Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs

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SUMMARY

To study population kinetics during primary *Ascaris suum* infections, 3 groups of 52 pigs each were inoculated with 100, 1000, or 10000 infective eggs. In all groups, the majority of larvae was found in the liver on day 3 post inoculation (p.i.) and in the lungs on day 7 p.i. Liver white spots, caused by migrating larvae, were most numerous at day 7 p.i., whereafter they gradually healed, and only low numbers of granulation-tissue type white spots and lymphonodular white spots persisted at days 21–56 p.i. Independent of dose level, 47–58 % of the inoculated eggs were recovered as larvae in the small intestine on day 10 p.i., but most larvae were eliminated at days 17–21 p.i. This elimination started earlier and removed a higher percentage of the worms with increasing inoculation dose, resulting in small strongly aggregated worm populations by day 28 p.i. (k of the negative binomial distribution was low: 0·2–0·4) without significant differences between groups. Thus, overdispersion, which is a characteristic of both porcine and human ascarosis, is found here under experimental conditions where aggregation factors like host behaviour, transmission rate, host status etc have been partly or totally controlled.

Key words: pigs, *Ascaris suum*, dose rate, migration, overdistribution, white spots.

INTRODUCTION

The migratory route of *Ascaris suum* in its natural host, the pig, is well known, and many experimental infection studies have been carried out with this host–parasite model. Nevertheless, only a few authors have systematically investigated the migratory patterns of the larvae through the liver and lungs and the return to the small intestine (e.g. Roberts, 1934; Kelley, Olsen & Hoerlein, 1957; Douvres, Tromba & Malakatis, 1969; Douvres & Tromba, 1971). One good reason is that *A. suum* eggs and migrating larvae are very small, making it extremely difficult and time consuming to recover the young immature stages from the internal organs of a large host like the pig. Therefore, Roberts (1934) and Kelley *et al.* (1957) only obtained relative distributions of larvae within the different organs without describing the migration quantitatively. Douvres *et al.* (1969) and Douvres & Tromba (1971) estimated the total numbers of larvae within the internal organs at different days after inoculation with known numbers of infective eggs, but because they examined subsamples by direct microscopy, the post-mortem procedures were so labourious that they only examined one pig per time of post-inoculation and their latest days of necropsy were 15 and 11 p.i., respectively. Therefore, when a newly developed agar gel technique made it relatively easy to isolate with high efficacy minute *Ascaris* larvae from large volumes of minced liver tissue and small intestinal contents (Slotved *et al.* 1996, 1997), the present study was carried out to obtain a quantitative description of the migratory phase and the small intestinal worm populations until patency in pigs inoculated with low, medium and high doses of infective eggs.

Earlier studies have indicated an inverse relationship between dose rate and the number of established adult worms (Roneus, 1971; Andersen *et al.* 1973; Jørgensen *et al.* 1975), but several later studies in our laboratory using low dose rates have not resulted in the expected high number of adult worms (e.g. Roepstorff & Murrell, 1997; Roepstorff, unpublished observations), and therefore an additional objective was to study the establishment of adult populations as a function of dose rate.

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MATERIALS AND METHODS

Experimental pigs

Danish Landrace/Yorkshire/Duroc cross-bred male

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and female pigs were obtained from a specific-pathogen-free (SPF) herd. Repeated faecal examinations had indicated that all weaners produced in the herd for several years had been helminth-free and no excretion of eggs was observed in the pigs selected for this study. A total of 156 pigs was evenly distributed into 3 groups (I, II and III) according to weight, sex and litter origin. Similarly, 10 groups of 5 pigs (2 females and 3 males) were established from each main group. To guard against the inconvenience that might be occasioned by untimely deaths, 2 extra pigs were included in each main group. At the start of the experiment the weaned pigs were 11–16 weeks old and weighed 33·2 ± 5·2 kg (mean ± S.D.). The pigs were allowed to adapt to pens and fodder for 2 weeks prior to inoculation.

House and feeding

Each of the 3 experimental groups occupied 4 large cages, and pigs slaughtered on the same day were evenly distributed among the 4 cages. The cages had totally slatted floors and no straw bedding and had never been used before. Separate tools and boots were used for each group in the daily routine work. The pigs were fed a standard feed regimen and the ration was adjusted every second week according to body weight. The diet consisted of ground barley, soy meal, minerals and vitamins, a diet previously shown to facilitate establishment/development of helminth infections, including *A. suum* (Petkevicius *et al*. 1995; Bjørn, Roepstorff & Nansen, 1996).

Parasite

The CEP-strain of *A. suum* (the strain used for most studies at the Centre for Experimental Parasitology) was isolated in the winter of 1993 from a small organic farm, which utilized only outdoor rearing facilities. *A. suum* eggs were isolated from fresh faeces and embryonated in 0·1 M H_2SO_4 for 3 months (in the dark at room temperature), conditions which have been shown to result in fully infective eggs (Oksanen *et al*. 1990). A few weeks before inoculation, the infectivity of the eggs was tested in 2 pigs and 30 % of the embryonated eggs inoculated were recovered as migrating larvae in the lungs on day 7 p.i. Just prior to inoculation the doses were adjusted to the desired numbers of fully embryonated eggs.

