Anamnestic responses in pigs to the *Taenia solium* TSOL18 vaccine and implications for control strategies

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SUMMARY

Specific antibody responses were assessed in pigs immunized with the *Taenia solium* vaccine TSOL18. Anti-TSOL18 responses were compared 2 weeks after secondary immunization, where the interval between primary and secondary immunization was 4, 8, 12, 16 or 20 weeks. All animals responded to the vaccine and there was no diminution in antibody responses in animals receiving their second injection after an interval up to 20 weeks. Pigs receiving vaccinations at an interval of 12 weeks developed significantly increased antibody responses compared with animals receiving immunizations 4 weeks apart ($P = 0{\cdot}046$). The ability to deliver TSOL18 vaccination effectively where the revaccination schedule can be delayed for up to 12–16 weeks in pigs increases the options available for designing *T. solium* control interventions that incorporate TSOL18 vaccination.

Key words: *Taenia solium*, pig, vaccine, TSOL18, anamnestic response.

INTRODUCTION

Taenia solium is the aetiological agent of neurocysticercosis in humans and may be associated with as many as 29% of cases of seizure disorders in endemic areas (Ndimubanzi *et al*. 2010). *Taenia solium* has been identified as one of a small number of human disease causing agents that have the potential to be eradicated (Anonymous, 1992; Schantz *et al*. 1993). The World Health Organization identifies neurocysticercosis as a Neglected Tropical Disease and has prioritized *T. solium* as the focus for new control initiatives (World Health Organization, 2015).

The full life cycle of *T. solium* is only perpetuated among poor people living in the world's poorest countries, where pigs roam freely and human defecation occurs in places where pigs have access to their faeces. A number of potential interventions are available for *T. solium*; however few, if any, specific interventions have led to a sustained decrease in the parasite's transmission (Lightowlers, 2013).

Pigs act almost exclusively as the natural animal intermediate host for *T. solium*. Two key intervention measures have been developed for pigs that have the potential to prevent *T. solium* transmission and thereby break the parasite's life cycle. Treatment of infected pigs with 30 mg kg^{-1} oxfendazole kills all cysticerci present in the muscle tissues (Gonzalez *et al*. 1997; Sikasunge *et al*. 2008). Vaccination of pigs with the TSOL18 recombinant antigen induces a high level of protection from subsequent exposure to the parasite (Flisser *et al*. 2004; Gonzalez *et al*. 2005). In a field trial carried out in Cameroon, a combination of both TSOL18 vaccination and a single treatment of pigs with oxfendazole eliminated *T. solium* transmission (Assana *et al*. 2010).

A limitation to interventions for *T. solium* that target pigs is that swine do not generally have a breeding season and hence new, susceptible individuals are born constantly into the pig population. There remains a gap in knowledge concerning the principles about how to apply vaccination and chemotherapy in pigs under field conditions in a way that would be effective, feasible and sustainable, and which could be adapted to take into account local cultural and pig management practices. In a review of the predicted effectiveness of a number of potential intervention scenarios for pigs, Lightowlers (2013) identified a schedule involving 4-monthly treatment of pigs with both TSOL18 and oxfendazole as being likely to achieve a high level of disease control, whilst minimizing the

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number of interventions that would be required on an annual basis. However, it was not possible at that time to recommend a vaccination scenario involving an interval of more than 4 weeks because there was no knowledge about whether or not a longer interval between primary and secondary immunizations with TSOL18 would lead to an adequate immune response to the vaccine.

The available evidence indicates that a principal, if not the only, immune mechanism induced in pigs by the TSOL18 vaccine is the lysis of the early developing parasite by specific circulating antibody and complement (Lightowlers, 2006). Specific antibody titres to TSOL18 have been used to evaluate responses to the vaccine that are associated with protection (Kyngdon *et al*. 2006; Assana *et al*. 2010). Here we present the results of an immunization trial carried out in pigs where the animals received booster immunizations at different time intervals after the primary vaccination, and evaluate the different vaccination schedules by assessing the anti-TSOL18-specific antibody titres that were induced.

