HOST AGE INFLUENCE ON THE INTENSITY OF EXPERIMENTAL *TRICHURIS SUIS* INFECTION IN PIGS

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Summary:

The impact of age-related resistance on the regulation of population dynamics of adult *Trichuris suis* was investigated in an experimental pig model. Helminth-naïve pigs varying in age from five weeks to four years were infected with *T. suis* to determine susceptibility to infection. Sows had a significantly lower establishment of adult *T. suis* worms compared with weaner pigs. Adult worm populations were highly overdispersed in both sows and grower pigs contrasted by a more even distribution among weaner pigs. Sows had significantly lower worm fecundities compared to weaner and grower pigs; *T. suis* from grower pigs, in turn, had reduced fecundity compared to worms in weaner pigs. In conclusion, we provide the first controlled experimental evidence that age-related resistance to *T. suis* occurs in pigs.

KEY WORDS : *Trichuris suis*, whipworm, pig, age-related resistance, single infection.

Résumé : INFLUENCE DE L'ÂGE SUR L'INTENSITÉ DE L'INFECTION EXPÉRIMENTALE DES PORCS PAR *TRICHURIS SUIS*

L'impact de la résistance liée à l'âge dans la dynamique de la population et l'épidémiologie de Trichuris suis *a été étudié expérimentalement chez le porc. Des porcs non porteurs d'helminthes et âgés de cinq semaines à quatre ans ont été inoculés par* T. suis *afin d'en étudier la susceptibilité à l'infection. Les taux d'infestation par les vers adultes sont très significativement plus bas chez les truies que chez les porcelets. La population de vers adultes est très dispersée chez les truies et les porcs à l'engrais, ce qui n'est pas le cas chez les porcelets. La fécondité des vers est significativement plus faible chez les truies que chez les porcelets et porcs à l'engrais ; chez ces derniers, les vers adultes sont également moins féconds que chez les porcelets. En conclusion, la preuve expérimentale de l'influence de l'âge en matière de résistance à l'infestation par* T. suis *est apportée pour la première fois.*

MOTS CLÉS : Trichuris suis, *trichure, porc, résistance liée à l'âge, infestation unique.*

The whipworm *Trichuris suis* (Schrank, 1788) is an intestinal nematode parasite of pigs (Powers *et al.*, 1959; Jacobs & Dunn, 1969; Pattison *et al.*, 1980). Adult worms reside in the caecum and colon, either in the intestinal contents or with part of their anterior ends embedded superficially in the mucosa (Jenkins, 1970). The prepatent period is 41 to 45 days and the parasite has a life-span seldom exceeding four to five months (Powers *et al.*, 1960).

Pigs are often used as models for human helminth infections because of similarities with respect to digestive anatomy and many aspects of physiology (Miller & Ullrey, 1987). *Trichuris suis* is very similar to the human whipworm *T. trichiura* with respect to life-cycle and morphology (Beer, 1976). Therefore, experimental *T. suis* infection in the pig is considered to be a good model for *T. trichiura* in humans, which affects at least 1,049 million people worldwide (Stephenson & Holland, 1987; Crompton, 1999).

Although ingestion of multiple, small infective egg doses in pigs and humans occurs in naturally contaminated environments, most pig experimental studies have employed high infective doses, i.e. 15,000 to 400,000 infective *T. suis* eggs given to pigs weighing approximately 20 kg (Powers *et al.*, 1960; Beer *et al.*, 1971; Batte & Moncol, 1972; Beer & Lean, 1973; Beer *et al.*, 1973; Batte *et al.*, 1977; Hale & Stewart, 1979). Hitherto, no studies have included adult animals and few have employed moderate infective doses e.g. 3×50 infective eggs per kg body weight (Mansfield & Urban, 1996).

The aim of the present study was to investigate experimentally age-related resistance to *T. suis* in pigs.

MATERIALS AND METHODS

ANIMALS AND FEEDING

Eight sows and 16 specific-pathogen-free cross-bred (Danish Landrace/Yorkshire/Duroc) castrated pigs of varying age (see below) were purchased from a helminth-free experimental farm. The sows were kept on soiled ground, with no previous

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contamination of *T. suis,* and access to a straw-bedded house. The younger pigs were housed in concrete floored pens with straw-bedding. A standard barley- and wheat-based diet was fed to both weaner and grower pigs (crude protein: 17 %) and sows (crude protein: 15 %). The physiological requirements for essential amino and fatty acids, macro- and microminerals were fulfilled (Chwalibog, 1997). The daily feed ration to pigs was adjusted on a weekly basis. From the commencement of faecal egg excretion at week six post infection (p.i.) until necropsy (week 10 p.i.) the estimated mean daily feed intake of weaner and grower pigs were 0.6-1.1 kg and 1.6-2.0 kg, respectively, while sows were fed the same daily feed quantity (2.8 kg) throughout the study. The pigs had free access to water via drinking nipples.

