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Transmission Dynamics of Helminth Parasites of Pigs on Continuous Pasture: *Ascaris suum* and *Trichuris suis*

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Abstract—Roepstorff A. & Murrell K. D. 1997. Transmission dynamics of helminth parasites of pigs on continuous pasture: *Ascaris suum* and *Trichuris suis*. *International Journal for Parasitology* 27: 563–572. In Denmark, alternative outdoor production systems for pigs are becoming more frequent, and information on the transmission of *Ascaris suum* and *Trichuris suis* under continuously grazed pasture conditions is needed. A group of pigs was turned out on a pasture in May 1993 (Year 1 of the study), inoculated with 200 eggs of *A. suum* and 1000 eggs of *T. suis*, and followed parasitologically. A non-experimentally infected group of pigs was similarly turned out on the same pasture the following year (Year 2) and again followed parasitologically. Pasture infectivity was measured using helminth-naïve tracer pigs. During the summer of Year 1, *A. suum* eggs became infective within 4–6 weeks on the pasture. However, transmission was moderate until August of Year 2, when a pronounced increase in transmission occurred. After 2 months on the pasture, the continuously exposed pigs in summer seasons of both Years 1 and 2 harboured small overdispersed populations of adult *A. suum*, moderate numbers of liver white spots, and high specific IgG responses. These parasitological measures on chronically exposed pigs did not, however, correlate well with pasture infectivity or with each other. In contrast, the liver inflammation and specific IgG responses (but not the intestinal *A. suum* burdens) of the tracer pigs were highly correlated ($P = 0.0001$) and appeared to better reflect pasture infectivity. The inoculated pigs excreted *T. suis* eggs by the late summer of Year 1, but no transmission took place before August of Year 2. Thus, the *T. suis* population of infective eggs built up very slowly. The results indicate that *T. suis* eggs may survive for a considerable time, however. The study results reveal that *A. suum* and *T. suis* eggs are much more resistant to environmental factors than free-living infective larvae of pig parasites such as *Oesophagostomum dentatum* and *Hyostrongylus rubidus*. Control of these parasites in outdoor systems will present more difficult challenges than that for parasites transmitted by free-living larvae. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: Ascaris suum; Trichuris suis; pigs; transmission; pasture infectivity; tracer pigs.

INTRODUCTION

The large roundworm, *Ascaris suum*, is commonly found in domestic pigs all over the world (Urquhart *et al.*, 1987) and it still occurs in even the most modern production units, although the presence of other species of helminths has been considerably reduced as a result of these modern management systems (reviewed by Roepstorff & Nansen, 1994). One of the most likely reasons for the persistence of high indoor prevalence rates of *A. suum* is the resistance of its eggs to adverse environmental factors and, consequently, their great longevity. However, these characters may also enhance the transmission of *A. suum* in outdoor pig-rearing systems. A relationship between outdoor facilities and high prevalence rates has been documented (Tharaldsen, 1972; Biehl, 1984). In previous studies in Denmark (Roepstorff *et al.*, 1992) *A. suum* was found to heavily parasitize 10–12-week-old pigs

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raised under outdoor conditions, indicating that significant transmission occurred soon after birth.

Trichuris suis is also transmitted as infective eggs, which are highly resistant and may remain infective for 11 years in outdoor conditions (Burden *et al.*, 1987). However, the eggs of this parasite develop very slowly (Alicata, 1935; Burden & Hammet, 1976, 1979) and this would greatly increase the risk of their being removed before infectivity is reached indoors, which may explain why whipworms are normally found only sporadically (Roepstorff & Nansen, 1994). In contrast, access to outdoor facilities had been shown to be associated with high *T. suis* prevalence rates (Alfredsen, 1983; Biehl, 1984), presumably because the long lived eggs remain in the environment, and this may result in clinical disease and even death (Jensen & Svensmark, 1996).

Thus, the outdoor environment appears to increase the risk for both ascarosis and trichurosis. Most studies on outdoor pigs, however, have dealt with helminth species such as *Oesophagostomum* sp. and *Hyostrongylus rubidus*, which are transmitted as free-living infective larvae and, in contrast, very little has been carried out on the transmission and epidemiology of *A. suum* and *T. suis* in the outdoor environment.

Outdoor pig production is becoming more common in Western Europe and there is a need for information on the epidemiology of *A. suum* and *T. suis* in pigs raised under pasture systems. The study described here was designed to provide information on infection patterns and pasture infectivity over a 3-year grazing cycle. The study also included *Oesophagostomum dentatum* and *Hyostrongylus rubidus*, and the data on these parasites have been reported separately (Roepstorff & Murrell, 1997).

MATERIALS AND METHODS

This study was designed to evaluate parasite transmission over a 3-year period (1993–1995, Years 1–3). Details on the pastures, climate determinations, and experimental animals and protocol have been described in a previous report (Roepstorff & Murrell, 1997), and these aspects will only be presented briefly here.

Parasites. The CEP-strains of both *A. suum* and *T. suis* (strains used for most studies at Centre for Experimental Parasitology) were isolated in the winter of 1993 from a small organic farm, which utilized only outdoor rearing facilities. Pigs had been kept on this farm for many years, and anthelmintics were never used. *A. suum* eggs were isolated from fresh faeces and embryonated in 0.1 N H_2SO_4 for 3 months (dark, room temperature), conditions which have been shown to result in fully infective eggs (Oksanen *et al.*, 1990). Infective eggs of *T. suis* were isolated from soil and passaged once in helminth-naïve pigs, and the eggs were isolated from faeces and embryonated in vermiculite, as described by Burden & Hammet (1976).

