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SHORT REPORT: VACCINATION OF PIGS TO CONTROL HUMAN NEUROCYSTICERCOSIS

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Abstract. *Taenia solium* taeniasis/cysticercosis is a zoonotic disease complex in which the pig is an obligate intermediate host. The infection is widespread, particularly in the developing world, and neurocysticercosis is a major cause of human neurologic disease where the parasite is endemic. Despite easy availability, effective anti-parasitic drugs have not been deployed effectively to control disease transmission. We have investigated a vaccine strategy to prevent parasite infection of the pig intermediate host. Such a strategy would interrupt the parasite's life cycle and eliminate the source of infection for humans. Two recombinant antigens selected from the parasite oncosphere life cycle stage were tested in vaccination trials in pigs that were challenged orally with *Taenia solium* eggs. Both antigens were highly effective in protecting the pigs against infection with the parasite (98.6% and 99.9% protection, respectively). No viable cysts were found in eight pigs vaccinated with one of the antigens. A recombinant subunit vaccine based on oncosphere antigens has the potential to improve the available control measures for *T. solium* and thereby reduce or eliminate neurocysticercosis.

The cestode parasite *Taenia solium* causes human neurocysticercosis, the most important cause of seizure disorders worldwide.[1] While most commonly occurring in the developing world, this disease also occurs in industrialized countries due either to the immigration of already infected patients or through infections acquired by direct or indirect contact with individuals from disease-endemic regions who harbor the adult parasite.[2] *Taenia solium* is transmitted from pigs, which harbor the larval parasites in their muscles as intermediate hosts, to humans, the definitive hosts who have adult tapeworms in their intestines. Pigs become infected by ingesting *T. solium* eggs in the feces of a person carrying the tapeworm. Humans become infected with intestinal tapeworms after ingesting raw or incompletely cooked infected pig meat. The clinical significance of the parasite to humans is not because of infection with the adult tapeworm, but because ingested tapeworm eggs leads to hematogenously disseminated larval cysts. When located in the central nervous system, these cysts cause neurocysticercosis, characterized by seizure disorders, psychiatric disturbance, hydrocephalus, and other neurologic conditions.

Transmission of *T. solium* has decreased or been eliminated in much of the first world over the last century as a result of improvements in general sanitation and public health. However, similar improvements in public living conditions are unlikely to occur in the short term in much of the developing world where cysticercosis is highly endemic, and other parasite control measures are required to have a more immediate impact on the prevalence of this disease. Neurocysticercosis is potentially eradicable[3] since inexpensive and highly effective anthelmintics are available that could potentially be used to eliminate human tapeworm infections. To date, these measures have not achieved sustainable control of *T. solium* transmission. One major barrier of the use of anthelmintics to control *T. solium* is that despite the elimination of tapeworm carriers by, for example, mass treatment of the population with drugs to remove tapeworms, pig infection will remain. This reservoir of infection can readily establish new tapeworm infections of humans and renewed disease transmission.

We have sought to develop a vaccine to prevent *T. solium* infection of pigs, thereby removing the source of *T. solium* tapeworm infections for humans.[4] Proteins contained within the parasite eggs (known as oncospheres) are known to be a source of protective antigens.[5,6] Furthermore, recombinant oncosphere antigens have been found to be highly effective as vaccines for prevention of infections with closely related parasites of sheep and cattle.[7] *Taenia solium* homologs of the protective antigens for these veterinary parasites have been identified[8,9] and recombinantly expressed in *Escherichia coli*.[4,7] These recombinant vaccines (TSOL18 and TSOL45), produced as glutathione-S-transferase (GST) fusion proteins, were recently found to have high efficacy in preventing pig infections in experimental trials in Mexico and Cameroon.[4] Here we describe efficacy results of a TSOL18 and TSOL45 vaccination trial of pigs in Peru, with *in vitro* correlates of immunity performed by immunoblotting.

