Exposure of sows to *Ascaris suum* influences worm burden distributions in experimentally infected suckling piglets

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SUMMARY

This paper reports on the influence of maternal exposure to *Ascaris suum* on worm burden distributions in experimentally infected piglets. In the first study, sows were inoculated before and during gestation (6 months, long-term exposure) with 10000 *A. suum* eggs twice weekly. In a second study, sows were inoculated during gestation only (3 months, short-term exposure) with increasing doses of eggs (10000–40000 eggs twice weekly). Helminth-naive sows served as controls in both studies. The third study used the same design as the short-term exposure study, but piglets from exposed and control sows were cross-suckled within 4 h of birth before colostrum uptake. All piglets were inoculated 2 or 3 times with 50 *A. suum* eggs on days 4 and 7 (and 14) after birth, and left with the sows. At 10 weeks of age all piglets were necropsied, and liver lesions and worm burdens were recorded. Surprisingly, in piglets born to long-term exposed sows, the prevalence of *A. suum* infection and the mean worm burden were significantly higher than those in piglets from control sows. In contrast, neither worm burdens nor prevalence were significantly different between piglets from short-term exposed sows compared with their controls. In the cross-suckling experiment, 67% of piglets suckling control sows harboured worms at slaughter, compared with 15% of piglets suckling exposed sows. Maximum likelihood analysis of worm burden distribution and the degree of parasite aggregation showed 3 distinctly different types of overdispersed distributions: worm counts in piglets from control sows, in piglets from short-term exposed sows and in piglets from long-term exposed sows. When the worm burden data were analysed including the cross-suckled piglets by biological mother, it appeared that the control and short-term distributions converged and that only the long-term exposure was significantly different. Overall, the degree of parasite aggregation in piglets infected with *A. suum* decreased with exposure of the sows. A non-linear relationship was observed between prevalence of infection and mean worm burden, which was different for piglets from exposed and control sows, and similar to relationships of this type that previously have been found in human *A. lumbricoides* infections. It was concluded that in porcine *A. suum* infections maternal exposure alters the distribution of worms in their offspring, in which the duration of exposure appeared to be an important influence. The results of the cross-suckling further suggest that maternal factors, e.g. antibodies, are transferred via colostrum.

Key words: *Ascaris suum*, piglets, maternal exposure, worm burden distribution, overdispersion.

INTRODUCTION

Experimental single infections with *Ascaris suum*, the roundworm of pigs, tend to result in small parasite populations (only 20–50% of pigs harbour adult worms), with immature worms being expelled soon after establishment (Jørgensen *et al*. 1975; Roepstorff *et al*. 1997). Similarly, only a minority of naturally exposed (Bernardo *et al*. 1990; Bøgh *et al*. 1994) or trickle inoculated pigs (Eriksen *et al*. 1992) harbour patent infections. However, recently Boes *et al*. (1998) reported that continuous exposure of growing pigs resulted in prevalences of 65% (trickle inoculation) and 84% (natural exposure), respectively. This leads to a consideration of two issues namely (a) the nature of immunity and (b) the effect of infection dynamics, particularly repeat (trickle) infections.

Pigs develop an acquired immunity to infection with *A. suum* that is characterized by a specific serum antibody response, eosinophilia, elimination of worms from the intestine and reduced larval migration after reinfection (Taffs, 1964; Eriksen *et al*. 1980; Eriksen, 1982; Urban & Tromba, 1984; Stewart *et al*. 1985). However, this immunity does not completely prevent infection upon challenge (Urban, Alizadeh & Romanowski, 1988; Jungersen *et al*. 1999; Helwigh & Nansen, 1999). Furthermore, although experimental inoculations of young pigs

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with *A. suum* eggs have resulted in acquired immunity (Kelley & Nayak, 1964; Eriksen *et al.* 1980), the level of resistance depends on the inoculation dose (Andersen *et al.* 1973; Jørgensen *et al.* 1975). Clearly, the conclusion is that immunity is dose dependent and only partially protective. Further, high *A. suum* prevalences in heavily exposed pigs, especially in the younger age groups, indicate that a naturally acquired immune response is not very efficient at preventing infection.