Protocol

Group 1 (continuously exposed pigs). Twenty-eight pigs were turned out on an experimental pasture (8000 m^2) in early May, Year 1, and inoculated twice by stomach tube with 100 eggs of *A. suum* (18 and 19 May, week 20) and with 500 eggs of *T. suis* (13 and 14 July, week 28). As reported elsewhere (Roepstorff & Murrell, 1997), the pigs were also inoculated with 1000 infective larvae of *O. dentatum* (week 20, mid-May) and 5000 larvae of *H. rubidus* (week 24, mid-June). Three pigs were removed at weeks 24, 28, 32 and 36 for necropsy and parasite analysis; the remaining 16 pigs of Gr. 1 were necropsied at week 40 (early October).

Group 4 (continuously exposed pigs). From late May 1994 of Year 2 (week 21) to early October (week 40) 28 pigs were continuously exposed to infection on the experimental pasture. They were necropsied for parasite analyses according to the protocol followed for Gr. 1 (Year 1).

Groups 2, 3, 5 and 6 (tracer pigs). Four groups of 15, 20, 21 and 3 pigs, respectively, were placed on a helminth-free pasture during the study. From May, Year 1, to October, Year 2 (and in July, Year 3), sets of at least 3 pigs were moved to the experimental pasture, where they were exposed to pasture contamination for 14 days. They were then held in a helminth-free indoor facility for 4 weeks to permit any larvae present to grow to macroscopic size. The pigs were then necropsied and their worm burdens determined.

Faecal samples. Faecal samples were collected rectally from all exposed pigs (Gr. 1 and 4, and exposed tracers) every 2nd week throughout the study. Samples from tracer pigs on clean pastures were obtained every 4th week. Faecal egg counts were determined by the concentration McMaster technique (sensitivity: 20 eggs g^{-1} (epg)), as described by Roepstorff & Nansen (in press).

Soil samples. By walking a W-route across the pasture, samples of surface soil were collected randomly from the experimental pasture weekly (summer) or fortnightly (winter) and from the clean pastures every 4th week. Sixteen grams of soil sample were mixed with tap water, and treated as for faecal samples, except that several McMaster chambers were counted; this gave a sensitivity of 1 egg g^{-1}.

Antibody assay. The swine sera were tested for IgG antibodies specific against *A. suum* excretory/secretory antigens which were obtained from *in vitro*-cultivated 2nd stage larvae (L2/L3-ES); the indirect ELISA technique employed was described in detail by Lind *et al.* (1993). Briefly, the wells were coated with L2/L3-ES antigen at a concentration of 2.5 $\mu g\,ml^{-1}$, pig plasma was diluted 10^{-3} and horseradish peroxidase conjugated rabbit–anti-swine-immunoglobulin (DAKO, code P164) was diluted 10^{-4}. Positive sera (for *A. suum* or *O. dentatum*) and normal pig serum controls were included in all tests along with a dilution series of an *A. suum*-positive serum pool. After correction for plate-to-plate variation, an optical density (O.D.) cut-off value for discrimination between *Ascaris*-positive and *Ascaris*-negative sera was set to O.D. = 0.280 (the mean + 5 S.D. of 23 *A. suum*-negative/*O. dentatum*-positive sera).

Necropsy. The pigs were not offered any food at the day of necropsy. They were euthanized by either a captive bolt pistol or by CO_2 suffocation, followed by exsanguination. The small intestine, the liver and the lungs were immediately removed. The unopened small intestine was emptied by pressing luke-warm tap water through it twice. All intestinal contents were washed with a stream of water on a 212-μm

sieve, and the retentate was stored in 0.9% NaCl at 4°C overnight, and then transferred to a large Petri dish held over a light table. The worms were recovered and measured to the nearest millimetre. The worms were differentiated as to developmental stage according to the criteria of Pilitt *et al.* (1981). The 5th stage worms were aged according to length criteria as follows: "Young adults": ♂♂, <120 mm; ♀♀, <150 mm; and "Large adults": ♂♂, ≥120 mm; ♀♀, ≥150 mm. The pig's liver and the lungs were examined for macroscopically visible lesions at the day of slaughter, and liver white spots of the diffuse granulation-tissue type as well as the lymphonodular type (Roneus, 1966) were enumerated. As described for post mortem recovery of *O. dentatum* from pigs by Roepstorff & Murrell (1997), subsamples of 20% of the large intestine contents were poured through a 212-μm sieve, and the rinsed intestinal wall was then incubated in saline overnight. The incubation fluid was then sieved (20 μm). All retained samples were fixed in iodine for later microscopical examination for *T. suis*.

Calculations and statistics. All calculations were performed using the SAS® Release 6.04 software package. Pearson's correlation coefficient was used to evaluate the linear relationship between the intestinal worm burdens (log transformed), the faecal egg output (log transformed), the numbers of diffuse liver white spots (log transformed) and the ELISA O.D.s for the continuously exposed pigs (exclusive of Gr. 1 pigs necropsied at weeks 24 and 28 and Gr. 4 pigs necropsied at weeks 27 and 31) and for the tracer pigs (exclusive of Gr. 2 pigs, necropsied at weeks 28 and 32). Thus, the continuously exposed pigs were tested only after 12–14 weeks of infection/exposure, and the tracer pigs were tested only after they had grazed on the infective experimental pasture.