MATERIALS AND METHODS

Pigs

Fifty, healthy Landrace-Pietran cross pigs approximately 12 weeks of age, comprising half-male and half-female animals were allocated randomly to 5 equal treatment groups, T1–T5. The animals were given feed and water *ad libitum* and housed indoors at a research farm under commercial-style hygienic conditions. Group sizes were determined based on prior research (Kyngdon *et al*. 2006) where 34 pigs developed a geometric mean anti-TSOL18 titre of 4634 (geometric S.D. = 0·978); we estimated that to have 80% power and 95% confidence interval (CI) of detecting a statistically significant difference, if there was a 4-fold difference between any of the treatment groups and the control group (interval between primary and secondary immunization of 28 days), we would require a minimum of 9 animals per treatment group. Allowing for inaccuracy in the assumptions underlying these estimates and/or the death of animals, we elected to allocate 10 pigs per treatment group.

TSOL18 vaccine

The TSOL18 cDNA sequence was cloned into *Pichia pastoris* GS115 using standard protocols (Invitrogen, USA). Secreted TSOL18 protein was harvested following *P. pastoris* bioreactor culture in fermentation basal salts media (Stratton *et al*. 1998). The culture supernatant was passed through a series of filters to remove yeast solids, concentrated by tangential flow filtration and dia-filtered using phosphate-buffered saline. Filter sterilized TSOL18 antigen was quantified by Coomassie dye-binding assay and densitometry of Coomassie-stained SDS polyacrylamide gels. Vaccine was prepared as a 1 mL dose containing 150 μg TSOL18 protein plus ISA206 adjuvant (SEPPIC) and 0·01% Thiomersol.

Vaccinations and sera

All pigs received a primary immunization intramuscularly in the neck on day 0. The five different treatment groups received identical secondary immunizations after 4, 8, 12, 16 or 20 weeks (T1–T5, respectively). Serum samples were obtained from each animal prior to vaccination and 2 weeks after the second immunization and were stored frozen for up to 9 months at −20 °C or below, prior to being assayed in enzyme-linked immunosorbent assay (ELISA). Experimental procedures were consistent with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

ELISA and statistical analyses

Anti-TSOL18-specific IgG titres were determined using a TSOL18 maltose-binding protein fusion as antigen and serial serum dilutions at 1:100, essentially as described by Kyngdon *et al*. (2006). Antibody titres were compared between groups by comparing $\log_2$-transformed (geometric) mean titres for the treatment groups followed by linear regression with back-transformation of results for interpretation, and making no distributional assumptions with the Wilcoxon rank sum test on the raw data.

RESULTS

All animals were serologically negative (optical density <1·0 at 1:200 dilution) prior to vaccination. Anti-TSOL18-specific antibody titres in individual animals 2 weeks after the second vaccination with TSOL18 are detailed in Table 1 and Fig. 1, and the data summarized in Table 2. All animals exhibited an anamnestic response after receiving their second vaccination. Antibody titres in groups receiving the second immunization more than 4 weeks after the primary immunization developed higher mean titres than pigs receiving their two immunizations 4 weeks apart. Comparison of antibody titres between animals receiving their second injection after an interval of 4 weeks, with groups receiving their second immunization at a later time point are presented in Table 3. Animals immunized at an interval of 12 weeks had 3·1 times the antibody titre of those immunized at an interval of 4 weeks (95% CI: 1·02, 9·38; $P = 0·046$; Wilcoxon rank sum test $P = 0·049$). Other comparisons were not statistically significant.

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Table 1. Individual animal anti-TSOL18-specific IgG titres in sera collected 2 weeks after the second immunization

Animal number	Sampling day	V1/V2 interval (weeks)[a]	Anti-TSOL18 titre	Animal number	Sampling day	V1/V2 interval (weeks)[a]	Anti-TSOL18 titre
1	42	4	400	31	126	16	14 000
2	42	4	1400	32	126	16	15 000
3	42	4	2800	33	126	16	600
4	42	4	2600	34	126	16	11 000
5	42	3	1650	35	126	16	1000
6	42	4	3250	36	126	16	850
7	42	4	6450	37	126	16	2500
8	42	4	700	38	126	16	10 000
9	42	4	15 600	39	126	16	600
10	42	4	750	40	126	16	1400
11	70	8	11 700	41	154	20	11 000
12	70	8	5500	42	154	20	5500
13	70	8	2600	43	154	20	5500
14	70	8	5800	44	154	20	11 000
15	70	8	1700	45	154	20	450
16	70	8	6000	46	154	20	13 500
17	70	8	2500	47	154	20	325
18	70	8	6000	48	154	20	ND[b]
19	70	8	17 200	49	154	20	1800
20	70	8	1200	50	154	20	240
21	98	12	3000				
22	98	12	8000				
23	98	12	22 000				
24	98	12	15 600				
25	98	12	10 500				
26	98	12	30 000				
27	98	12	9000				
28	98	12	1800				
29	98	12	1600				
30	98	12	1400				

[a] V1, primary immunization. V2, secondary immunization.
[b] No data.