Using high doses, trickle infections tend to result in higher prevalences than single infections, and do not always appear to elicit the same degree of expulsion (Boes *et al.* 1998). Consequently, it would appear that the immune response is modulated by the pattern of exposure. One feature of experimental studies with *A. suum* to date is that in both single and trickle infections, efforts are made to secure hosts that have no previous exposure to the parasite. This is imperative given the desire to study the development of the immune response. However, early transmission of *A. suum* to piglets within the first few weeks of life is common in traditionally managed pig herds (Raynaud, Sennelier & Irisarri, 1975; Roepstorff, 1991) and in organic pig herds (Roepstorff *et al.* 1992), and most hosts in endemic environments/populations will be born to exposed mothers, suggesting that for the purposes of studying the epidemiology and transmission dynamics, current experimental results may be misleading.

The study presented here aims to integrate early information provided by Kelley & Nayak (1965) with recent findings concerning *A. suum* population dynamics (Boes *et al.* 1998). Kelley & Nayak (1965) showed that some immunity to experimental *A. suum* infection can be transferred from hyper-immunized sows to suckling piglets via colostrum. However, their study differed from the present investigation in 3 aspects. Firstly, these authors focused on migrating larvae from the lungs of piglets that were necropsied 2 or 4 weeks post-inoculation. It has been shown that upon infection with *A. suum* most pigs will mount an immune response strong enough to rapidly expel the worms from the small intestine before the infection reaches patency (Jørgensen *et al.* 1975; Roepstorff *et al.* 1997). However, it is not known if maternal immunity influences establishment of patent *A. suum* infections. Secondly, Kelley & Nayak (1965) administered high challenge doses to test the immune response of experimental piglets, while a low challenge dose seems more realistic and has been shown to be more infective in young pigs (Andersen *et al.* 1973). Thirdly, Kelley & Nayak (1965) immunized the sows only for a relative short period, namely during gestation, while recently it was suggested that both magnitude and duration of exposure may have a significant affect on the prevalence and distribution of *A. suum* infection in pigs (Boes *et al.* 1998).

The purpose of this study was to investigate if exposure of sows to *A. suum* influences subsequent experimental *A. suum* infections in their piglets, compared with piglets from helminth-free control sows, in experiments that differed in exposure duration.

MATERIALS AND METHODS

Experiment 1 : long-term exposure

In the first study 9 Danish Landrace/Yorkshire/Duroc cross-bred female pigs were purchased at approximately 3 months of age from a specific pathogen-free (SPF) farm, that has been shown to be helminth free. Five pigs were turned out on pasture and trickle inoculated with 10000 *A. suum* eggs per pig in the feed twice weekly from the age of 3 months for 6 months (until 3 weeks prior to farrowing). The remaining 4 pigs were kept as parasite-naive controls on a separate pasture. When they were approximately 6 months old, the young sows in each group were served by a parasite naive Danish Landrace/Yorkshire/Duroc cross-bred boar (1 boar per group). Three weeks before farrowing, the infected sows were treated with anthelmintic (albendazole, Valbazen®, Pfizer) mixed in the feed to remove infection and prevent contamination of the farrowing units. One week before farrowing, the exposed sows were washed thoroughly to remove infective eggs from their skin and all sows were moved to individual outdoor farrowing units.