Experimental protocol

Pigs in Groups I, II and III were inoculated by stomach tube with 100, 1000 and 10000 embryonated eggs, respectively. Five pigs from each group were killed on days 3, 7, 10, 14, 17, 21, 28, 35, 42, and 56 p.i. to determine the worm burdens in the livers, lungs, and small intestines. Faecal samples were collected every second week from 2 weeks before inoculation until day 28 p.i. and thereafter weekly. Blood was sampled 2 weeks prior to inoculation and each week during the course of infection.

Necropsy

The pigs were not offered any food at the day of necropsy. They were killed by a captive bolt pistol followed by exsanguination. The small intestine, the liver and the lungs were immediately removed. The small intestine was divided in 4 equal sections (section 1: 0–25 % from the pylorus, section 2: 25–50 %, section 3: 50–75 %, section 4: 75–100 %), which were handled separately. Each section was opened, and the mucus, but not the mucosa, was scraped off twice by pulling the intestinal wall gently through 2 sticks. Any large worms were immediately isolated. Thereafter the intestinal wall was washed in lukewarm physiological saline (0·9 % NaCl), and the washing water was gently poured through a 20 μm sieve. The intestinal contents, the mucus and the material retained on the sieve were mixed in a beaker, and physiological saline was added to a total volume of 500 ml. Thereafter, the ascarid larvae were isolated by the agar gel technique, described in detail by Slotved *et al*. 1997. In short, the sample was immediately mixed with 500 ml of 2 % agar (43–48 °C), poured out on 3 disposable cotton cloths and allowed to solidify for a few mins at room temperature. These agar gels were incubated in physiological saline overnight at 38 °C. The following day, nearly all larvae (> 97 %, Slotved *et al*. 1997) had migrated out of the gels, and an almost clean suspension of small ascarid larvae could be collected on a 20 μm sieve.

The liver and the lungs were examined for macroscopic lesions, especially superficial granulation-tissue type white spots (GT–WS) and lymphonodular white spots (LN–WS) (Roneus, 1966), and subsequently cut into 3–5 mm pieces in a kitchen blender. After thorough mixing, 2 samples of 25 % each of the total weight were taken. One 25 % sample was immediately mixed with an equal volume of 2 % agar and handled as described above for the small intestinal contents. The other 25 % sample was macrobaermannized, i.e. the minced tissue was placed on a double layer of gauze in physiological saline overnight (38 °C), and the next day all the sediment, including any ascarid larvae, was washed on a 20 μm sieve. The comparison of the 2 alternative methods for handling liver and lungs has been presented in detail by Slotved *et al*. (1996), and these authors concluded that the agar gel technique was superior to the macrobaermann technique with respect to isolation of larvae from the livers, while macrobaermannization was the tech-

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nique of choice with respect to the lungs. Therefore, only the results of the most suitable methods will be presented here.

Laboratory procedures

Faecal egg counts were determined by a concentration McMaster technique with a lower detection limit of 20 eggs per g (epg) (Roepstorff & Nansen, 1997). 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 ELISA technique employed has been described in detail by Lind *et al.* (1993). Briefly, the wells were coated with L2/L3–ES antigens at a concentration of 2·5 μg/ml, pig plasma was diluted 10^{-3}, and horseradish peroxidase-conjugated rabbit-anti-swine-IgG (DAKO) was diluted 10^{-4}. *A. suum*-positive sera 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 (OD) cut off value for discrimination between *Ascaris*-positive and *Ascaris*-negative sera was set to OD = 0·139 (the mean + 5 S.D. of sera from 32 known *A. suum*-negative pigs).

Samples of worms (from all organs) were fixed with iodine (80 g iodine and 400 g potassium iodine in 800 ml of distilled water) and stored. Before counting using a stereo-microscope, the samples were poured into Petri dishes and decolourized with sodium thiosulphate.

Calculations and statistics

All calculations were performed using the SAS® Release 6.11 software package. The existence of overdispersion was evaluated by the variance to mean ratio, which has to be $\gg 1$ to declare overdispersion (Anderson, 1993). The parameter k of the negative binomial distribution is another measure of the degree of aggregation and is calculated as $k = \text{mean}^2/(\text{variance} - \text{mean})$, where mean and variance are calculated on total worm burdens. Differences in total worm burdens, numbers of GT–WS, faecal egg counts and ELISA ODs between experimental groups were analysed by Student's t-test by day p.i. Pearson's correlation coefficient was used to evaluate the linear relationship between the inoculation dose (log transformed), the intestinal worm burdens (log transformed), the faecal egg counts (log transformed), the number of GT–WS (log transformed), and the ELISA ODs for the pigs necropsied day 56 p.i.