RESULTS

No clinical signs of nematode infections were observed in the pigs. At turnout in Year 1 (May 1993), the experimental pasture had a lush grass cover. Due to the pigs' rooting behaviour, the area became denuded of vegetation during the summer and the autumn of Year 1, and remained a dirt lot for the whole of Year 2. The late summer of Year 1 was rainy, but otherwise normal, and the winter periods of Year 1, early Year 2 was almost normal, with some periods of freezing. The summers of both Years 2 and 3 were very hot and dry. More detail on climate and pasture appearance can be found in Roepstorff & Murrell (1997).

Ascaris suum

Continuously exposed pigs—Year 1. The intestinal worm counts from serial necropsies of continuously exposed pigs (Gr. 1) (Table 1, Fig. 1) reveal that the initial inoculations with 2 × 100 eggs at week 20 (Year 1) resulted in a range of 0–120 young adult *A. suum* 4 weeks later, which decreased to 0–49 large ascarids 8 weeks post-inoculation (p.i.). Thereafter only low numbers of mostly mature adults were found in the Gr. 1 pigs. The adult worms were overdispersed within the host population, as the variance to mean ratio was high (12.1 for the 16 pigs slaughtered at week 40). The first *A. suum* eggs were found in faeces of Gr. 1 pigs 6 weeks p.i. and the number of eggs excreted rose rapidly to a mean of approximately 600 epg at week 8 p.i., after which the excretion level was constant (Fig. 2). Only a few hepatic white spots were observed in the Gr. 1 pigs at necropsy on weeks 24–32, but thereafter the pigs had a mean of 9 diffuse white liver spots and 4 lymphonodular white spots at necropsy (Fig. 1). The ELISA results show that the mean IgG response increased rather slowly until week 28; it then increased rapidly. All pigs seroconverted between weeks 24 and 32 (weeks 4–12 p.i.).

Tracer pigs—summer Year 1. Only 5th stage adult worms were recovered from Gr. 2 tracers. The large majority of the worms measured 80–150 mm. The data from these tracer pigs indicate that the first transmission of infective eggs on the experimental pasture occurred at weeks 30–32 (Table 1, Fig. 3). Subsequently, a variable low number (0–26) of young ascarids was found in the Gr. 2 tracers, and the number of hepatic white spots observed was always low (Fig. 3). The ELISA test results on the sequential sets of tracers (Fig. 4) show that the antibody responses increased throughout the season.

Tracer pigs—winter Year 1–2. A low number of young ascarids was found in 55% of the Gr. 3 winter tracers (exposed October of Year 1 to April of Year 2), but hepatic white spots were rarely recorded. Only 5 out of 20 pigs in this period seroconverted (Fig. 4).

Continuously exposed pigs—Year 2. The uninoculated pigs of Gr. 4 were turned out on to the experimental pasture in May of Year 2 (week 21) to graze continuously. Continuously exposed pigs, necropsied on weeks 27 and 31 (6 and 10 weeks after turnout) harboured low numbers of ascarids, mostly young adults (Table 1). After this time, all worms recovered were mature adults. All pigs necropsied at weeks 31, 35 and 39 harboured at least 3 ascarids, while only 10 of the 16 pigs necropsied at week 43 harboured adult worms (the variance to mean ratio was 29.5). The first positive faecal egg examinations occurred at week 29 (8 weeks after turnout); the mean epg gradually increased to 1200–1500 by the autumn period. The number of diffuse hepatic white spots was highest at the last 2 autumn necropsy intervals (mean: 55 and 68, respectively). Nearly all pigs had lymphonodular white spots from week 31 onwards (Fig. 1). The ELISA tests show that the first seroconversions occurred by 6 weeks after turnout.

Table 1—The *Ascaris suum* worm counts (mean and min.–max.) of all groups of continuously exposed pigs and tracer pigs. The Gr. 1 pigs were experimentally infected in week 20 (total of 200 infective eggs) to initiate parasitic contamination of the experimental pasture, while all other groups were infected through natural exposure

	Continuously exposed pigs					Tracer pigs		
Week[a] (date)	No. of pigs	Young adult[b] *A. suum*		Large adult[b] *A. suum*		Weeks[c] of exposure (date)	No. of pigs	Total *A. suum*[d]
				Year 1				
		Group 1					Group 2	
24 (15 June)	3	40	(0–120)	0	(—)	22–24 (1 June–15 June)	3	0 (—)
28 (13 July)	3	<1	(0–1)	16	(0–49)	26–28 (29 June–13 July)	3	0 (—)
32 (10 Aug.)	3	0	(—)	4	(0–8)	30–32 (27 July–10 Aug.)	3	9 (0–26)
36 (7 Sept.)	3	3	(0–7)	5	(0–10)	34–36 (24 Aug.–7 Sept.)	3	4 (0–11)
40 (5 Oct.)	16	<1	(0–1)	4	(0–26)	38–40 (21 Sept.–5 Oct.)	3	5 (0–10)
				Year 1–Year 2				
							Group 3	
						43–45 (26 Oct.–9 Nov.)	3	1 (0–3)
						47–49 (23 Nov.–7 Dec.)	3	3 (1–5)
						51–1 (21 Dec.–4 Jan.)	3	3 (0–5)
						3–5 (18 Jan.–1 Feb.)	3	<1 (0–1)
						7–9 (15 Feb.–1 Mar.)	3	1 (0–3)
						13–15 (29 Mar.–12 Apr.)	5	2 (0–7)
				Year 2				
		Group 4					Group 5	
27 (6 July)	3	2	(0–5)	0	(—)	21-23 (25 May–8 June)	3	5 (4–7)
31 (3 Aug.)	3	7	(3–11)	2	(1–4)	25–27 (22 June–6 July)	3	12 (0–37)
35 (31 Aug)	3	2	(0–3)	3	(1–6)	29–31 (20 July–3 Aug.)	3	21 (7–43)
39 (28 Sept.)	3	2	(0–5)	6	(2–8)	33–35 (17 Aug.–31 Aug.)	3	3 (2–6)
43 (26 Oct.)	16	1	(0–5)	8	(0–59)	37–39 (14 Sept.–28 Sept.)	5	<1 (0–1)
						41–43 (12 Oct.–26 Oct.)	4	<1 (0–1)
				Year 3				
							Group 6	
						27–29 (5 July–19 July)	3	9 (0–26)