Experiment 2 : short-term exposure

In the second study 7 young sows, approximately 6 months old and purchased at the above-mentioned SPF farm, were treated with progestagen (Regumate®, Hoechst–Roussel) to induce heat synchronization. Subsequently, all sows were artificially inseminated with semen from the same parasite-free boar and housed in individual pens with straw bedding. Four sows were randomly selected to be trickle inoculated through the feed during gestation with increasing doses of *A. suum* eggs, which was chosen to obtain an exposure that was quantitatively comparable to that of the long-term exposed sows. The inoculations were as follows: 10000 eggs twice weekly from week 3 to week 8 of gestation; 20000 eggs twice weekly in weeks 9 and 10; and 40000 eggs twice weekly in weeks 11 and 12 of gestation. The remaining 3 sows were kept as uninfected controls. Three weeks before farrowing the exposed sows were treated with anthelmintic (fenbendazole, Panacur®, Hoechst) in the feed for 2 consecutive days to remove infection. Two weeks before farrowing the sows were moved to individual farrowing pens.

Experiment 3 : cross-suckling

Cross-suckling was performed using 4 sows that were purchased at the above-mentioned SPF farm. Two sows served as controls while the remaining 2 sows were exposed to *A. suum* infection during gestation as described in Exp. 2. Anthelmintic treatment and housing were similar to that in Exp. 2. Farrowing was induced by i.m. cloprostenol (Estrumat®, Mallinckrodt) and immediately after birth piglets were removed from the sows and kept in straw bedding under a red heating lamp for a maximum of 4 h. After 4 h half of the piglets from control sows were selected randomly and returned to their biological mother while the other half were placed with an exposed sow, and vice versa. The condition of the piglets and their acceptance by the sows were monitored until 2 h after crossing.

Inoculation of piglets

The piglets born to exposed and control sows in the first experiment were inoculated twice within the first week of life (on days 4 and 7 after birth) with 50 *A. suum* eggs that had been isolated from pig faeces and embryonated as described by Roepstorff *et al.* (1997). The eggs were administered orally on the base of the tongue with a plastic syringe. In the second and third experiment, all piglets were inoculated as described above on 3 occasions: days 4, 7 and 14 after birth. The low inoculation dose was chosen because low numbers of *A. suum* eggs have been shown to give more patent infections in young pigs (Andersen *et al.* 1973; Jørgensen *et al.* 1975). In all 3 studies the piglets were treated prophylactically on day 4 with a subcutaneous injection of 150 mg iron (Ferridex®, Rosco) to prevent anaemia. All piglets were left with the sows for the remainder of the study period and necropsied at 10 weeks of age.

Sampling procedures

Faecal samples were taken from the sows in all 3 experiments at regular intervals before farrowing, 1 week before farrowing and 1 week after farrowing to monitor infection status. Faeces were collected from the piglets at weeks 6, 8 and 10 post-inoculation (p.i.) and analysed using a concentration McMaster method with a lower detection limit of 20 eggs g^{-1} faeces (Roepstorff & Nansen, 1998). At week 10 p.i. the piglets were necropsied; the number of liver white spots and the degree of liver fibrosis were recorded, and adult and immature worms (>2 cm) were recovered from the small intestine of the piglets, counted and sexed.

Statistical analysis

Due to the aggregated nature of *A. suum* infection the raw parasitological data required non-parametric analysis; litter means, however, were normally distributed. Therefore, worm burdens (WB) and liver white spots (WS) were compared between groups (control versus exposed) using litter means (Student's *t*-test) and group medians (Mann–Whitney *U*-test). The prevalence of infection in exposed and control piglets was compared with Fisher's Exact test. Within exposure groups, differences between litters in WB and WS were tested using the non-parametric Kruskal–Wallis (KW) Analysis of Variance. The degree of overdispersion of the worm burden distributions was calculated using the maximum likelihood estimate of k, the parameter of the negative binomial distribution which tends towards 0 as parasite aggregation increases (Bliss & Fisher, 1953; Anderson & May, 1991).