RESULTS

During days 16 and 21 p.i., 3 pigs from Group I died of either acute haemorrhagic enteritis (2 pigs) or diffuse fibrinous peritonitis (1 pig), but no clinical symptoms or temporary anorexia were observed in any other pigs. Because of the deaths, only 4 pigs from Group I were necropsied at day 35 p.i. In Groups II and III, the 2 extra pigs were necropsied together with the last pigs on day 56 p.i. (Table 1).

Worm counts

The worm counts in the liver, lungs and the small intestine are presented in Table 1. Larvae were regularly found in the livers from day 3 to day 14 p.i., but the recoveries were always low in Groups II and III (max. 8% and 6%, respectively), while unexpectedly high recoveries were observed in the Group I pigs as late as days 10 p.i. (72%) and 14 p.i. (42%). The first few larvae were found in the lungs already on day 3 p.i., but by day 7 p.i. the majority of larvae were found in the lungs (33, 32 and 40% of the inoculation doses, respectively), whereafter some larvae were still present in the lungs on day 10 p.i. (14, 8 and 10% of the inoculation doses, respectively). Few larvae were recovered in the small intestine on day 7 p.i., but on day 10 p.i. the large majority of larvae had reached the intestine, representing 47, 58 and 56% of the inoculation doses, respectively. Comparable high intestinal worm burdens were obtained on day 14 p.i. (37, 69 and 50% of the inoculation doses, respectively), while considerable reductions in numbers were observed on day 17 p.i. in the high dose groups (46, 21 and 5% of the inoculation doses recovered, respectively). By day 17 p.i. and onwards, almost all ascarids were found in the small intestine, and means of 1–41 worms were found on days 28–56 p.i. without significant differences between groups. At this late stage of the infection, 58% of the Group I pigs, 41% of the Group II pigs, and 32% of the Group III pigs harboured worms.

Overdistribution of worm burdens

For all groups of pigs, irrespective of inoculation dose and day of necropsy, the 'variance to mean ratio' was much greater than 1, indicating overdispersed worm burdens. The parameter k of the negative binomial distribution (Table 1) was found to be quite high in all experimental groups during migration and the early intestinal phase (k = 3·6–54·2, except for the few larvae recovered in Group I on day 3 p.i.). In almost all groups, k was less than 1 after day 17 p.i. and 0·2–0·4 by day 28 p.i. and onwards, indicating a gradual increase in the degree of aggregation.

Location of worms within the pig

The relative distributions of worms in the liver, the lungs and the small intestine, and in the 4 sections of the small intestine during the course of infection were largely identical for the 3 dose groups and the

Table 1. Worm counts in the livers, lungs and the small intestines and total numbers of white spots (granulation-tissue type and lymphonodular) in the livers of pigs infected once with either 100, 1000 or 10000 infective eggs of *Ascaris suum*

(The coefficients of variance and the *k* values of the negative binomial distribution were calculated on the basis of the total worm burdens in all organs.)

Exp. group	Day p.i.	No. of pigs	White spots	Worms in the liver	Worms in the lungs	Worms in the small intestine	Total worm burden mean ± S.D.	Percentage infected pigs	*k* value*
I	3	5	3	4	2	0	6 ± 7	60	1·1
(100 eggs)	7	5	23	13	33	< 1	46 ± 14	100	13·9
	10	5	2	72	14	47	133 ± 71	100	3·6
	14	5	15	42	0	37	79 ± 32	100	6·7
	17	5	3	0	0	46	46 ± 27	100	3·2
	21	5	7	1	0	5	6 ± 10	80	0·4
	28	5	1	1	0	16	17 ± 27	60	0·4
	35	4	2	0	0	7	7 ± 13	50	0·3
	42	5	4	0	0	20	20 ± 31	60	0·4
	56	5	0	0	0	41	41 ± 88	60	0·2
II	3	5	19	79	2	0	81 ± 31	100	7·2
(1000 eggs)	7	5	135	83	318	2	403 ± 117	100	12·3
	10	5	38	46	77	578	700 ± 98	100	54·2
	14	5	37	17	0	694	711 ± 127	100	32·7
	17	5	19	0	0	212	212 ± 280	100	0·6
	21	5	5	0	0	8	8 ± 10	60	0·6
	28	5	14	0	0	8	8 ± 17	40	0·2
	35	5	4	0	0	1	1 ± 1	40	1·8
	42	5	15	0	0	5	5 ± 11	40	0·3
	56	7	10	0	0	20	20 ± 37	43	0·3
III	3	5	296	643	11	0	654 ± 258	100	6·5
(10000 eggs)	7	5	396	23	4013	4	4040 ± 743	100	29·8
	10	5	325	54	954	5559	6566 ± 1811	100	13·2
	14	5	146	14	10	4994	5019 ± 2089	100	5·8
	17	5	24	3	0	470	473 ± 397	100	1·4
	21	5	26	0	0	100	100 ± 112	100	0·8
	28	5	22	0	0	3	3 ± 7	40	0·2
	35	5	11	2	1	1	3 ± 7	20	0·2
	42	5	9	0	0	36	36 ± 80	20	0·2
	56	7	15	0	0	4	4 ± 8	43	0·2