[a]Week of necropsy.
[b]*A. suum* adults were aged according to length, as follows: young adults: ♂♂: <120 mm, ♀♀: <150 mm; large adults: ♂♂: ≥120 mm, ♀♀: ≥150 mm.
[c]Weeks of exposure to the contaminated pasture (the pigs were necropsied 4 weeks after exposure).
[d]The majority of worms were 80–150 mm in length, no egg producing females found.

although the last pig of the group did not seroconvert until week 37 (week 16 post-turnout).

Tracer pigs—summer Year 2. During the summer months of Year 2, Gr. 5 tracers, exposed to infection in June and July, acquired more ascarids (range 0–43 worms) than subsequent tracers exposed from August to November (Table 1). The numbers of diffuse and lymphonodular white spots, however, exhibited a contrasting trend and increased to very high levels in the last 3 tracer subgroups (Fig. 3). The antibody responses of the Gr. 5 tracer pigs (Fig. 4) also increased dramatically during the late summer (exposure in August–October) (Fig. 4). The serological responses often decreased a little after the tracers had been housed in a helminth-free environment for 4 weeks.

Soil samples. Flotation of soil samples from the experimental pasture (from August of Year 1 to October of Year 2) revealed <1 unembryonated *A. suum* egg g^{-1} of soil; however, 2/3 of all eggs found were recovered in a single sample.

Tracer pigs—summer Year 3. The 3 final tracer pigs (Gr. 6) picked up a large number of overwintered eggs during the dry summer of Year 3, and the number of liver white spots was also relatively high (Table 1, Fig. 3). Likewise, the antibody levels in these pigs were also high (Fig. 4).

Correlations between measures of A. suum *infection.* In the continuously exposed pigs, the number of intestinal worms was positively correlated with egg shedding ($P = 0.0001$), but there were no significant correlations between egg counts and worm counts vs antibody levels, egg counts and worm counts vs liver white spots, and antibody levels vs liver white spots. In the tracer pigs the number of intestinal ascarids was not significantly related to the number of liver

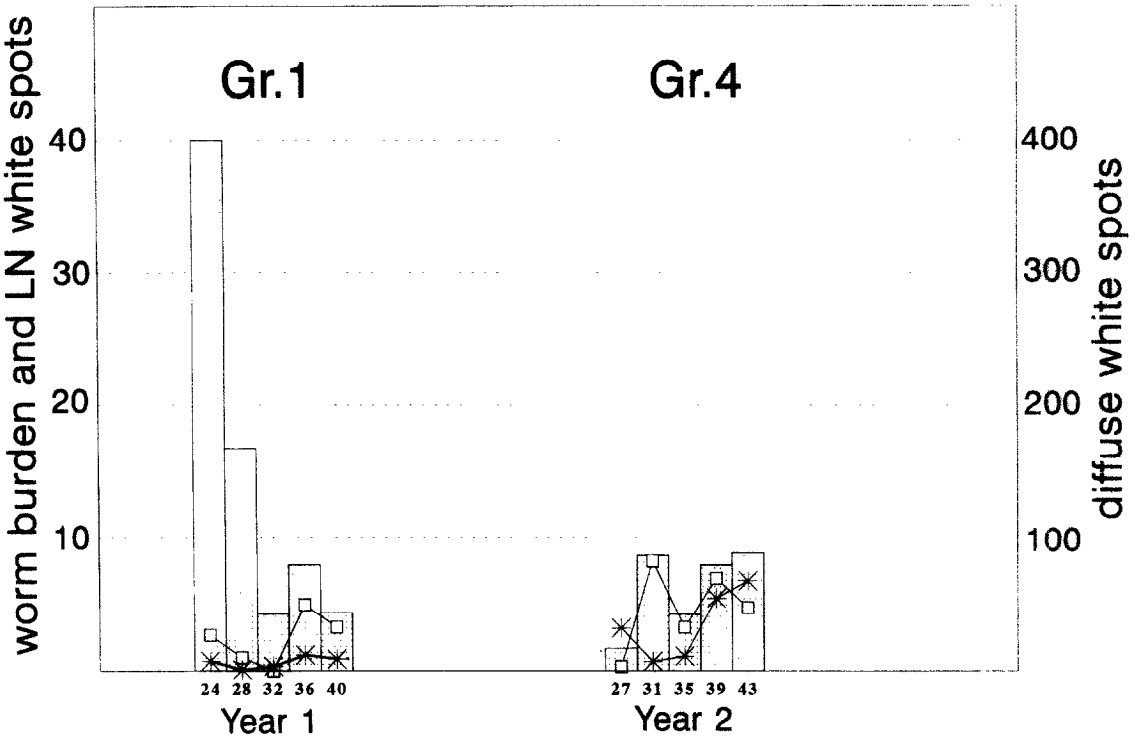

Fig. 1. Mean number of intestinal *A. suum* (columns, left *y*-axis), mean number of lymphonodular white spots (□———□, left *y*-axis) and mean number of diffuse (granulation-tissue type) white spots (*———*, right *y*-axis) in continuously exposed pigs (Gr. 1 and 4). Three pigs were necropsied at the week indicated, except for weeks 40 (Gr. 1) and 43 (Gr. 4), when 16 pigs were necropsied.

white spots or to the pig antibody levels. However, antibody levels and liver white spots had a strong positive correlation ($P = 0.0001$).