Due to the small number of piglets born to each sow, litter-wise estimation of negative binomial parameters is not feasible. Consequently, the approach adopted is to use the relationship between k and the mean burden for each litter and estimate the value of k between and within different experiment and treatment combinations (Medley *et al.* 1993; Billingsley *et al.* 1994). Previous investigations of parasite heterogeneity have suggested that there is a linear relationship between k and the mean burden such that k increases (heterogeneity decreases) as mean worm burden increases for a variety of parasites (Guyatt *et al.* 1990; Lwambo, Bundy & Medley, 1992; Medley *et al.* 1993). In a modification of these procedures, models of overdispersion, based on the negative binomial distribution, were fitted as follows.

If $p(w \mid m, k)$ is the probability of observing w parasites from a negative binomial distribution with mean m and parameter k, then the total log-likelihood for the data is calculated as:

$$l = \sum_X \sum_T \sum_i \sum_j \ln\{p(w_{jiXT} \mid m_{iXT}, k)\},$$

where w_{jiXT} is the number of worms found in piglet j of litter i in treatment T ($=E$ or C for exposed or control respectively) and experiment X ($=S$ or L for short-term or long-term exposure respectively), and m_{iXT} represent the mean burden for each litter. By maximizing this log-likelihood, values of k can be estimated for different subsets of the data and differentiated by appropriate subscripts. A single negative binomial is fitted to all data combined (minimal model) and additional values of k introduced stepwise to separate experiment/treatment combinations. The fits are then compared by likelihood ratio tests (χ^2 test for model improvement).

We also investigate the relationship between the prevalence of infection and the mean worm burden per litter using a linear relationship of k with mean

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Table 1. Long-term exposure (Exp. 1): *Ascaris suum* infection in piglets from sows exposed to trickle infection before and during gestation (6 months) compared with piglets from helminth-naive sows

Group	Litter	*N*	Mean number of liver spots*	Mean worm count†	Variance	Prevalence (%)
Control	1	8	3·4	5·4	207	38
($n=31$)	2	4	2·0	4·3	72	25
	3	9	2·4	8·1	196	44
	4	10	2·7	17·5	277	80
Combined litters ($n=4$)		7·8	2·6	8·8	188	52
Exposed	5	9	6·6	7·0	43	78
($n=47$)	6	11	6·0	14·5	305	91
	7	11	1·2	12·4	75	91
	8	7	2·1	8·9	42	86
	9	9	2·6	12·7	21	100
Combined litters ($n=5$)		9·4	3·7	11·1	97	89

* White spots of the lymphonodular type.
† No significant litter effects within groups (Kruskal–Wallis ANOVA, $P>0·05$).

worm burden (m), as suggested by Guyatt *et al.* (1990), of the form:

$$k_{XT} = a_{XT} + b_{XT} m_{jXT}.$$

Again, the optimal fit is determined by likelihood ratios, and the overall fit of the negative binomial assumption is tested by comparing expected and observed frequencies (χ^2).

RESULTS

Long-term exposure

The mean litter size was 8·0 for the 4 control sows (32 piglets) and 9·8 for the 5 exposed sows (49 piglets). Litter sizes were not significantly different between control and exposed sows ($P>0·3$). However, 2 piglets from exposed sows and 1 from a control sow died within the first 2 weeks of the experiment. Two of the 5 exposed sows were excreting eggs during the infection period but after anthelmintic treatment all sows had zero egg counts 1 week before farrowing.

Table 1 shows litter size, mean number of liver white spots, mean worm burden and prevalence of infection for litters of control and exposed sows, recorded at necropsy. Eggs appeared in the faeces of piglets in both groups at week 8 p.i. At necropsy, the numbers of WS – of the lymphonodular type – in litters from exposed sows (mean WS: 3·7, median WS: 2) were not significantly higher than those in litters from control sows (mean WS: 2·6, median WS: 2) ($t=0·852$, D.F.$=7$, $P=0·427$; $U=655·0$, $P=0·455$). The range of white spot counts was 0–10 in the control group, compared to 0–21 in the exposed group. There was significant variation in WS numbers between litters in the exposed group (KW-statistic$=19·31$, $P<0·01$).