* For formula, see Materials and Methods section.

combined data are shown in Fig. 1. On day 10 p.i., when the larvae had returned to the small intestine, they were found primarily in section 2 and adjacent sections, while negligible numbers occurred in section 4. Four days later, very few larvae were found in section 1, while the majority were found in sections 3 and 4. Coinciding with the reduction in worm recovery and the increase in overdispersion, this backward movement continued on days 17 and 21 p.i., when few larvae were found in sections 1 and 2. In contrast, the small overdispersed worm populations found at day 28 p.i. and onwards, were located in section 2 and the adjacent sections, except a few worm populations which were located more distally (2 pigs of Group III at days 35 and 56 p.i., respectively).

Faecal egg counts and serum antibody levels

The faecal examinations (Fig. 2A) showed that 4 out of 34 pigs excreted eggs in faeces on day 42 p.i. (1 pig in Group I and 3 pigs in Group II and none in Group III), whereafter the egg counts increased in all groups. The highest mean epg level was observed in Group I and the lowest in Group III; there were, however, no significant differences in the egg excretion between groups ($P > 0{\cdot}15$). The specific anti-*Ascaris* IgG response (Fig. 2B) increased very slowly in the Group I pigs, of which max. 20% were seropositive (OD > 0·139) on any sampling day. In Group II, 13–67% of the pigs had ODs above the cut off value on days 14–56 p.i., but the antibody level was only significantly higher in Group II compared to Group I on days 14–35 p.i. ($P < 0{\cdot}05$). The antibody response in the Group III pigs was highest on days 14–28 p.i., whereafter the mean OD declined gradually. From day 21 p.i. and onwards 50–86% of the Group III pigs were seropositive. In the period 14–35 days p.i., the ODs of the Group III pigs were significantly higher than in the Group I

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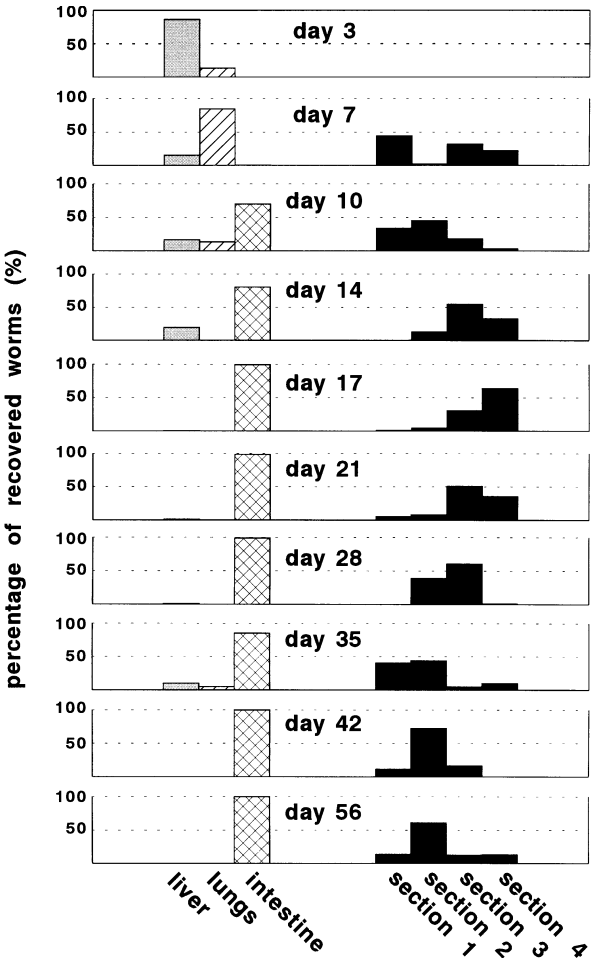

Fig. 1. The 3 left columns represent percentages (%) of recovered *Ascaris suum* found in the livers (light columns), the lungs (hatched columns), and the small intestine (cross-hatched columns). The 4 right columns (dark columns) represent the percentages (%) of recovered intestinal worms found in the 4 equal sections of the small intestine (sections 1, 2, 3, and 4). The pigs were serially necropsied after a single inoculation with either 100, 1000, or 10000 infective *A. suum* eggs at day 0, and data for these 3 dose groups are combined. All values are arithmetric means. For number of pigs, see Table 1.

pigs ($P < 0{\cdot}01$) and the Group II pigs ($P < 0{\cdot}05$), while no significant differences could be observed later in the course of infection.