Trichuris suis

The Gr. 1 pigs were inoculated with 1000 infective eggs of *T. suis* in mid-July of Year 1, and 840 worms were recovered per pig 8 weeks later (Table 2), at which time the mean egg count was about 800 epg (Fig. 2). Thereafter, the egg counts declined sharply and only 33 worms were recovered per pig in early October. No tracer pigs became infected with this parasite during the summer of Year 1 and the winter of Year 1–2. The continuously exposed pigs of Gr. 4 (summer of Year 2) were negative for *T. suis* until late September, when pigs necropsied at that interval had a mean of 84 worms. In late October the Gr. 4 pigs had a mean of 172 worms. This pattern was repeated in the Gr. 5 tracer pigs (also the summer of Year 2), which were negative until exposed in August–October. However, the tracer pigs generally harboured higher worm burdens than the continuously exposed pigs. The tracer pigs of Gr. 6 acquired a mean of 212 *T. suis* during exposure in July of Year 3. Only 1 out of 32 soil samples from the experimental pasture contained eggs of *T. suis* (unembryonated). The number of intestinal *T. suis* in the continuously exposed pigs was positively correlated with the egg output ($P = 0.0001$).

DISCUSSION

Ascaris suum

The first excretion of *A. suum* eggs occurred on the experimental pasture in the first half of July of Year 1 (Fig. 2) and consequently the first tracer pigs became infected in late July–early August. This showed that *A. suum* eggs may embryonate on a pasture within 4–6 weeks during a normal Danish summer, as occurs under indoor conditions in England (Connan, 1977; Stevenson, 1979) and Sweden (Nilsson, 1982). The winter tracers and the pigs grazing the experimental pasture in the early summer of Year 2 revealed that some infective eggs overwintered, and the summer tracers of Year 2 showed that transmission increased dramatically in August of Year 2. This August increase in transmission may have been the result of maturation of overwintered eggs or rapid development of new eggs or both. The first possibility is supported by M. L. Larsen's (M.Sc. thesis, University of Copenhagen, 1996) observations that *A. suum* eggs

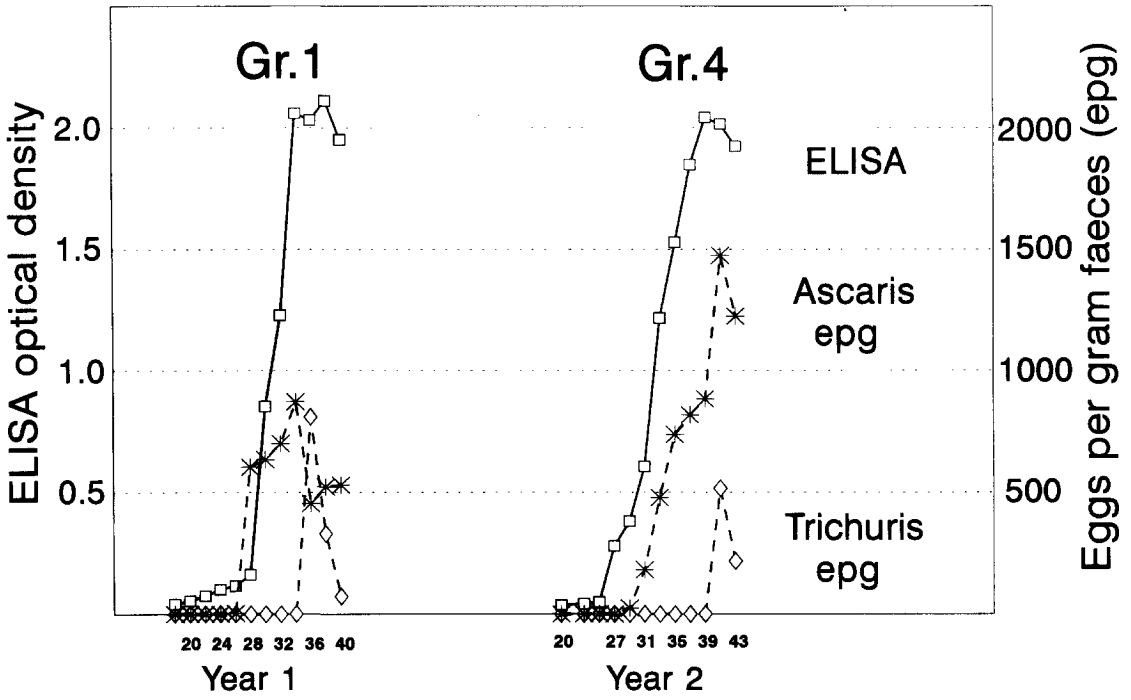

Fig. 2. Faecal egg counts (eggs g^{-1} faeces) of *A. suum* (*———*, right *y*-axis), faecal egg counts (eggs g^{-1} faeces) of *T. suis* (◇---◇, right *y*-axis), and mean ELISA response (specific IgG response against *A. suum* L2/L3-excretory–secretory antigens, □———□, left *y*-axis) of continuously exposed pigs (Gr. 1 and 4). All results are mean values of 28 pigs (at the start of the grazing season) to 16 pigs (at the end of the grazing season). Sera with O.D.s > 0.280 are regarded as *A. suum*-positive.

in faeces, deposited on short grass or in bare soil in October–March, survived in measurable numbers and embryonated during the following summer, a scenario described for indoor conditions (Stevenson, 1979; Nilsson, 1982); almost all *A. suum* eggs deposited on the pasture during the drought in July 1994 in Larsen's concurrent study died very quickly. However, this does not rule out the possibility that new eggs developed rapidly during the drought in protected environments (e.g., the wallowing facility).

