Vaccination of Rats and Pigs against *Trichinella spiralis spiralis* using the Subspecies, *T. spiralis nativa*

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ABSTRACT

Rats and pigs were vaccinated against *Trichinella spiralis spiralis* either by feeding infective larvae of the subspecies, *Trichinella spiralis nativa* in musculature or by gavage. The number of larvae established in the musculature of vaccinated nonchallenged and vaccinated challenged rats and pigs were negligible and statistically comparable, while highly significant infections were established in the nonvaccinated challenged rats and pigs. High vaccination doses of *T. spiralis nativa* gave virtually complete protection to challenge with *T. spiralis spiralis* in pigs. The results of one trial in rats with a lower vaccination dose of larvae suggest that there is a minimal vaccination dose of larvae required to elicit marked resistance to challenge. The low numbers of muscle larvae established due to the high vaccination doses of larvae confirm the low infectivity of the subspecies, *T. spiralis nativa* in rats and pigs.

Key words: *Trichinella spiralis spiralis*, *Trichinella spiralis nativa*, vaccination, resistance.

RÉSUMÉ

Cette expérience consistait à vacciner des rats et des porcs contre *Trichinella spiralis spiralis* en leur faisant manger de la viande parasitée par des larves infectantes de la sous-espèce *Trichinella spiralis nativa* ou en les gavant de ces larves. Le nombre de larves qui s'enkystèrent dans les muscles des rats et des porcs vaccinés, soumis ou non à une infection de défi, s'avéra négligeable et comparable du point de vue statistique, tandis que plusieurs larves s'enkystèrent dans les muscles des rats et des porcs témoins. Des doses vaccinales élevées de *T. spiralis nativa* conférèrent une protection presque totale contre une infection de défi des porcs avec *T. spiralis spiralis*. Le résultat d'une expérience chez les rats, avec une plus faible dose vaccinale de larves, suggéra la nécessité d'une dose vaccinale minimale de larves, pour obtenir une résistance marquée à une infection de défi. Le fait que peu de larves s'enkystèrent après la vaccination avec une forte dose de larves, confirme l'infectivité négligeable de la sous-espèce *T. spiralis nativa* pour les rats et les porcs.

Mots clés: *Trichinella spiralis spiralis*, *Trichinella spiralis nativa*, vaccination, résistance.

INTRODUCTION

Trichinella spiralis spiralis, in common with many metazoan parasites, evokes a powerful immune response (1). Animals can be immunized against the intestinal phase of the infection resulting in structural and functional changes in the worms followed by expulsion of the worm population from the intestine (1). Exposure to a primary infection confers strong immunity to reinfection.

Immunity to *T. spiralis* infections has been accomplished by ionizing radiation to sterilize larvae that will nevertheless grow into the adult form. Another approach has been termination of infections with an anthelmintic. Recently swine were immunized with partially purified stichosome antigens derived from *T. spiralis* muscle larvae which induced moderate levels of resistance to challenge inoculation (2).

Trichinella spiralis nativa, found in arctic mammals, has been shown to have a low infectivity for rats and swine (3,4,5,6,7). In consideration of the demonstrated low infectivity of *T. spiralis nativa* for rats and swine, investigations were carried out to determine if vaccination with this subspecies would protect rats and swine against challenge, with *T. spiralis spiralis* which readily establishes in these species.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Wistar laboratory white rats were purchased from a commercial animal breeder. York x Landrace pigs of both sexes weighing approximately 20 kg were purchased from a commercial producer. Guinea pigs, used to propagate *T. spiralis nativa* larvae for vaccination purposes, were raised in the laboratory. All experimental animals were fed commercially prepared food. The Canadian Council on Animal Care guidelines outlined in "Guide to the Care and Use of Experimental Animals, Volume I" were followed.