Experimental design

Eight newly weaned pigs (five weeks of age; 10 ± 1 kg), eight grower pigs (12 weeks of age; 33 ± 2 kg) and eight sows (four years of age; 237 ± 21 kg) were included in the experiment. Infective *T. suis* eggs were originally isolated in 1996 from soil on a small organic farm and subsequently passaged in worm-free pigs. The eggs used in the present experiment were isolated from faeces of pigs, embryonated in vermiculite according to the method described by Burden & Hammet (1976) and subsequently stored at 10° C. The pigs were inoculated orally via a stomach tube with 50 infective eggs per kg body weight on each of three consecutive days. This ensured that comparable infection levels could be obtained in relation to physical size of the intestine. Faecal samples were collected from the rectum of each pig at the time of infection and then weekly starting five weeks p.i. Egg counts were evaluated using a concentration McMaster Technique as described by Roepstorff & Nansen (1998), with a detection limit of 20 eggs per gram (e.p.g.). Egg counts from sows and grower pigs (x) were adjusted by multiplying the e.p.g. with the following factor: (mean daily feed volume (x)/mean daily feed volume (weaner))/(initial mean body weight (x)/initial mean body weight (weaner)) (Stephenson & Holland, 1987).

Necropsy and worm recovery

All pigs were slaughtered 10 weeks p.i. Feed was withheld on the day of necropsy. *Trichuris suis* worms were recovered according to the method described by Roepstorff & Murrell (1997). The large intestine was divided into five sections, as follows, starting at the caecum: 1) caecum; 2) 0-20 % of the total length of colon; 3) 21-40 %; 4) 41-60 % and 5) 61-100 % including rectum. The sections were opened with scissors and the intestinal wall was gently washed to liberate *T. suis.* This procedure ensured that all worms were removed. The suspension of intestinal contents and intestinal wall washings was homogenized by vigorous stirring, and two representative subsamples in total 10 % of volume were washed over a sieve of mesh size 212 µm. Retained samples of worms were fixed in iodine (80 g iodine and 400 g potassium iodide in 800 ml of distilled water) for later isolation of *T. suis.* Samples were transferred to a Petri dish held over a light table and decolourized with a 30 % sodium thiosulphate solution. Worms visible with the naked eye were recovered with a pair of tweezers, stored in a 9:1 mixture of ethanol (70 % v/v) and glycerol (6 % w/w), enumerated and differentiated according to developmental stage and sex (Beer, 1973).
Percentage worm establishment was defined as the percentage of the infective eggs recovered as worms. Body lengths of intact worms was measured by means of a stereo-microscope and a digital analysis system (Microvision®, DTI, Denmark); for those pigs harbouring more than 10 worms of each sex, 10 female and 10 male worms were randomly selected for measurement. For those pigs harbouring female worms, fecundity was estimated by dividing e.p.g. at the day of necropsy by the number of females recovered.

Statistical analysis

Percentage worm establishment and faecal egg counts for each host group were compared using the non-parametric Mann-Whitney test or Student's *t*-test (worm fecundity). For worm distribution in intestinal sections, proportion of female *T. suis* and worm length, one-way analysis of variance (ANOVA) or Kruskal-Wallis's test were used. Worm burden was included as a covariate in analysis of length. Longitudinal analysis of faecal egg production was carried out by estimating the area under the curves of individual pigs. It was assumed that the weekly counts represented mean daily counts for the whole week. The prevalences of infection (no. infected/no. of pigs) of *T. suis* at necropsy were compared between groups using Fisher's Exact test. The parameter of the negative binomial distribution, k, was estimated as k = mean^2/(variance-mean); k decreases as overdispersion increases. Mean and variance were calculated on total worm burdens.