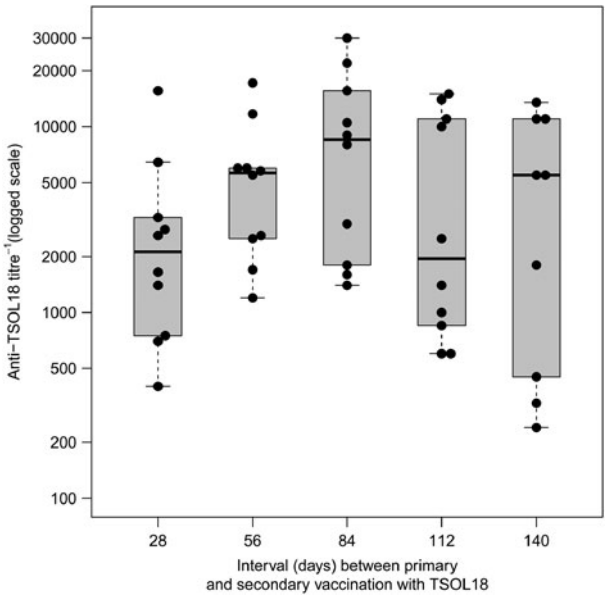

Fig. 1. Boxplots of individual animal anti-TSOL18-specific IgG titres in the sera of pigs collected 2 weeks after the second immunization with the TSOL18 vaccine.

DISCUSSION

Similar anti-TSOL18 antibody titres were recorded for pigs that received their second TSOL18 immunizations at intervals of 4–20 weeks to the anti-TSOL18 titres recorded in pigs involved in previous studies (Kyngdon *et al*. 2006; Assana *et al*. 2010). Until now, results have been published from 5 different TSOL18 vaccine trials in pigs that have involved an experimental challenge infection with *T. solium* eggs, as well as two field trials with the vaccine (Lightowlers, 2013). The immunization protocol used in these trials has involved, predominantly, two immunizations given at an interval of 4 weeks or less. While this vaccination schedule is extremely effective in inducing protective immune responses, implementing it in pigs in communities where *T. solium* is transmitted would be likely to present logistical difficulties. As the full lifecycle of *T. solium* is transmitted almost exclusively among the poorest people living in the poorest countries, the major challenges for control measures involve minimizing cost while maximizing effectiveness and sustainability. The availability of more flexible

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Table 2. Summary of anti-TSOL18- specific IgG titres in sera of vaccinated pigs collected 2 weeks after the second immunization with the TSOL18 vaccine

Treatment group (*n*)	V1/V2 interval (weeks)	Anti-TSOL18 IgG antibody titre^{-1}				
		Mean[a]	Median	Geomean (S.D.)[b]	Min[c]	Max[d]
T1 (10)	4	3560	2125	2024 (2·99)	400	15 600
T2 (10)	8	6020	5650	4453 (2·31)	1200	17 200
T3 (10)	12	10 290	8500	6272 (3·10)	1400	30 000
T4 (10)	16	5695	1950	2750 (3·89)	600	15 000
T5 (10)	20	5479	5500	2444 (5·09)	240	13 500

[a] Arithmetic mean.
[b] Geometric mean titre with geometric S.D..
[c] Minimum titre.
[d] Maximum titre.

Table 3. Back-transformed linear regression outputs of $\log_2$(anti-TSOL18-specific IgG titres) by interval between primary and secondary vaccination with the TSOL18 vaccine

Treatment group	V1/V2 interval (weeks)	Coeff. (S.E.)[a]	*T*	95% CI[b]	*P*
T1	4	1·00	–	(Reference category)	
T2	8	2·20 (1·54)	1·43	0·73, 6·66	0·16
T3	12	3·10 (1·50)	2·06	1·02, 9·38	0·046
T4	16	1·36 (2·43)	0·56	0·45, 4·11	0·58
T5	20	1·21 (3·66)	0·33	0·39, 3·77	0·74
Constant	–	2024 (103)	–	925, 4430	–

[a] Regression coefficient with standard error (constant represents geometric mean of group T1, and other groups' coefficients represent fold changes in group geometric means compared with group T1).
[b] Confidence interval.

vaccination schedules using TSOL18 would address some of the logistical difficulties associated with implementation of pig vaccination for *T. solium* control.

