Further evaluation of the synthetic peptide vaccine S3Pvac against *Taenia solium* cysticercosis in pigs in an endemic town of Mexico

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SUMMARY

Taenia solium cysticercosis is a parasitic disease frequently affecting human health and the pig industry in many developing countries. A synthetic peptide vaccine (designated S3Pvac) against porcine cysticercosis has been developed previously as an aid to interrupt transmission and has been shown to be effective. The results of the present study support the effectiveness of the vaccine under endemic field conditions. However, given the time-frame of the vaccination trial, no changes in the local levels of transmission were detectable before and after vaccination using sentinel pigs. Thus, this investigation shows the limited usefulness of single vaccination as the sole means of interrupting *Taenia solium* transmission in an endemic region.

Key words: *Taenia solium*, cysticercosis, control, vaccination, field evaluation.

INTRODUCTION

Taenia solium taeniasis/cysticercosis is a major parasitic disease of humans and/or pigs which seriously affects human health and the economy in developing countries in Latin America, Asia and Africa (Sciutto *et al*. 2000; Carabin *et al*. 2006). As it is progressively being recognized as a key disease (Boa *et al*. 2003; Phiri *et al*. 2003), the development of more effective control measures against cysticercosis is needed. As an adjunct to other measures, the vaccination of pigs against cysticercosis promises to be a useful preventative and control tool, because of the role that pigs play in the life-cycle of *T. solium* and the life-styles of rural people in endemic regions (Sciutto *et al*. 2000). Vaccination of the porcine (intermediate) host could reduce or eliminate the transmission to humans (definitive and accidental intermediate host), thus decreasing the environmental contamination with *T. solium* eggs and diminishing the impact of transmission and disease, particularly neurocysticercosis in humans (Huerta *et al*. 2001; González *et al*. 2005).

Various whole or subunit vaccine candidates with promising protective effects against porcine cysticercosis have been reported, but their effectiveness has been assessed mostly experimentally rather than under complex field conditions (Nascimento *et al*. 1995; Molinari *et al*. 1997; Plancarte *et al*. 1999; Cai *et al*. 2001; Wang *et al*. 2003; Flisser *et al*. 2004; Manoutcharian *et al*. 2004; Gonzalez *et al*. 2005). Such a vaccine requires testing in local breeds of pigs, reared under extensive (free-range) conditions, being exposed to famine, stress and various diseases other than cysticercosis. Recently, a vaccine to protect against porcine cysticercosis, based on 3 synthetic-peptides (designated S3Pvac) present in all stages of development of *T. solium*, was developed and shown to be effective in mice against *Taenia crassiceps* (see Toledo *et al*. 1999, 2001) and in pigs against *T. solium* (see Manoutcharian *et al*. 1996; Huerta *et al*. 2001). Its protective capacity (50% reduction in prevalence levels and 98% reduction of intensity of infection)

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Table 1. Percentage of free-range pigs with cysticercosis included in the vaccination trial in the town of Cuentepec in Mexico

(Prior to the vaccination trial, sentinel pigs (Group S1) were randomly selected and examined for cysticerci (cysts). The vaccination trial included pigs which were administered the adjuvant saponin (Group V0), and pigs which were injected once (Group V1) or twice (Group V2) with S3Pvac in saponin and then examined for cysts 4–8 months later. Two additional examinations for cysts in sentinel pigs were performed 8 (Group S2) and 22 (Group S3) months after the termination of the vaccination trial.)

Group of pigs	Group description	Date of inspection for cysticerci by tongue examination	No. of pigs with cysticerci/total no. of pigs examined (% with cysticercosis)	
S1	Sentinel; prior to vaccination	April–May 2001	4/29	(13·8)
			8/29	(27·6)°
V0	Saponin	June–October 2001	2/20	(10·0)
V1	S3Pvac (1 dose) + Saponin	June–October 2001	2/48	(4·2)
V2	S3Pvac (2 doses) + Saponin	June–October 2001	3/98	(3·1)
S2	Sentinel; after vaccination	July 2002	24/200	(12·0)
S3	Sentinel; after vaccination	September 2003	8/50	(16·0)

° Also examined for cysts by necropsy.

was demonstrated against naturally-acquired cysticercosis in piglets free-ranging in Tepetzitzintla, Puebla, Mexico (Huerta *et al*. 2001).