Liver white spots

At necropsy, the maximum number of GT–WS was observed on day 7 p.i. in all dose groups (Fig. 2C) being strongly positively correlated with the dose level (Group I: 23 GT–WS; Group II: 135 GT–WS; Group III: 396 GT–WS). On days 3, 7, and 10 p.i., significant differences ($P < 0{\cdot}05$ or $0{\cdot}01$) in the numbers of GT–WS were found for all combinations of groups (except Groups I and II at day 3 p.i.). When calculating the maximum number of GT–WS as a percentage of the inoculation dose, negative correlations with dose level were found at day 7 p.i. (Group I: 23%; Group II: 14%; Group III: 4%; $P < 0{\cdot}05$ for Group I *vs.* Group III and Group II *vs.* Group III). The livers with the highest GT–WS counts (300–500 GT–WS) were more or less white in colour and counts of more than approximately 200 GT–WS were not precise because large spots presumably consisted of several confluent spots. The majority of the GT–WS observed on day 10 p.i. were clearly in the process of healing (less white and distinct than at day 7 p.i.), and from day 14 p.i. and onwards a reduction in the number of GT–WS was observed in all experimental groups, and no significant differences in numbers between groups were found after day 10 p.i. From day 21 p.i. and onwards only low numbers of clearly visible GT–WS were observed, but these late GT–WS counts are not precise, as counting such faded GT–WS is quite subjective. Thus, very faint GT–WS were often observed without being counted. The LN–WS counts are much more exact and these lesions first appeared 10 days p.i., whereafter low numbers, positively correlated with the inoculation dose, persisted throughout the experiment (Fig. 2D).

Correlations between measures of infection

In the 19 pigs necropsied on day 56 p.i., the number of intestinal worms was positively correlated with egg shedding ($P = 0{\cdot}0001$) and antibody level ($P = 0{\cdot}01$), but there were no significant correlations between worm counts *vs.* GT–WS or LN–WS, egg counts *vs.* GT–WS, LN–WS or IgG response, and antibody level *vs.* GT–WS or LN–WS. None of the parasitological measures were significantly correlated with the inoculation dose.

DISCUSSION

Worm counts during the migration phase

The *A. suum* larvae had essentially the same migration characteristics at all 3 dose levels. Thus, 4–8% of the administered eggs were found as larvae in the liver on day 3 p.i. (the majority of the recovered larvae), on day 7 p.i. 33–40% of the inoculated eggs were isolated from the lungs (the majority of the recovered larvae), and on day 10 p.i. 47–58% of the inoculated eggs had succeeded in establishing as larvae in the small intestine. There were no indications of any differences between groups inoculated with different egg doses regarding rate and success of migration, and the time schedule for the migration corresponds to that previously observed in single-dose infected pigs (Roberts, 1934; Kelley *et al.* 1957; Douvres *et al.* 1969; Douvres & Tromba, 1971). While Roberts (1934) and Kelley *et al.* (1957) only presented relative distributions without information on inoculation

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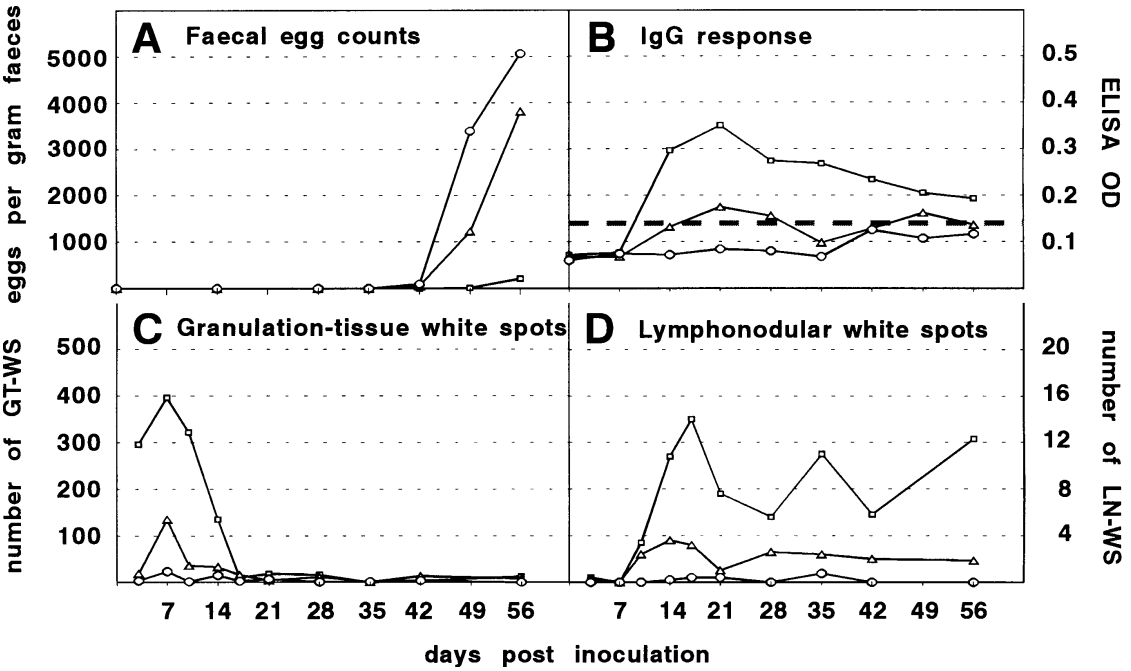