The median but not mean worm burdens recovered at slaughter from the small intestine of piglets born to exposed sows (mean WB: 11·1, median WB: 11) were significantly higher ($U=522·0$, $P=0·035$; $t=0·744$, D.F.$=7$, $P=0·481$) than those found in piglets from control sows (mean WB: 8.8, median WB:1). The high variances in Table 1 and the frequency distributions in Fig. 1A and C show that worm counts in both groups were aggregated. No significant within-group differences in worm counts were found between the litters in either exposed or control groups. The prevalence of infection in piglets from exposed sows was 89%, which was significantly higher than the 52% prevalence in piglets from control sows ($P=0·001$).

Short-term exposure

A total of 77 piglets were born to 7 sows (mean litter size: control sows 9·3, exposed sows 12·3). Litter sizes were not significantly different between control and exposed sows ($P>0·1$). However, 5 piglets were stillborn and in the first week after birth, 6 piglets died due to enteropathogenic *Escherichia coli* infection which caused moderate to severe diarrhoea in all piglets. As a result, only 22 piglets suckling control sows and 43 piglets suckling exposed sows were inoculated and sampled. The piglets were treated penwise for 4 consecutive days during the first 2 weeks of life with sulphadiazine plus trimethoprim (Norodine®, Scanvet) and electrolyte solution was available *ad libitum*. Because the diarrhoea also occurred at the days when the inoculations with *A. suum* eggs were given, each piglet received a third dose of 50 eggs on day 14 after birth, when the diarrhoea had ceased.

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Table 2. Short-term exposure (Exp. 2): *Ascaris suum* infection in piglets from sows exposed to trickle infection during gestation (3 months) compared with piglets from helminth-naive sows

Group	Litter	*N*	Mean number of liver spots*	Mean worm count†	Variance	Prevalence (%)
Control	1	6	1·7	2·0	11	33
(*n* = 22)	2	7	1·3	27·1	244	86
	3	9	0·2	22·3	620	67
Combined litters (*n* = 3)		7·3	1·1	17·1	292	64
Exposed	4	11	0·3	1·1	4	46
(*n* = 43)	5	9	0·7	3·4	14	60
	6	11	7·0	12·9	247	73
	7	12	2·4	10·7	74	83
Combined litters (*n* = 4)		11	2·6	7	85	66

* White spots of the lymphonodular type.
† In the exposed group, there was a significant difference between the litter with the highest and lowest mean worm burden (Kruskal–Wallis ANOVA, $P < 0{\cdot}05$).

Table 3. Exp. 3: *Ascaris suum* infection in piglets born to sows exposed to trickle infection during gestation (3 months) and piglets born to helminth-naive sows, and the influence of cross-suckling (transfer of piglets from exposed to control sows and vice versa)

Group	Sow (suckling mother)*	Sow (biological mother)	Number of piglets	Mean number of white spots	Mean worm count	Variance	Prevalence (%)
Control	A	A	3	1·0	31·3	862	100
(*n* = 21)	A	D	9	3·0	5·3	157	33
	B	B	5	1·2	13·6	295	80
	B	C	4	0·5	20·0	376	100
Combined litters			10·5	1·4	14·1	423	67
Exposed	C	C	3	1·7	0·0	0	0
(*n* = 13)	C	B	2	1·5	0·0	0	0
	D	D	4	1·3	0·0	0	0
	D	A	4	0·3	0·5	0·2	50
Combined litters			6·5	1·2	0·1	0·1	15

* A and B: control sows; C and D: exposed sows.

All sows had zero egg counts 1 week before farrowing. The mean numbers of liver white spots, mean worm burdens and prevalence of infection found in the piglets at necropsy are shown in Table 2. *Ascaris* eggs were detected in the faeces of the piglets 8 weeks p.i. At necropsy, low numbers of lymphonodular liver white spots were recorded. The numbers of WS in piglets suckling control sows (mean WS: 1·1, median WS: 0, range: 0–6) were not significantly different from those found in piglets suckling exposed sows (mean WS: 2·6, median WS: 1, range: 0–18) ($t = 0{\cdot}826$, D.F. = 5, $P = 0{\cdot}438$; $U = 341{\cdot}5$, $P = 0{\cdot}067$), but significant variation in white spot numbers was observed between litters in the exposed group (KW-statistic = 29·88, $P < 0{\cdot}01$).