After the first eggs of *A. suum* had embryonated on the pasture in late July–early August of Year 1, approximately half of the exposed tracer pigs harboured a low number of young intestinal ascarids at necropsy (Table 1). However, it is difficult to draw conclusions about seasonal variation in the transmission rate on the basis of intestinal worm counts alone. The tracers were exposed for a fixed period of time followed by 4 weeks in quarantine, and therefore their course of infection may resemble experimental primary infections, which have been shown often to result in the expulsion of immatures from the small intestine during the 3rd week of infection, resulting in an almost dose-independent number of large immatures and adults (Roepstorff, unpublished). Therefore, a correlation may not be expected between pasture infectivity and the intestinal worm burdens 4–6 weeks later; in fact, if any relationship exists, it may even be negative, as described by Jørgensen *et al.* (1975). This is in contrast to the results from the same tracer pigs exposed to *Oesophagostomum dentatum* and *Hyostrongylus rubidus*, which had greater correlations between *post mortem* worm counts and pasture contamination (Roepstorff & Murrell, 1997).

Better indicators for environmental contamination and transmission may be antibody determinations and liver white spot estimations. The present ELISA technique is very sensitive in repeatedly infected pigs, which typically seroconvert within 2–4 weeks depending on dose level (Lind *et al.*, 1993). The serological responses of the tracer pigs in this study (Fig. 4) indicate that a moderate, but continuous, transmission took place in the autumn of Year 1, followed by a lower transmission in the winter and spring. By August–October of Year 2 a very high transmission rate occurred, and tracers exposed during the 3rd summer (July 1995) had a more moderate transmission level. Hepatic white spots also reflect the migration of immature ascarids (Roneus, 1966), and in singly exposed pigs, white spots indicate larval migration within the previous 2–3 weeks (Roepstorff, unpublished). Eriksen *et al.* (1992) showed that the number

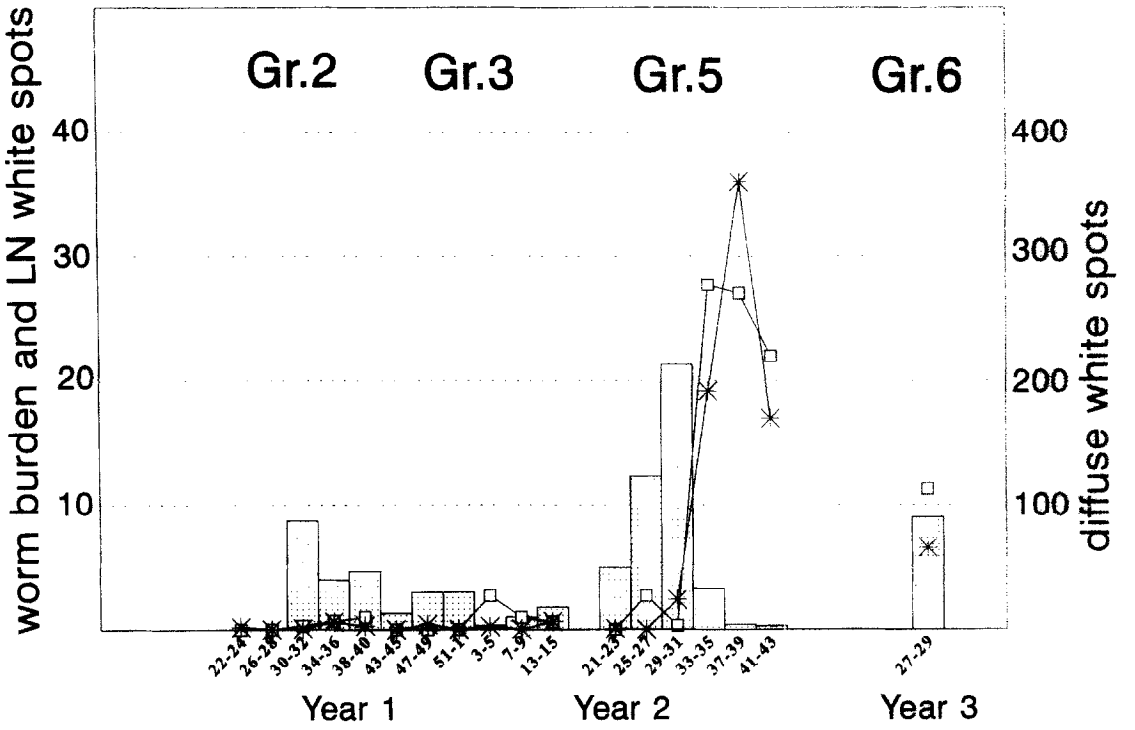

Fig. 3. Mean number of intestinal *A. suum* (columns, left *y*-axis), mean number of lymphonodular white spots (□——□, left *y*-axis), and mean number of diffuse (granulation-tissue type) white spots (*——*, right *y*-axis) in tracer pigs. The *x*-axis shows the weeks of exposure to the contaminated pasture. There were 3 pigs per necropsy, except for weeks 19 (Gr. 3) and 43 (Gr. 4), when 5 pigs were necropsied.

of liver white spots increased until weeks 6–9 under continuous exposure, and thereafter gradually declined to low levels, even though reinfection continued. Based on this finding, the very low numbers of liver white spots observed in the tracer pigs throughout Year 1 and early Year 2 are interpreted as the result of continuous light to moderate infections, which resulted in the majority of the liver white spots resolving prior to necropsy. In contrast, the large number of white spots in late summer and autumn of Year 2 indicate intense transmission with severe liver inflammation, too intense to be resolved prior to necropsy. Thus, the ELISA results and the liver white spot data of the tracer pigs both support the interpretation that pasture transmission varied seasonally, and that such measurements are more sensitive indicators of pasture infectivity than the intestinal worm burdens alone.