Fig. 2. *Ascaris suum* faecal egg counts (A), anti-*A. suum* L2/L3–excretory/secretory IgG response measured as optical density (OD) by ELISA (B), numbers of granulation-tissue white spots (GT–WS) in the liver (C), and numbers of lymphonodular white spots (LN–WS) in the liver (D) in pigs single-infected with either 100 (○), 1000 (△), or 10000 (□) infective *A. suum* eggs at day 0. The cut-off level (OD = 0·139) for discriminating between negative and positive optical densities of the ELISA is indicated by a broken line in B. All values are arithmetric means. For numbers of pigs, see Table 1.

doses and worm numbers recovered, Douvres *et al.* (1969) inoculated their pigs with 100000 infective eggs and by examination of 1 pig per post-inoculation day of necropsy they recovered 4–11 % of the inocula in the livers at days 3–4 p.i., 12–30 % in the lungs at days 5–8 p.i. and 1–22 % in the small intestine at days 9–15 p.i. Thus, the recoveries of Douvres *et al.* (1969) were smaller in the lungs and small intestine than in the present experiment, which may be explained by the higher dose rate (see below) and perhaps a more unreliable counting method (direct microscopy of subsamples of blended tissue). Douvres & Tromba (1971) inoculated their pigs with only 1300 infective eggs and the migratory pattern they found by necropsy of a total of 7 pigs on days 1–11 p.i. was very similar to the results of Douvres *et al.* (1969), and again they only found 13–26 % of the inoculation dose as larvae in the small intestine at days 9–11 p.i. The present study took advantage of the newly developed agar-gel method for isolation of clean suspensions of larvae from blended liver tissue (Slotved *et al.* 1996) and small intestinal contents (Slotved *et al.* 1997), while a macrobaermann technique was used for the blended lungs (Slotved *et al.* 1996). These methods are fast and have high efficacies (Slotved *et al.*, 1996, 1997), and therefore, we were able to recover the very small larvae rather easily from large samples (total worm counts of intestinal contents). An unexpectedly large number of larvae was found in the livers until day 14 p.i., especially in Group I. Correspondingly, Roberts (1934) observed larvae in the liver until day 10 p.i. after single-dose infections. These late liver larvae may, however, have failed to migrate further, as they seemingly didn't result in subsequent isolation of any larvae from the lungs. We do not have any explanation of the relatively high number of larvae in the livers of the Group I pigs on days 10 and 14 p.i., but the possibility exists that significant numbers of larvae may have been trapped in the livers of all 3 groups of pigs and that they only were released and able to move actively out of the agar gels in the low dose group because of the less severe inflammatory tissue reactions.

The apparently meaningless migration of some nematode larvae, including *Ascaris* larvae, within their final hosts has most often been explained as a phylogenetic reminiscence (e.g. Smyth, 1994). However, Read & Skorping (1995) have demonstrated that nematodes exploiting tissue habitats during development are, on average, bigger and grow faster than their closest relatives that develop wholly in the gastrointestinal tract. Therefore, these authors suggested that migration may be a life-history strategy that confers selective advantage despite the fact that adult establishment rates are typically lower for species with larval migration. Read & Skorping (1995) proposed that migrants run the risk of getting lost or trapped in inappropriate host tissues, but the present study showed that migration succeeded for approximately 50 % of the inoculated eggs and that *A. suum*, therefore, does not seem to be hampered by

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the fast larval growth in the more benign tissue habitat.

Worm counts in the small intestine

After having arrived in the small intestine between days 7 and 10 p.i., the sizes of the intestinal worm burdens were largely constant (approximately 50% of the inoculation dose) until day 14 p.i. (Groups II and III) or day 17 p.i. (Group I). Douvres *et al.* (1969) found considerable numbers of larvae in the large intestine by day 10 p.i.; this early elimination may explain the variable and low worm burdens in the small intestine of their pigs inoculated with 100000 eggs. This finding fits with the present observation that the onset of larval expulsion from the small intestine depended on the dose rate, starting earlier in Group II and especially Group III than Group I. The contents of the caecum and colon were not examined in the present experiment, however, the expulsion was indicated by the posteriorly located worm populations on days 14–21 p.i. By day 28 p.i. only small populations were recovered and they were primarily located in section 2 and adjacent sections.