To be broadly applicable, a vaccine against cysticercosis should also be effective under a variety of conditions in which the disease occurs naturally and under different transmission/infection pressures. For this reason, the vaccine S3Pvac was assessed in Cuentepec (Morelos), a densely populated rural town in Mexico with a high prevalence of porcine cysticercosis (Morales *et al*. 2002). This town differs significantly in socio-economic, cultural and climatic characteristics from Tepetzitzintla (*cf*. Morales, 2003) in which this vaccine was tested previously (Huerta *et al*. 2001).

MATERIALS AND METHODS

The synthetic peptide vaccine, S3Pvac

The S3Pvac vaccine consists of the following synthetic peptides: GK1 (amino acids [aa]; (GYYYPSDPNTFYAPPYSA); KETc1 (APMSTPSATSVRG) and KETc12 (GNLLLSCLG). The peptides were prepared commercially (Invitrogen Corporation). Each of these peptides was shown to be 95% pure, based on analysis using high-pressure liquid chromatography employing analytical C18 reverse-phase columns (3·9 × 150 nm; Delta Park, Waters). The aa sequence of each peptide was verified by protein sequencing using a pulsed liquid-phase sequencer (Applied Biosystems). Each dose of vaccine per pig comprised 250 μg of each of the peptides KETc1, KETc12 and GK1 as well as saponin (500 μg). The vaccine was stored at 4 °C.

Community selected as the study site

Cuentepec, Temixco, in the State of Morelos, México, was selected because the epidemiological conditions in this town favour the transmission of *T. solium*. In this town, there are (i) a high prevalence of porcine cysticercosis (Morales *et al*. 2002), (ii) defaecation by humans in open latrines, (iii) open-range pig rearing, (iv) extensive domestic slaughtering of pigs and local consumption of uninspected and cysticercotic pork (Morales *et al*. 2002; Morales, 2003; Fleury *et al*. 2006). Also, Cuentepec differs from the site of the previous trial of vaccine S3Pvac (Huerta *et al*. 2001) in that it is less isolated socially, more densely populated and represents a more humid, temperate and fertile rural area (http://www.elocal.gob.mx/work/templates/enciclo/morelos/Municipios/17018a.htm).

Surveys of cysticercosis in sentinel pigs before and after vaccination

'Sentinel pigs' (7–12 months of age; both sexes) from Cuentepec, living under the same conditions as those included in the vaccination trial, were randomly selected and inspected for cysticercosis before and after the trial. Prior to the vaccination trial (April–May, 2001), 29 sentinel pigs were slaughtered and examined for cysticercosis by tongue inspection and by whole-body necropsy, according to the procedures described elsewhere (Gonzalez *et al*. 1990). After the termination of the vaccination trial in October 2001, 2 other evaluations of cysticercosis in sentinel pigs (Groups S2 and S3) were conducted by tongue inspection (200 pigs in July 2002, and 50 different pigs in September 2003) (see Table 1).

Vaccination trial

A total of 476 piglets that had reached approximately 2 months of age in the period between June and October of 2001 were considered for inclusion in the study. Ultimately, 381 piglets (80%) were included;

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the other 95 belonged to owners who refused to provide pigs for the trial. Each piglet included was tagged with a microchip, and its sex and its owner's name and address were recorded. Pigs were first injected (subcutaneously at the base of an ear) at 2 months of age. The following groups of control and vaccinated piglets were formed: Group V0, 1 control pig in every 2 litters received saponin alone ($n=32$); Group V1, 1 piglet from each litter received 1 dose of the S3Pvac vaccine at ($n=95$); Group V2, the rest of the piglets ($n=254$) received 2 doses of S3Pvac (the second injection was given 1 month after the first).

Diagnosis of cysticercosis in pigs

The diagnosis of porcine cysticercosis was conducted *in vivo* by tongue inspection for subepithelial cysts. This is considered the most practical method available; it achieves a sensitivity of 25–75%, and a specificity of up to 100% (Viljoen, 1937; Gonzalez *et al*. 1990; Sciutto *et al*. 1998). Diagnosis was performed 4–8 months after the termination of the vaccination trial, because, in rural communities, most pigs are consumed or sold at 7–12 months of age. If pigs were found to be cysticercotic, their owners were informed about the diagnostic finding and advised not to consume uncooked pork or sell infected pigs.

Questionnaire

Twice during the study, in September 2000 and May 2001, owners were asked to answer a questionnaire designed to 'track' possible changes in factors affecting the prevalence of porcine cysticercosis in the community (e.g., governmentally implemented treatment programmes of humans, or the exchange of healthy for sick pigs) or to its estimation (e.g., biased sale of diseased pigs, slaughter or consumption of cysticercotic or vaccinated pigs).