TRICHINELLA STRAINS

A *T. spiralis nativa* isolate originally recovered from a wolf killed near Nain, Labrador (56° 40′N, 62° 00′W) in April, 1980 and passaged through foxes, cats and ferrets at various times was used to vaccinate rats and pigs in this study. A *T. spiralis spiralis* isolate originally recovered in 1974 from a pig originating near Halifax, Nova Scotia (44° 43′N, 63° 26′W) and maintained in rats was used to challenge the rats and pigs.

VACCINATION TRIALS

Trials 1 and 2 were carried out in rats and trial 3 in swine. Animals were vaccinated and/or challenged either by feeding infected musculature (Trial 1) or infective larvae by gavage (Trials 2 and 3) as outlined in Tables I, II and

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III. Number of infective larvae in muscle tissue fed to rats was determined by pepsin-digestion of comparable muscle tissue. Larval doses given by gavage were calculated by determining the mean number of larvae/mL in three aliquots of the larval mixture. Each trial had three groups of six animals each. In trials 1 and 2, rats were challenged 30 days after vaccination or the second vaccination in the case of group 2 in trial 2. In trial 3, pigs were challenged 28 days after vaccination. In trials 1 and 2, rats were killed 30 or 31 days following vaccination or challenge. In trial 3, pigs were killed 28 days after vaccination or challenge.

Infections in rats and pigs were determined by digesting 20 and 400 (or 500) g composite samples of musculature, respectively, in a 1% pepsin-1% HCl mixture. Muscles included in the samples were tongue, masseter, diaphragm, intercostals, rectus abdominis and psoas.

Statistical analysis of the significance of the results in all trials were carried out using the nonparametric Wilcoxon two sample test.

RESULTS

TRIAL 1

The results are presented in Table I. The nonchallenged vaccinated group 1 rats had negligible infections with an average of 0.15 larvae per gram (1a/g) of musculature. The vaccinated challenged group 2 rats had a mean infection of 229.8 la/g of musculature while the nonvaccinated challenged group 3 rats had a mean infection of 431 la/g of musculature. Vaccinating rats with *T. spiralis nativa* significantly reduced ($P \leq 0.021$) the number of *T. spiralis spiralis* larvae established in the musculature when challenged 30 days later.

TABLE I. *Trichinella* Infections Established in Vaccinated Nonchallenged, Vaccinated Challenged, and Nonvaccinated Challenged Rats

Group	Rat	Vaccination Dose (*T. spiralis nativa*)	Challenge Dose (*T. spiralis spiralis*)	Larvae/g of Musculature
1	1	630	-	0.1
	2	630	-	0.1
	3	630	-	0.5
	4	630	-	0.15
	5	630	-	0
	6	630	-	0.05
2	1	630	1025	292.0
	2	630	1025	118.5
	3	630	1025	304.0
	4	630	1025	9.7
	5	630	1025	475.5
	6	630	1025	179.0
3	1	-	1025	443.5
	2	-	1025	372.0
	3	-	1025	415.5
	4	-	1025	531.0
	5	-	1025	424.5
	6	-	1025	399.5

TABLE II. *Trichinella* Infections Established in Vaccinated Challenged, Revaccinated Challenged and Nonvaccinated Challenged Rats

Group	Rat	Vaccination Dose (*T. spiralis nativa*)	Challenge Dose (*T. spiralis spiralis*)	Larvae/g of Musculature
1	1	2000	1000	6.3
	2	2000	1000	7.1
	3	2000	1000	0.2
	4	2000	1000	7.3
	5	2000	1000	1.5
	6	2000	1000	0.1
2	1	2000[a]	1000	0.2
	2	2000[a]	1000	4.1
	3	2000[a]	1000	0.6
	4	2000[a]	1000	2.3
	5	2000[a]	1000	60.0
	6	2000[a]	1000	0.2
3	1	-	1000	610.0
	2	-	1000	440.0
	3	-	1000	1130.0
	4	-	1000	860.0
	5	-	1000	550.0
	6	-	1000	480.0