New opportunities have been created for controlling *T. solium* transmission by pigs following development of the TSOL18 vaccine and discovery of the cystocidal effects of oxfendazole (Gonzales *et al.* 1996); however, it remains unclear what would be the most effective method to apply these tools. The TSOL18 vaccine is effective in preventing new *T. solium* infections in vaccinated animals but is not expected to affect *T. solium* cysticerci that may be already present in an animal's tissues when it is vaccinated (Lightowlers, 2010). Treatment of pigs with 30 mg kg^{-1} oxfendazole will kill all cysticerci present in the muscle tissues (Gonzales *et al.* 1996; Sikasunge *et al.* 2008). A 21-day withholding period is required after treatment of pigs with oxfendazole from the only current supplier of oxfendazole registered for use in pigs for treatment of cysticercosis (MCI Santé Animale, Morocco). Compliance with the withholding period would be unlikely in the poor and poorly educated communities where control of *T. solium* transmission is needed. In an experimental field trial, Assana *et al.* (2010) used a combination of vaccination and a single oxfendazole treatment, which eliminated *T. solium* transmission by the animals involved in the trial. The combined use of both vaccination and oxfendazole treatment in young pigs avoided the difficulties created by the requirement for a withholding period after oxfendazole treatment because use of the drug was restricted to very young animals that were unlikely to be consumed. However, Assana *et al.* (2010) used a vaccination schedule, two doses 4 weeks apart, based on previous experimental challenge vaccine trials; one which might be difficult to implement as part of an on-going *T. solium* control programme. Here we have shown that antibody responses to the TSOL18 vaccine are not diminished in pigs in which the secondary immunization is given up to 20 weeks after the primary injection of vaccine. Indeed responses to TSOL18 are significantly greater in pigs receiving two immunizations 12 weeks apart, compared with the responses seen in animals immunized at a 4 weekly interval.

Antibody responses in pigs following two immunizations with TSOL18 given 1 month apart have been described, as have the responses seen in animals in each of the vaccination trials that have involved experimental challenge infections with *T. solium* (Kyngdon, 2005, 2006). There is no clear relationship between the peak IgG antibody titre

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raised to TSOL18 and the level of protection observed in individual pigs. However, all animals in all experiments have been either completely protected against infection or almost completely protected. The lowest peak anti-TSOL18 antibody titre recorded for any of the vaccinated and protected animals was 700 (Kyngdon *et al*. 2006). The rate of decline in the IgG titre seen each week following a peak antibody titre determined 2 weeks after the second injection in pigs not exposed to *T. solium* infection (Kyngdon, 2005) is described by the equation:

$$\text{Titre} = 10^{[-0{\cdot}11589^{*}(\text{week}\,-\,2)]} \times (\text{week 2 titre})$$

In the study described here, the group of pigs which received vaccinations 12 weeks apart developed a mean peak antibody titre of 10 290. This would be predicted to provide a titre of ⩾700 for 12 weeks following the second immunization. Protection of at least this duration is also indicated by the results obtained during the TSOL18 field trial undertaken in Cameroon. In that trial, the pigs which were vaccinated at approximately 2 and 3 months of age were protected completely against infection with *T. solium* caused by natural exposure to the parasite's eggs at least until they received a subsequent booster immunization approximately 3 months later (Assana *et al*. 2010). Hence, the antibody response induced in pigs following immunizations with TSOL18 that were given at approximately 3 monthly intervals would be predicted to provide continuing protection for pigs after the second injection. This prediction will be tested in field studies involving local pigs breeds naturally exposed to *T. solium* infection.

The data presented here support the likelihood that TSOL18 vaccination could be applied effectively in pigs where the interval between primary and secondary immunizations is 3–4 months. This finding increases the options available for designing *T. solium* intervention programs that use the vaccine and suggests that a 3- or 4-monthly intervention schedule, as proposed by Lightowlers (2013), may be effective for prevention of *T. solium* transmission by pigs.

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