Statistical analysis

Data were processed in Excel 7.0 (Microsoft). Statistical analyses were performed using the Instat Software Program (GraphPad, San Diego, California), EPIINFO 6.0 (CDC Atlanta, Georgia, 2002). The Fisher's exact test was used (95% confidence level).

RESULTS

The percentages of sentinel pigs detected to be cysticercotic by tongue inspection were 13·8% (April–May, 2001, Group S1), 12% (July 2002, Group S2) and 16% (September 2003, Group S3) (Table 1); the differences between these percentages were not statistically significant. The estimate of porcine cysticercosis by necropsy in the sentinel pig group S1 was 27·6%, which is significantly greater than that estimated by tongue inspection in the same pigs (13·8%); thus, tongue inspection had a sensitivity of 50% compared with that of necropsy (data not shown).

The vaccination trial included 381 piglets, 166 (44%) of which were recovered 4–8 months later, at the time of inspection, whereas 215 (56%) were reported to be sold or missing by their owners. Table 1 shows the percentages of pigs with cysticercosis included in the vaccination trial. In Group V0, 2/20 (10%) pigs were found to be infected, whereas 4·2% (2/48) and 3·1% (3/98) were found to be infected in Groups V1 and V2, respectively. There was no statistical difference in the percentage of cysticercotic pigs between Groups V1 and V2 ($P>0·05$). When pooled, the percentage of cysticercotic pigs in the vaccinated groups (V1 and V2) was 3·4% (5/146). Although the percentage of cysticercotic pigs was less than 50% of that of the saponin group (V0), statistical analysis did not support a significant difference between them, likely to be due to the difference in sample sizes ($P=0·2$; OR = 0·31, CI [0·05–1·76]). However, the 3·4% (for cysticercotic, vaccinated pigs) was significantly different from the percentage of cysticercotic sentinel pigs in Groups S1, S2, and S3, before or after the vaccination trial (Table 1).

DISCUSSION

The results from this field trial carried out in the rural town of Cuentepec, Mexico, support the protective effect of the vaccine S3Pvac against porcine cysticercosis reported previously for the endemic village of Tepetzitzintla (Huerta *et al*. 2001), despite significant epidemiological, ecological and social differences between the two villages.

The percentage of cysticercotic pigs found in the different groups of sentinel pigs, before and after the vaccination study, did not change significantly. The apparent stability of the prevalence in sentinel pigs from the same town suggests that a single vaccination is not sufficient to decrease the impact of *T. solium* transmission at the community level, possibly because the number of human carriers of adult *T. solium* under the local, rural life-style is not affected in such a short a time-frame of diminished cysticercus supply. Thus, the beneficial effects of vaccinating pigs may be obscured on a larger scale if other risk factors remain unchanged (i.e. the tapeworms' long life-span assures ready transmission of infective eggs to a new generation of naïve pigs). This resembles the situation that exists for *Taenia ovis*, *Taenia hydatigena* and *Echinococcus granulosus* control programmes for which, after thorough therapeutic intervention, levels of cysticercosis in sheep are promptly re-established through the persistence or new arrival of dogs infected with adult

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worms (*cf*. Gemmell, 1978; Cabrera *et al*. 2002). The time lag between a reduction in the prevalence of porcine cysticercosis and the prevalence of human carriers of adult *T. solium* may impact on the usefulness of vaccination in controlling or preventing this disease, if not sustained. Also, there is a need for a simultaneous implementation of other measures in control programmes seeking to rapidly and thoroughly interrupt transmission; these include specific and general health educational programmes, the elimination of human carriers of adult *T. solium* in the community, encouragement of confined (indoor) instead of free-range pig farming, improvement of pork inspection and cooking methods, discouragement of the sale of cysticercotic pigs to traffickers, etc.

Notwithstanding their complications (costs, labour intensiveness and loss of pigs) and the imprecision of tongue inspection for cysticercosis, field testing of vaccines against cysticercosis is critical in the evaluation of their true effectiveness and usefulness. To be applicable, a vaccine must be effective not only under experimental conditions but, importantly, must be effective in the diverse and changing natural conditions of transmission. The factors involved in the latter situation are complex and include mixed pig breeds, variable exposure to *T. solium* material from the environment, possible seasonal changes in levels of transmission, variable pigs' health status, stress due to famine and various infectious and non-infectious diseases, and the different life-styles and migration patterns linked to the human population. Clearly, much more work is required to optimize prevention and control programmes involving vaccination against porcine cysticercosis in endemic areas.

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