[a]Vaccinated twice with 1000 larvae 21 days apart

TRIAL 2

The results are presented in Table II. Both group 1 (single vaccinated, challenged) and group 2 (double vaccinated, challenged) rats developed extremely low infections with mean muscle infections of 3.7 and 11.2 la/g of musculature. The infections established in both groups were not significantly different. On the other hand, the group 3 (nonvaccinated, challenged) rats developed mean muscle infections of 678.3 la/g. The findings in both vaccinated groups were highly significantly different ($P < 0.001$) from the nonvaccinated group.

TRIAL 3

The results are presented in Table III. Both the vaccinated nonchallenged group 1 and the vaccinated challenged group 2 pigs developed negligible muscle infections while the nonvaccinated challenged group 3 pigs developed muscle infections with a mean of 32.2 la/g of musculature. There was no significant difference between the infections in the vaccinated nonchallenged and vaccinated challenged groups. A highly significant difference ($P \leq 0.001$) was present between the vaccinated challenged group 2 and nonvaccinated challenged group 3 pigs.

TABLE III. *Trichinella* Infections Established in Vaccinated Nonchallenged, Vaccinated Challenged and Nonvaccinated Challenged Pigs

Group	Pig	Vaccination Dose (*T. spiralis nativa*)	Challenge Dose (*T. spiralis spiralis*)	Larvae/g of Musculature
	1	23000	-	0.436
	2	23000	-	0.002
1	3	23000	-	0.006
	4	23000	-	0
	5	23000	-	0.062
	6	23000	-	1.040
	1	23000	5500	0.01
	2	23000	5500	0.0075
2	3	23000	5500	0
	4	23000	5500	0.12
	5	23000	5500	0.02
	6	23000	5500	0.0025
	1	-	5500	95.3
	2	-	5500	22.5
3	3	-	5500	5.4
	4	-	5500	21.2
	5	-	5500	43.0
	6	-	5500	5.6

DISCUSSION

Vaccinating with large doses again demonstrates the low infectivity of *T. spiralis nativa* for rats and pigs compared to the infections established with the subspecies, *T. spiralis spiralis* (6,7). Vaccinating pigs with large doses of *T. spiralis nativa* induced highly significant resistance or virtually complete protection to challenge with *T. spiralis spiralis*. Revaccinating rats, three weeks after the initial vaccination with a second dose of 1000 larvae offered no advantage over a single vaccination of 2000 larvae. It would seem that there is a minimal vaccination dose of larvae required before maximal resistance to challenge develops based on the findings in trial 1. The virtually complete protection of rats and pigs achieved when vaccinated with high doses of *T. spiralis nativa* against *T. spiralis spiralis* challenge does demonstrate that it is possible to successfully vaccinate against trichinosis. The minimal dose of *T. spiralis nativa* required to consistently give a high degree of protection in rats and in swine is not known.

The actual mechanism by which resistance is achieved through vaccination is not completely understood. It has been suggested that two immune barriers impeding worm production appear to be involved; fecundity (reproductive ability) of the worms is affected and some mechanism that effectively interferes with the invasion or establishment of newborn larvae in musculature (8).

The use of vaccination as a method of controlling trichinosis in swine has been recently reviewed (8). It is unlikely to be practical because of the relatively small numbers of infected swine although it may be a viable option within foci with a high prevalence of infection that are not controlled by other preventative measures such as cooking of garbage and rodent control (8). The results of this study certainly demonstrated that it is possible to protect swine by vaccination with only negligible numbers of muscle larvae becoming established. It is not known if the few larvae observed in the vaccinated challenged swine resulted from the vaccination with *T. spiralis nativa* or the challenge with *T. spiralis spiralis*. It has been noted that swine containing less than one larva of *T. spiralis* per gram of musculature are generally not considered capable of causing clinical trichinosis in man (9).

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