In previous studies in which pigs were inoculated with varying numbers of infective eggs and slaughtered after day 30 p.i. an inverse relationship between dose rate and worm burden has been observed (Roneus, 1971; Andersen *et al.* 1973; Jørgensen *et al.* 1975). For example, Andersen *et al.* (1973) found means of 32, 14 and 1 worms when giving 50, 500 and 5000 eggs, respectively. One of these studies was very preliminary with only 1 pig per dose level (Roneus, 1971), while the documentation of the other studies was limited to 3 groups of 3 pigs (Andersen *et al.* 1973), and 2 groups of 5 pigs (Jørgensen *et al.* 1975). Nevertheless, the 3 cited studies confirm each other, and low inoculation doses have since been included in experimental protocols at our laboratory when patent worm burdens were needed. In one experiment, inoculation of 20 pigs with 50 infective eggs each resulted in only 1 mature worm being recovered 10–12 weeks later (Roepstorff, unpublished observations) and in another experiment doses of 200 infective eggs resulted in small, variable numbers of patent worms in half of the pigs (data from Roepstorff & Murrell, 1997). Several more inoculations with small numbers of eggs in our laboratory have given similar results, and it is therefore well documented that low doses of eggs most often result in variable and low numbers of adult worms, far from the 100% infected low-dose pigs of Andersen *et al.* (1973) and Jørgensen *et al.* (1975). By administration of 300 or 600 infective eggs, Forsum, Nesheim & Crompton (1981) were able to establish patent infections in all inoculated 3 to 4-week-old pigs showing that more uniform worm burdens may be obtained under some experimental conditions. In a trickle infection experiment with dose rates of 25 or 500 eggs given twice weekly, Eriksen *et al.* (1992) found that 30% and 20%, respectively, of groups of 20 pigs harboured varying low numbers of adult worms 10–13 weeks after the first inoculation. These data showed that no significant differences existed between these dose levels and that establishment of patent worms in trickle infections may be very similar to that of most single infections. In the present experiment, no statistically significant differences in worm burdens were found among the 3 groups after day 28 p.i., but when looking at the numbers of infected pigs there seemed to be an inverse relationship between dose level and prevalence of worms.

It is commonly found that helminths are aggregated within the host population and that this aggregation fits a negative binomial distribution with the parameter k, which varies inversely with the degree of parasite aggregation (Anderson, 1993). Values of k close to zero imply a high degree of aggregation with the majority of parasites being harboured by a few hosts (Anderson, 1993). Values for k were quite high ($k = 3$–54) in all dose groups of the present experiment before the expulsion of worms started; however, it decreased during expulsion, becoming 0·2–0·4 by day 28 p.i. and onwards, which indicates an increasing degree of aggregation. According to Anderson & Gordon (1982), factors which generate aggregation include heterogeneity in host behaviour influencing the uptake of infective stages, spatial heterogeneity in the distribution of infective stages, and heterogeneity in effective immunity within the host population (due either to past experiences of infection, other parasitic species within the host or genetic constitution). Therefore, it is interesting that a high degree of aggregation in the *A. suum*/pig system has been obtained in the present study with most of these aggregation factors excluded. Host behaviour had no impact on transmission, the infective stages were evenly distributed to pigs within groups, there were no uncontrolled infections with *Ascaris* or any other helminths, and the genetic constitution is expected to be more uniform than in freely mating populations of hosts although the genetics are not fully controlled. Furthermore, factors which generate underdispersion (non-random even distribution of parasites within the host population) include host immunological processes and density-dependent processes (Anderson & Gordon, 1982). Both types of processes have been shown to occur in *Ascaris*-infected pigs (e.g. Eriksen, 1981; Jørgensen *et al.* 1975; Croll *et al.* 1982; and the present results, showing a positive correlation between inoculation dose and parasite mortality). Nevertheless, a high degree of overdispersion is normally found in both naturally and artificially infected pigs (J. Boes, personal communication) as well as in humans

infected with *A. lumbricoides* (e.g. Guyatt *et al.* 1990), and the present controlled experiment indicates that overdispersion may be characteristic of the relationship between *Ascaris* and its host. This point of view is supported indirectly by studies in humans which show that the relationships between prevalence of infection and the mean worm burden for *A. lumbricoides* in different geographical areas fit the same negative binomial distribution, i.e. have the same low k value, independent of the level of infection (prevalence and intensity of infection) in the communities (Guyatt *et al.* 1990). Therefore, the underlying mechanisms of the aggregated distribution of *Ascaris* in its natural hosts need to be further investigated.

The ELISA responses

Relatively marked IgG antibody responses only occurred for some weeks in the pigs given 10000 eggs. The present ELISA was developed by Lind *et al.* (1993) who found that pigs trickle-infected with either 25 or 500 *A. suum* eggs twice weekly (see Eriksen *et al.* 1992) seroconverted 4 and 2 weeks after the initial inoculation in which they received only 200 and 2000 infective eggs, respectively. The explanation of the poorer IgG responses in the present experiment may be that single infected pigs only experienced the stage-specific antigens for a limited period of time (see Kennedy & Qureshi, 1986). This observation fits with the slow seroconvertion of pigs single-dosed with 200 eggs (Roepstorff & Murrell, 1997), and with the occasional findings of seronegative pigs with patent infections (Bøgh *et al.* 1994; Roepstorff, unpublished observations). Nevertheless, the two latter studies showed that the present ELISA setup is very useful in practice, presumably because natural transmission is a continuous process (corresponding to trickle infections) and not a single event, and the present results merely indicate that when natural transmission is low, false negative ELISA results may occur.