Twenty six three-month-old pigs (14 males and 12 females, Pietrain/Landrace mixed breed) were used in the vaccine trial. All vaccinations were given with 1 mg of Quil A (Superflos Biosector, Vedbaek, Denmark) as adjuvant. Two groups of eight pigs each were vaccinated with either TSOL18 or TSOL45. TSOL18 was given as a GST fusion protein[4] (two 200-μg doses spaced four weeks apart). TSOL45 was given as two injections of 200 μg as a GST fusion protein[4] spaced four weeks apart, and an additional injection of 200 μg as a recombinant maltose binding protein (MBP) fusion protein[4] two weeks later. Five control pigs were immunized with 200 μg of recombinant GST only (two doses spaced four weeks apart), and a second group of five control pigs received successively two immunizations of 200 μg of GST spaced four weeks and one immunization two weeks later with 200 μg of recombinant MBP. Ten days after their final immunization, all pigs received an oral challenge infection with one full gravid *T. solium* proglottid obtained from treatment of tapeworm carriers in the previous three weeks and stored in glyc-

erol. Twelve weeks after the challenge infection, pigs were humanely killed and the entire carcass and brain were assessed for the presence and viability of cysticerci.[6] Parasites were classified as viable if a defined cystic structure with liquid content was still present, and degenerate if this had been replaced by semi-solid contents or an inflammatory scar. The study was reviewed and approved by the Animal Ethics Committee of the School of Veterinary Medicine, Universidad de San Marcos, Lima, Peru and the Johns Hopkins School of Public Health. Details of the immunization schedule and parasite/cyst loads of pigs after challenge infection are shown in Table 1.

Numerous viable cysticerci were found in each of the 10 control pigs (mean = 1,634 cysts per animal, median = 1,429, range = 69–5,336). No viable cysts and four degenerated parasites were found in TSOL18-vaccinated animals (mean = 0.5 cysts per animal, median = 0, range = 0–2). A single viable cyst and 178 degenerated parasites were found in TSOL45-vaccinated pigs (mean = 22.4 cysts per animal, median = 2.5, range = 0–98). Five of eight pigs vaccinated with TSOL18 and three of eight vaccinated with TSOL45 were found to be completely free of any infection. These findings show that TSOL18 and TSOL45 vaccines induced a high level of protection (99.98% and 98.6%, respectively; $P = 0.002$, by Mann-Whitney test).

To determine whether the TSOL18 or TSOL45 vaccines induced antibodies that recognized native *T. solium* oncosphere proteins, sera from vaccinated and control pigs were analyzed by immunoblot, using an assay we have previously published.[6] Antisera against TSOL18 reacted with a single antigen of approximately 20 kD, while antisera raised to TSOL45 identified three prominent bands with sizes of 35, 38, and 39 kD, and showed less prominent reactivity against 20-kD and 25-kD antigens. Pre-immunization control sera showed a thin reactive band at 20 kDa, indistinguisable in altitude from the 20 kDa antibody band obtained in response to TSOL18 (Figure 1). The size of the above native antigens is larger than the sizes predicted by the associated cDNAs, possibly due to glycosylation at predicted N-linked glycosylation sites.[8,9] The detection of several oncosphere antigens with antisera raised against TSOL45 is consistent with the previously published observation that the gene encoding this protein is transcribed into three differentially spliced mRNAs.[8] The antigens recognized by antisera against TSOL45 appeared to correspond to relatively prominent components detected by antisera raised against whole oncosphere extract (lane 4 in Figure 1).[6] However, the component detected with antisera raised against TSOL18 did not correspond to any of the dominant specificities detected by pigs vaccinated and protected[6] by immunization with whole oncosphere extract, possibly due to cleavage of a larger antigen.

This study confirms and extends the utility of the TSOL18 and TSOL45 vaccine recombinant proteins as a vaccine capable of preventing pig infection by *T. solium*. These results are consistent with those recently reported in Mexico and Cameroon.[4] Of note, Western immunoblot provided an ex-

TABLE 1

Numbers of *Taenia solium* cysticerci in vaccinated (n = 16) and control (n = 10) pigs and the level of protection afforded by vaccination*