RESULTS

Only adult *T. suis* worms were recovered and the worm counts are presented in Table I. The percentage worm establishment was lower in sows compared with weaner pigs (P = 0.023), but there was no significant difference between either weaner versus grower pigs or grower pigs versus sows. Worm populations in grower pigs and sows were highly overdis-

Group	Egg dose	Intestinal section							Estb
		S1	S2	S3	S4	S5	Total	C. Total	(%)
W	1,326	110	230	240	90	10	680	680	51.3
k = 18.6	1,674	30	250	0	250	50	580	580	34.7
	1,653	120	150	180	70	0	520	520	31.5
	1,467	200	130	80	50	10	470	470	32.0
	1,260	70	130	210	10	0	420	420	33.3
	1,620	50	180	90	50	10	380	380	23.5
	1,347	160	130	40	20	10	360	360	26.7
Mean	1,478	106	171	120	77	13	487	487	33.3
(S.D.)	(172)	(61)	(50)	(91)	(81)	(17)	(115)	(115)	(8.9)
G	5,103	800	960	350	140	20	2,270	688	44.5
k = 0.8	4,653	500	420	320	200	580	2,020	612	43.4
	5,103	380	640	190	550	40	1,800	545	35.3
	5,103	220	250	140	250	80	940	285	18.4
	4,500	0	0	10	80	10	100	30	2.2
	4,950	0	0	10	0	10	20	6	0.4
	4,950	0	0	0	0	0	0	0	0
	5,553	0	0	0	0	0	0	0	0
Mean	4,989	238	284	128	153	93	894	271	18.0
(S.D.)	(319)	(300)	(364)	(147)	(187)	(199)	(998)	(303)	(20.2)
S	34,164	3,600	9,810	220	70	160	13,860	585	40.6
k = 0.3	37,779	6,020	3,990	660	190	400	11,260	475	29.8
	31,317	40	650	130	150	60	1,030	43	3.3
	38,217	0	10	10	0	0	20	1	0.1
	35,808	0	0	0	0	0	0	0	0
	35,259	0	0	0	0	0	0	0	0
	31,536	0	0	0	0	0	0	0	0
	40,407	0	0	0	0	0	0	0	0
Mean	35,561	1,208	1,808	128	51	78	3,271	138	9.2
(S.D.)	(3,202)	(2,316)	(3,514)	(230)	(78)	(142)	(5,786)	(244)	(16.3)

Table I. – Number of *Trichuris suis* recovered from the large intestine of weaner pigs (W), grower pigs (G) and sows (S) infected with 3 × 50 infective eggs per kg body weight and necropsied 10 weeks p.i. (S1) section 1 (caecum). (S2) section 2 (0-20 % of the total length of colon). (S3) section 3 (21-40 %). (S4) section 4 (41-60 %). (S5) section 5 (61-100 % including rectum). Total: all five sections. Corrected total to body weight. Infective egg dose administered orally. Percentage establishment (no. of worms recovered/no. of infective eggs administered). The parameter *k* of the negative binomial distribution.

persed ($k < 1$) contrasted by a more even distribution among weaner pigs (Table I). A higher percentage of the worm burden was recovered from intestinal section two of sows compared with younger pigs ($P = 0.006$) (Fig. 1); no significant difference was detected in the other sections. Neither prevalence of infection, proportion of female *T. suis* nor worm length differed significantly between groups (Table II); further, worm burden was not a significant covariate in analysis of worm length. Faecal egg excretion in some of the pigs started six weeks p.i. (Fig. 2). Worm fecundity at 10 weeks p.i. was lower in sows compared with both weaner and grower pigs ($P < 0.001$); *T. suis* from grower pigs also had reduced fecundity compared with weaner pigs ($P = 0.008$) (Table II). Longitudinal analysis revealed a lower total faecal egg excretion in sows compared with both weaner pigs ($P = 0.001$) and grower pigs ($P =$

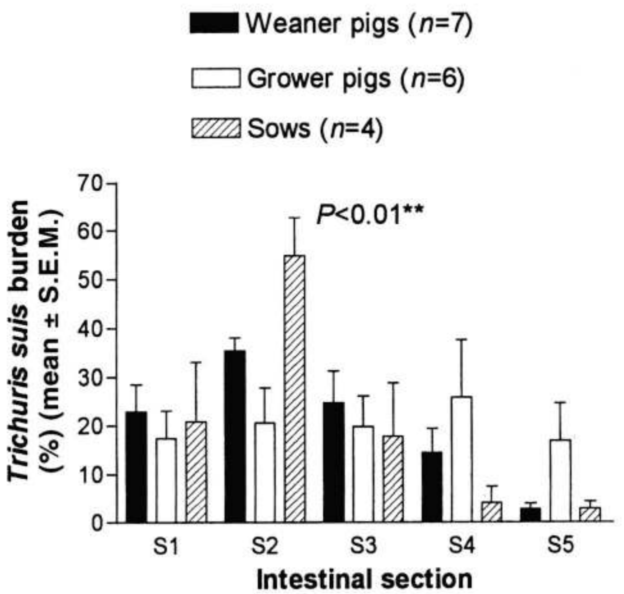

Fig. 1. – Comparison of the relative distribution (%) (mean ± S.E.M.) of *Trichuris suis* in sows, weaner and grower pigs. (S1) caecum. (S2) 0-20 % of the total length of colon. (S3) 21-40 %. (S4) 41-60 %. (S5) 61-100 % including rectum. A higher percentage of the worm burden was found in section two of sows compared with younger pigs ($P = 0.006$).