After some weeks of exposure, the adult *A. suum* recovered from the continuously exposed pigs of both Gr. 1 and 4 were strongly overdispersed (high variance to mean ratios) within the host population, a common observation in many helminth infections, including single *A. suum* infections (e.g., Petkevičius *et al.*, 1995; Roepstorff, unpublished). The finding of very few young adults during the late summer and autumn of Years 1 and 2 and the absence of an increase in total worm counts, indicate that infective eggs on the pasture did not contribute significantly to the intestinal worm population, once the pigs had been infected. This corresponds to the results of Eriksen *et al.* (1992), who found very few migrating larvae 3–14 weeks after the first inoculation in repeatedly infected pigs, irrespective of whether the pigs harboured an existing population of reproducing adults or not, and it indicates that adults may somehow evade an intestinal immune response that is effective against invading larvae.

The numbers of diffuse white spots were quite low in the Gr. 1 pigs (Year 1), and low to moderate in the Gr. 4 pigs (Year 2). However, many of the pigs (Gr. 1) in the autumn of Year 1 and nearly all Gr. 4 pigs of Year 2 developed livers with numerous scars, presumably representing recovered white spots, comparable to livers recovered from continuously trickle-infected pigs reported in Eriksen *et al.* (1992). In singly infected pigs lymphonodular white spots develop considerably later during the course of infection, but persist much longer (Roepstorff, unpublished). Thus the rather consistent number of lymphonodular white spots of the continuously exposed pigs (Gr. 1 and 4) reflect the chronic character of the infections. From

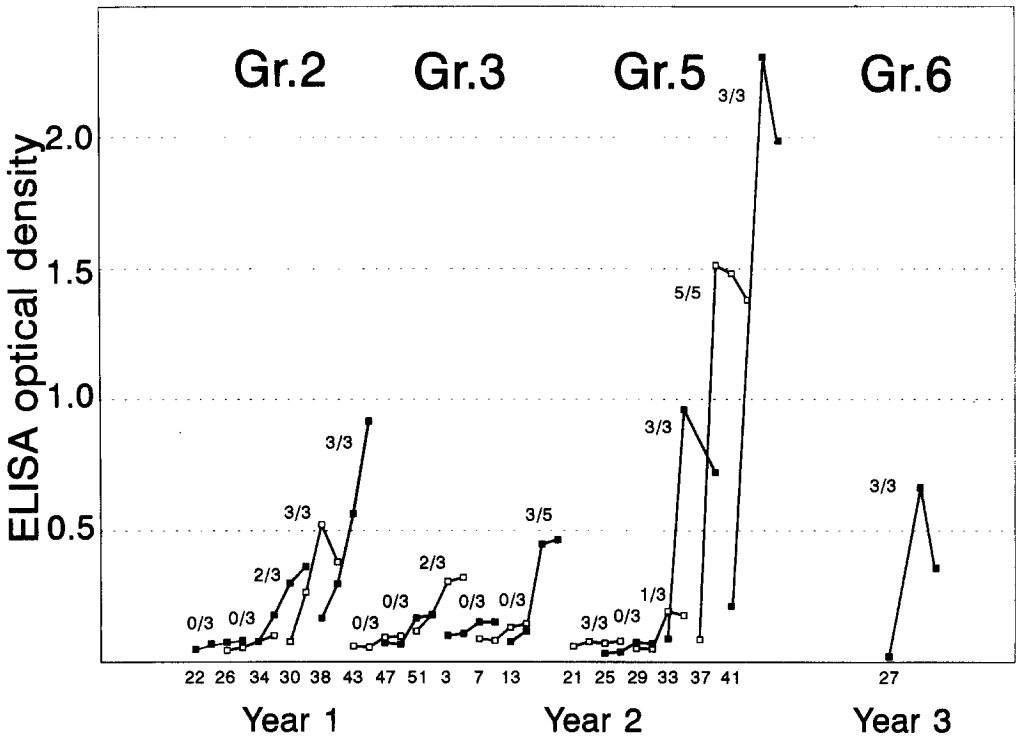

Fig. 4. Mean ELISA response (specific IgG response against *A. suum* L2/L3-excretory–secretory antigens) of individual subsets of tracer pigs from the day of introduction to the contaminated pasture until the day of necropsy, 6 weeks later. All subsets consisted of 3 pigs, except for 5 tracers exposed in weeks 13–15 (Gr. 3) and 5 tracers exposed in weeks 37–39 (Gr. 5). Sera with O.D.s > 0.280 are regarded as *A. suum*-positive. The fraction of pigs, that seroconverted before necropsy, are shown for each subset.

the data on worm population distribution, recovered livers, and high antibody levels, it is understandable that no significant correlations existed between these 3 measures in the continuously exposed pigs. This lack of correlation should be considered when interpretating the results of prevalence studies where chronic infections are involved.