Liver white spots

Liver lesions, caused by migrating ascarid larvae, are commonly designated 'white spots'. Roneus (1966) described 3 morphological and histological types, namely small and large granulation-tissue type white spots (GT–WS) and lymphonodular type white spots (LN–WS). GT–WS are mesh-like lesions, characterized by clearly visible grey-white interlobular septa (increased amounts of connective tissue), the thickening of which decreased peripherally. The large GT–WS have a central compact grey-white tissue mass and Roneus (1966) suggested that they are formed around trapped larvae, while he believed that the small ones without a compact centre are generated along the migration routes of the larvae. Macroscopically, however, the borderline between small and large GT–WS is not sharp, and no attempts to differentiate them were made in the present study. Previous studies have shown that GT–WS may appear by day 3 p.i. (Oldham & White, 1944; Roneus, 1966) and that they may have disappeared at day 40 p.i. (Roneus, 1966). LN–WS are round, sharply defined pearl-like semi-transparent nodules of lymphocytes and according to Roneus (1966) they are developed from large GT–WS. In single *Ascaris*-infected pigs, LN–WS first appeared at days 10–13 p.i. (Schwartz & Alicata, 1933; Roneus, 1966), i.e. later than the GT–WS, and Roneus showed that they may persist until day 70 p.i., while they had disappeared at days 90–170 p.i. However, the results of Oldham & White (1944) may have been influenced by the uncontrolled *Ascaris* infections, and Roneus (1966) only examined a total of 7 pigs during days 3–170 p.i., and therefore the available documentation on white spot occurrence during the course of single infections is scarce. Nevertheless, the present results confirm these sparse results, as the numbers of GT–WS had a maximum at day 7 p.i., whereafter the large majority had disappeared by day 17 p.i., leaving a low number of GT–WS and LN–WS for the remaining period.

The late GT–WS may be explained by the few migrating larvae which were isolated from the livers as late as days 28 and 35 p.i. It is unlikely that these late larvae originated from uncontrolled reinfection with naturally embryonated eggs as strict precautions were taken. It is more likely that a few eggs, at inoculation, may have contaminated the pens either directly or after having passed through the pigs without hatching, but if this was true, we should expect 10-fold differences in late GT–WS and late migrating larvae between groups, but that was not the case. Therefore, it remains unsolved whether the late GT–WS and the late migrating larvae isolated from the livers originated from a few lately obtained infective eggs or from larvae which had survived for a considerable time within the host without normal development.

Both GT–WS and LN–WS are well known from slaughterhouse examinations of naturally infected pigs, and 24% of the superficial white spots have been found to be LN–WS by both Roneus (1966) and Bindseil (1967) (recalculation of the data of Bindseil, as he did not recognize LN–WS as caused by parasites). Thus, the livers of naturally infected pigs resemble the late primary infections of the present experiment, indicating that fatteners at normal slaughter weight have often passed the acute phase of liver white spot formation.

In the 3 dose groups, the numbers of white spots were positively correlated with the numbers of eggs administered in the early phase of infection. A

similar relationship has previously been observed in single infections (Eriksen *et al*. 1980) and multiple/trickle infections (Eriksen *et al*. 1992; Yoshihara *et al*. 1983). But when comparing the number of administered eggs and the number of larvae which later succeeded in establishing in the small intestine, the numbers of white spots were relatively low in Groups II and III. By slicing whole livers Roneus (1966) found that approximately half of the white spots present were superficial and half were situated deep in the liver tissue. If this ratio holds true for the present experiment, the maximum of 23 superficial white spots in the Group I pigs at day 7 p.i. corresponds to a total of approximately 46 white spots, a value which fits nicely with the 47 established larvae in the small intestine at day 10 p.i. In Group II and especially Group III, the white spot counts were relatively smaller (even when multiplied by 2 to correct for hidden spots) than the numbers of successfully migrating larvae, presumably indicating that considerable numbers of white spots are confluent.

In sum, the present study throws new light on the population kinetics of migrating *A. suum* larvae in pigs, indicating that elimination of worms after single infections with 100, 1000 or 10000 eggs takes place during days 14–21 p.i., i.e. after the larvae have reached the small intestine. The result of this elimination is small overdispersed populations of intestinal worms, characteristic of both porcine and human *Ascaris* infections. Further studies are urgently needed on these small intestinal worm populations to elucidate the mechanisms by which some worms are able to evade the massive intestinal elimination.

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