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Brief Communication

Establishment and Growth of Trichinella Muscle Larvae in Iron Supplemented Mice

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Previous studies have shown that iron is important for the survival of *Trichinella spiralis* muscle larvae in vitro (*Theodoropoulos & Greve* 1986a) and the pathogenesis of trichinellosis in swine (*Theodoropoulos & Greve* 1986b). Specifically iron-deficient media reduce the survival of *T. spiralis* larvae *in vitro* while trichinous pigs exhibit reduced levels of total iron binding capacity and unsaturated iron binding capacity in blood serum, reduced iron concentration levels in the liver, and increased iron concentration levels in the spleen. Even though the cause of these iron changes in trichinous pigs is not clear, it may be related to the phenomenon of "nutritional immunity" which "restricts the availability of growth essential nutrients for use by parasitic micro-organisms" (*Kochan* 1977). In the converse situation, where iron is in excess, there is a greater susceptibility to parasitic infections as it has been shown in infections caused by *Plasmodium bergei* in rats (*Murray et al.* 1975) and *Entamoeba histolytica* in hamsters (*Diamond et al.* 1978).

The objective of the present study was to determine whether iron supplement in the host increases establishment and growth of *T. spiralis* muscle larvae.

Trichinella spiralis larvae (ISS004) were isolated from mouse muscle tissue by HCl-pepsin digestion and sedimentation (*Gable* 1996). Thirty three 10-12 g mice fed a commercial feed in the form of pellets were inoculated in groups with 100, 200, or 400 muscle larvae *per os* with a feeding needle (Table 1). The inoculum had a volume of 0.25 ml. Sixteen mice were supplemented with 0.01 ml of iron dextran (10% Fe^{+3}, FERRIDEX® VET, A/S ROSCO, Denmark) subcutaneously (SC) by injection at intervals of 3 to 4 days, starting 3 days before inoculation with *T. spiralis* larvae. Injection sites (inguinal and axillary regions) were rotated over the course of the trial. The remaining 17 mice were injected with 0.01 ml of Ringer's solution SC as placebo.

Thirty days after infection the mice were sacrificed by cervical dislocation and skinned. After removal of the abdominal organs the carcass was weighed. Following mincing of the carcasses with scissors, the *T. spiralis* larvae were freed from the minced muscle tissue collected from the carcasses of the mice by HCl-pepsin digestion. Larva counts were made for each mouse individually.

The growth of *T. spiralis* larvae was assessed by measuring the width, length, perimeter and surface area of individual *T. spiralis* muscle larvae images by using image analysis (Image Pro Plus™ 3.0 for Windows 95 by MEDIA CY-

Table 1. Intensity and size of *Trichinella spiralis* muscle larvae in iron supplemented (iron dextran) and control (Ringer's solution) mice.

	Inoculum								
	Low (100 larvae)			Medium (200 larvae)			High (400 larvae)		
	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n	$\bar{x}$	s.d.	n
Intensity (lpg)									
Iron supplement	$1754^{a,d}$	1097	5 mice	$3009^{b,d}$	1486	5 mice	$1657^{b,d}$	1503	7 mice
Control	$664^{a,c}$	257	5 mice	$1378^{b,d}$	850	5 mice	$3024^{b,d}$	1717	6 mice
Size (width in μm)									
Iron supplement	38.53^{e}	0.95	14 larvae	40.06^{e}	0.52	16 larvae	38.66^{e}	0.87	14 larvae
Control	36.32^{f}	1.06	12 larvae	37.61^{f}	0.63	16 larvae	37.16^{f}	0.87	14 larvae

lpg: Larvae per gram mouse muscle tissue, $\bar{x}$: Mean value, s.d.: Standard deviation, n: Number of samples, a and b are significantly different ($p = 0.05$), c and d are significantly different ($p = 0.03$), e and f are significantly different ($p = 0.002$).

BERNETICS®, U.S.A.). The still image (4X light microscopic magnification) of each individual larva was examined. Width, length, perimeter and surface area of each individual *T. spiralis* muscle larva image were recorded. Measurement data were sent by the Dynamic Data Exchange (DDE) method to be saved in a spreadsheet (Excel® by Microsoft Plus for Windows 95®) for statistical analysis.

The effect of iron supplement on parasitic burden of mice (lpg) was analyzed through a 2 way ANOVA (*Neter et al.* 1980). Accordingly, all 4 variables regarding the growth of individual muscle larvae (width, length, perimeter and surface area) were also analyzed by a 2 way ANOVA.

The infection intensity and size of muscle larvae in the 6 experimental groups are presented in Table 1. The number of muscle larvae per gram of tissue (lpg) was found to be significantly lower in the low infection dose (100 larvae) than the other 2 levels of infection doses (200 and 400 larvae) ($p = 0.05$). The iron supplement had a significantly ($p = 0.03$) positive effect on establishment of muscle larvae. Thus, the difference in lpg between iron supplemented and control mice was highest in mice inoculated with 100 larvae, less expressed in mice inoculated with 200 larvae, and not significant in mice inoculated with 400 larvae.

Muscle larvae from mice given iron supplement were significantly wider ($p = 0.002$) than from mice given placebo. No statistically significant differences between groups were found for the other variables (length, perimeter, and surface area).

Competition for iron between animals and their parasitic micro-organisms plays an important role in the severity of infection, and experimentally or naturally induced hyperferremia increases host susceptibility to many bacteria, fungi, and protozoa (*Weinberg* 1978). The results of the present study indicated that iron supplement had effect on the establishment (lpg) and growth (width) of *T. spiralis* muscle larvae. The observations demonstrated that iron had a noticeable effect on the number of trichinous larvae established in muscle tissues at low-level exposure, and this effect decreases at higher levels of exposure. The high-level inoculation (400 larvae) appeared to be close to the limit where higher inoculum did not result in a higher establishment of muscle larvae. It was not possible from the present study to elucidate