The worm burdens in piglets from exposed sows (mean WB: 7·0, median WB: 3) were not significantly different from the worm counts in piglets from control sows (mean WB: 17·1, median WB: 9) ($t = 1{\cdot}393$, D.F. = 5, $P = 0{\cdot}222$; $U = 360{\cdot}0$, $P = 0{\cdot}118$). Similarly, the overall prevalence of 66% in piglets suckling exposed sows was not significantly different from that in piglets suckling control sows (64%) ($P > 0{\cdot}8$).

Cross-suckling

The mean litter sizes at birth were 8·0 for the control sows and 12·0 for the exposed sows, respectively. Litter sizes were not significantly different between control and exposed sows ($P > 0{\cdot}4$). However, because the sows were kept in the same stable as those in the short-term exposure study, all piglets also experienced *E. coli* diarrhoea, causing 6 fatalities. Therefore, only 13 piglets born to control sows and 21 piglets born to exposed sows were inoculated

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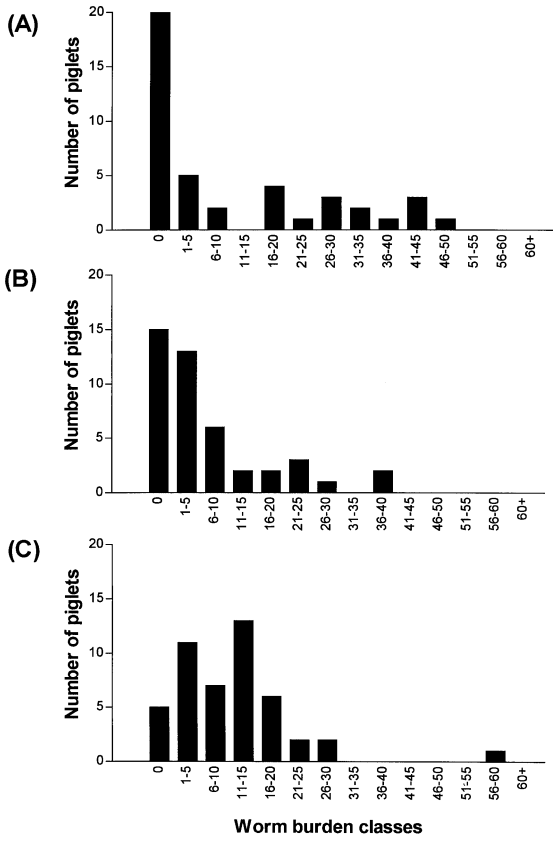

Fig. 1. Worm burden distributions in piglets experimentally infected with *Ascaris suum*. (A) The combined data from piglets from helminth naive sows serving as controls in the short-term or the long-term exposure study ($n=53$). (B) Piglets from sows that were exposed during gestation only with increasing doses of *A. suum* eggs twice weekly (short-term exposure, $n=44$). (C) Piglets from sows that were exposed before and during gestation with 10000 *A. suum* eggs twice weekly (long-term exposure, $n=47$).