Group	*n*	%	Female proportion	Length	Fecundity	Total e.p.g.
W	7	100	0.52 ± 0.05	31.8 ± 2.9	24.8 ± 9.4	27,952 (21,948-35,599)
G	8	75	0.53 ± 0.04	29.1 ± 1.8	3.0 ± 1.1	8,105 (2,234-29,343)
S	8	50	0.46 ± 0.04	31.0 ± 2.7	0.9 ± 0.6	165 (2-2,556)

Table II. – Prevalence (%) of infection (no. of infected/no. of pigs), proportion of female *T. suis*, worm length (mm), worm fecundity (e.p.g./no. of female *T. suis*) in sows (S), weaner (W) and grower (G) pigs at necropsy 10 weeks p.i. (mean ± S.D). Geometric mean (95 % confidence interval) total egg excretion from week six to 10 p.i.

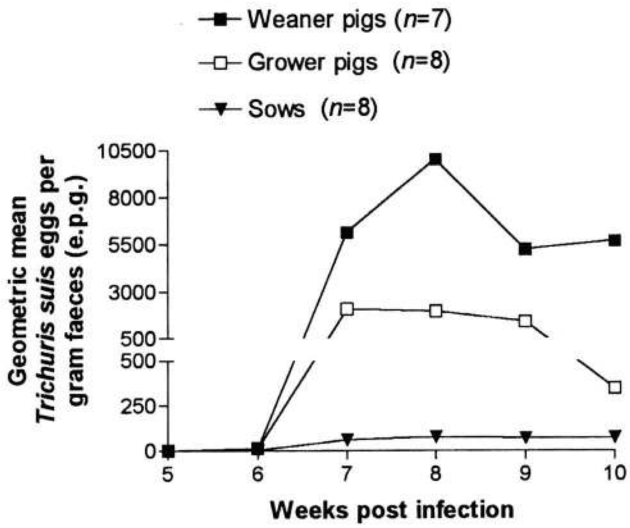

Fig. 2. – Geometric mean eggs per gram faeces (e.p.g.) from five to 10 weeks post infection in weaner pigs, grower pigs and sows infected with 3 × 50 eggs per kg body weight.
Egg counts were Log_{10} (× + 10)-transformed

0.018) (Table II). Moreover, total egg output was lower in grower compared with weaner pigs (P = 0.032). Faecal egg counts were lower in sows compared with weaner and grower pigs from week seven to 10 p.i. and from week seven to nine p.i., respectively (Fig. 2). Egg counts were lower in grower pigs compared with weaner pigs only at week eight and 10 p.i.

Looseness of faeces occasionally occurred in a number of pigs. One weaner pig died following inoculation.

DISCUSSION

Age-related resistance to *T. suis* infection has previously been suggested on the basis of a decrease in prevalence and infection intensity among older age groups (Powers *et al.*, 1959; Jacobs & Dunn, 1969). This epidemiological observation could be explained by many factors such as host nutritional status, acquired immunity, age-related resistance (e.g. innate immunity and gastrointestinal physiology) as well as behavioural (e.g. geophagia) determinants of exposure, which make it difficult to infer causality-effect relationships.

Obviously, *T. suis* larvae need to penetrate the epithelium and burrow into lamina propria of the large intestine. During this part of the life-cycle worms are vulnerable to various host defences. It is possible that a less efficient immune response and/or altered intestinal physiology may permit a better establishment and fecundity of *T. suis* in younger pigs compared with sows. The occurrence of overdispersed worm burdens in grower pigs and sows reflects that there was an increase in the number of refractory hosts, and consequently more skewed worm distributions with increasing age. In sows the worm burden was primarily located in the predilection sites of *T. suis*, i.e. caecum and proximal colon (Powers *et al.*, 1960). This may indicate that a more unfriendly intestinal habitat for worms existed in sows compared with younger pigs, which exhibited a more even worm distribution among intestinal sections.

In conclusion, we provide the first controlled experimental evidence that age-related resistance to *T. suis* occurs in adult pigs. The resistance reduces the establishment and survival for 10 weeks of adult worm populations, increases aggregation of worm populations, changes intestinal location of *T. suis*, and causes lower worm fecundity along with faecal egg excretion. This phenomenon may help to explain the characteristic decrease in intensity of *T. trichiura* infection in human populations with increasing age (Bundy & Cooper, 1989). The mechanisms responsible for age-related resistance need to be further investigated.

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