Trichuris suis

A high percentage (84%) of the inoculated *T. suis* eggs established in the Gr. 1 pigs, but their egg excretions lasted only for about 1 month and they were expelled 8–12 weeks post-inoculation, presumably as a result of acquired immunity (Powers *et al.*, 1959). Most eggs were deposited on the pasture in September of Year 1 and did not appear to become infective before August of Year 2, as none of the tracers or the continuously exposed pigs became infected before that time (Table 2). This interpretation is supported by the finding of Alicata (1935), who showed that *T. suis* requires high temperatures for embryonation, and by Burden & Hammet (1979) who found that although embryonation may succeed within 1 summer season, the large majority of eggs require between 62 and 90 weeks to complete their development. The plot study by Larsen (thesis cited above) confirmed this; the eggs of the CEP strain of *T. suis* require 1–2 summer seasons to reach infectivity. In contrast to the slow embryonation, infective eggs have been shown to survive for 11 years (Burden *et al.*, 1987), and therefore it is not surprising that significant numbers of *T. suis* were acquired by tracer pigs in July of Year 3 (Table 2). It is striking that the tracers in late Year 2 (Gr. 5) harboured larger worm burdens than the continuously exposed pigs of Gr. 4 (Table 2). Because Gr. 4 were more exposed to infective eggs than the tracers, a stronger host immune response developed that reduced the intestinal worm burdens to a level below that of the tracers. Jensen & Svensmark (1996) observed in a Danish pig herd severe clinical symptoms in a group of small pigs, turned out on an area with 50–245 *T. suis* eggs gram^{-1} soil. The continuously exposed pigs in Year 2 (Gr. 4) of the present experiment were larger (60–80 kg body weight mid-August) and did not pick up comparable numbers of eggs (< 1 egg g^{-1} soil). This may explain why the pigs were able to control their infections at subclinical levels. In pigs raised in outdoor organic farms (Roepstorff *et al.*, 1992), large differences

Table 2—The *Trichuris suis* worm counts (mean and min.–max.) of all groups of continuously exposed pigs and tracer pigs. The Gr.1 pigs were experimentally infected in week 28 (totally 1000 infective eggs) to initiate parasitic contamination of the experimental pasture, while all other groups were infected through natural exposure

Continuously exposed pigs						Tracer pigs			
Week[a] (date)	No. of pigs	Immature *T. suis*		Adult *T. suis*		Weeks[b] of exposure (date)	No. of pigs	Immature *T. suis*	
				Year 1					
		Group 1					Group 2		
24 (15 June)	3	0	(—)	0	(—)	22–24 (1 June–15 June)	3	0	(—)
28 (13 July)	3	0	(—)	0	(—)	26–28 (29 June–13 July)	3	0	(—)
32 (10 Aug.)	3	487	(180–860)	7	(0–20)	30–32 (27 July–10 Aug.)	3	0	(—)
36 (7 Sept.)	3	113	(40–240)	727	(600–800)	34–36 (24 Aug.–7 Sept.)	3	0	(—)
40 (5 Oct.)	16	0	(—)	33	(0–465)	38–40 (21 Sept.–5 Oct.)	3	0	(—)
				Year 1–Year 2					
							Group 3		
						43–45 (26 Oct.–9 Nov.)	3	0	(—)
						47–49 (23 Nov.–7 Dec.)	3	0	(—)
						51–1 (21 Dec.–4 Jan.)	3	0	(—)
						3–5 (18 Jan.–1 Feb.)	3	0	(—)
						7–9 (15 Feb.–1 Mar.)	3	0	(—)
						13–15 (29 Mar.–12 Apr.)	5	0	(—)
				Year 2					
		Group 4					Group 5		
27 (6 July)	3	0	(—)	0	(—)	21-23 (25 May–8 June)	3	0	(—)
31 (3 Aug.)	3	0	(—)	0	(—)	25–27 (22 June–6 July)	3	0	(—)
35 (31 Aug)	3	0	(—)	0	(—)	29–31 (20 July–3 Aug.)	3	0	(—)
39 (28 Sept.)	3	32	(10–75)	52	(40–70)	33–35 (17 Aug.–31 Aug.)	3	627	(205–925)
43 (26 Oct.)	16	98	(0–655)	174	(0–925)	37–39 (14 Sept.–28 Sept.)	5	966	(560–1815)
						41–43 (12 Oct.–26 Oct.)	4	768	(0–2440)
				Year 3					
							Group 6		
						27–29 (5 July–19 July)	3	212	(80–360)

[a]Week of necropsy.
[b]Weeks of exposure to the contaminated pasture (the pigs were necropsied 4 weeks after exposure).

between individual herds were observed. Only 1 out of 12 herds studied had heavy *T. suis* infections and clinical problems, while the pigs in the other herds appeared to control the parasite to subclinical levels, perhaps because pasture rotations systems were used. In contrast, the other common geohelminth, *A. suum*, which also has resistant and long-lived eggs, produces more eggs, which have a shorter embryonation time, features which may explain why *A. suum* seems to be more difficult to control in organic farm systems (Roepstorff *et al.*, 1992).

The present study emphasizes that helminths, which are transmitted by infective eggs, are difficult to control in outdoor pig rearing, because the eggs are long-lived and overwinter to a much greater extent than do the infective larvae-transmitted helminths *O. dentatum* and *H. rubidus* (Roepstorff & Murrell, 1997). On the other hand, recent observations on cohorts of *A. suum* and *T. suis* eggs have shown that they may still face very high mortality rates under field conditions (Larsen, thesis cited above), and therefore the biotic/abiotic factors that influence the development and survival of the eggs on pastures need to be identified. Such knowledge will help in the design of a biologically-based control strategy suitable for these farming systems.

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