	Cyst count by tissue										
	Skeletal muscle		Heart		Tongue		Brain				
Group	V	D	V	D	V	D	V	D	Total cysts	Total mean	Protection† %
Controls											
GST	2,313	341	28	82	23	20	3	0	2,810	1,634.3‡	–
GST	486	572	2	58	0	23	5	0	1,146		
GST	65	104	1	10	2	4	0	0	186		
GST	1,614	61	8	15	6	4	3	0	1,711		
GST	1,720	386	23	3	10	0	1	0	2,143		
GST/MBP	719	975	35	38	13	5	0	0	1,785		
GST/MBP	4,969	17	231	24	85	3	7	0	5,336		
GST/MBP	15	50	0	3	0	0	1	0	69		
GST/MBP	689	276	34	6	12	0	4	0	1,021		
GST/MBP	80	48	0	5	1	1	1	0	136		
Vaccinated											
TSOL 18	0	1	0	0	0	0	0	0	1	0.5	99.9
TSOL 18	0	2	0	0	0	0	0	0	2		
TSOL 18	0	1	0	0	0	0	0	0	1		
TSOL 18	0	0	0	0	0	0	0	0	0		
TSOL 18	0	0	0	0	0	0	0	0	0		
TSOL 18	0	0	0	0	0	0	0	0	0		
TSOL 18	0	0	0	0	0	0	0	0	0		
TSOL 18	0	0	0	0	0	0	0	0	0		
TSOL 45	0	4	0	0	0	0	0	0	4	22.3	98.6
TSOL 45	0	1	0	0	0	0	0	0	1		
TSOL 45	0	0	0	0	0	0	0	0	0		
TSOL 45	0	0	0	0	0	0	0	0	0		
TSOL 45	0	27	0	2	0	1	0	0	30		
TSOL 45	0	0	0	0	0	0	0	0	0		
TSOL 45	1	84	0	13	0	0	0	0	98		
TSOL 45	0	44	0	2	0	0	0	0	46		

* V = viable; D = degenerated; GST = glutathione-S-transferase; MBP = MBP = maltose binding protein.
† Calculated as the percentage reduction in the mean number of cysts in comparison with controls ($P < 0.01$, by Mann-Whitney test).
‡ Since statistical comparison between the two control groups indicated no significant difference ($P > 0.05$, by Mann-Whitney test), the data were combined for comparisons with other groups.

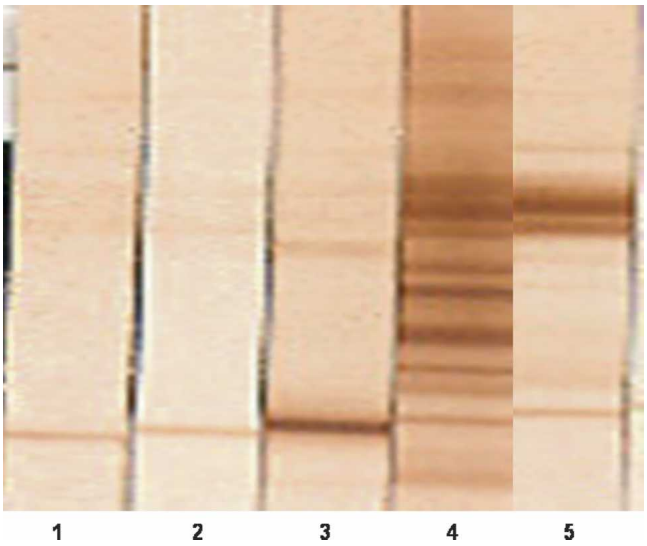

FIGURE 1. Immunoblots of *Taenia solium* oncosphere extract (0.56 μg per mm of gel) reacting with antisera from a pig vaccinated with glutathione-S-transferase only (lane 1), a non-immunized animal (lane 2), the same pig after vaccination with the recombinant TSOL18 (lane 3), a pig vaccinated with whole oncosphere extract (lane 4), and a pig vaccinated with TSOL45 (lane 5). Oncosphere components were separated by non-denaturing, 5–22.5% gradient sodium dodecyl sulfate–polyacrylamide gel electrophoresis. This figure appears in color at www.ajtmh.org

cellent *in vitro* correlate of protective immunity. The high degree of protective immunity induced by the two *T. solium* antigens described here in preventing experimental infection of pigs suggests that a practical and effective vaccine, based on disrupting the parasite life cycle, vis à vis the intermediate host, is achievable. With this strategy as an additional tool to control *T. solium* transmission, the goal of disease eradication should be considered achievable, particularly because the ability of these recombinant vaccines is reproducible in different countries and by different groups. Although operational and practical issues have to be addressed before this vaccine can be deployed, porcine vaccination is feasible to prevent new *T. solium* infections as part of eradication schemes.

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