cloned ribosomal DNA fragments and sequence amplification by the polymerase chain reaction. *Experimental Parasitology* **72**, 174–183.

ZARLENGA, D. S. & GEORGE, M. (1995). *Taenia crassiceps*: cloning and mapping of mitochondrial DNA and its application to the phenetic analysis of a new species of *Taenia* from Southeast Asia. *Experimental Parasitology* **81**, 604–607.