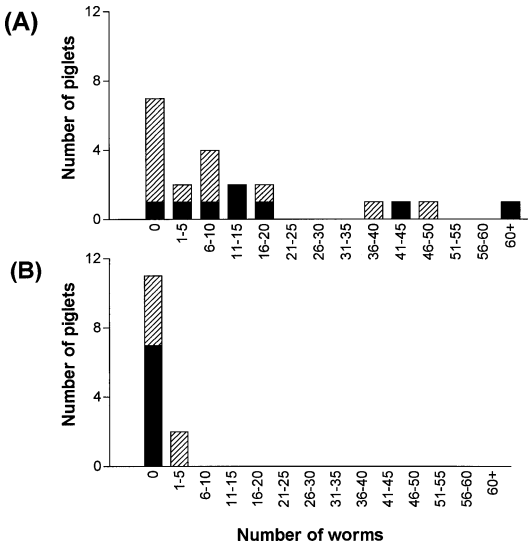

Fig. 2. Worm burden distribution in cross-suckled piglets from control sows and sows exposed during gestation with increasing doses of *Ascaris suum* eggs. (A) Piglets suckling control sows ($n=21$) and (B) piglets suckling exposed sows ($n=13$). The hatched bars indicate transferred piglets (from control to exposed sow and vice versa).

according to the procedure described in Exp. 2. In addition, the piglets received penwise treatment and electrolyte solution as described above.

Within 4 h after birth 6 piglets from control sows (biological mothers) were transferred to exposed sows (suckling mothers), while 13 piglets born to exposed sows were transferred to control sows. The design of the cross-suckling and the parasitological data are shown in Table 3. Worm burdens in piglets suckling control sows (mean WB: 14·1, median WB: 6) were significantly higher ($t=3·177$, D.F. $=6$, $P=0·026$; $U=52·5$, $P=0·003$) than those in piglets suckling exposed sows (mean WB: 0·1, median WB: 0). The overall prevalence in piglets suckling control sows (67 %) was significantly higher than that in piglets suckling exposed sows (15 %) ($P=0·011$).

Parasite distributions

The worm burden distributions of the piglets in Exps 1 and 2 are shown in Fig. 1A–C by treatment (exposed or control) and experiment (exposure duration). As the worm burden distributions in both control groups were very similar, they were combined into Fig. 1A. Visual inspection of Fig. 1 suggested that the pattern of overdispersion is different for the controls and each exposed group, i.e. 3 types of overdispersed distributions. The distribution in piglets from control sows is highly aggregated (Fig. 1A), whereas in piglets from exposed sows the degree of aggregation decreases while the frequency of light infections increases (Fig. 1B and C); this is more pronounced in the long-term exposure study (Fig 1C).

The worm burden distributions of the cross-suckled piglets in Exp. 3 are shown in Fig. 2A (piglets suckling control sows) and Fig. 2B (piglets suckling exposed sows), with an indication of the piglets' cross-suckling status. While the distribution of worms has the characteristic overdispersed form in piglets suckling helminth naive sows, all but 2 piglets suckling exposed sows were worm free.

For the analysis of overdispersion (k), 2 datasets containing the combined data of the 3 studies were created. Because the exposure duration of sows and the inoculation dose of the piglets were similar in the second and third study, the piglets involved in the cross-suckling (Exp. 3) were included in the short-term exposure group (S in Table 4). To investigate whether the distribution of infection in suckling piglets is influenced by maternal factors transferred before or after birth, worm burdens were then analysed grouping the cross-suckled piglets by recipient (first dataset) and biological mother (second dataset) (see Table 4).

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Table 4. Maximum likelihood estimates (k) for the degree of overdispersion of *Ascaris suum* distributions in piglets from exposed and helminth-naive sows, grouping cross-suckled piglets by either recipient or biological mother.

(One cross-suckled litter had no worms when grouped by suckling mother, so 1 piglet was nominally given a burden of 1 worm to be able to include the litter in the analysis. Models 2, 3 and 4 are compared with model 1, whereas models 5 and 6 are compared with model 4. If for example model 2 has one more parameter than model 1, twice the difference in negative log-likelihoods must be greater than $\chi^2(1;0{\cdot}95)=3{\cdot}84$ for model 2 to be significantly better at the 0·05 level (see Hilborn & Mangel, 1997). If models are not significantly different the model with the lowest number of parameters is the optimal model. The estimates for the negative binomial with the optimal fit are highlighted.)

	Recipient mother				Biological mother			
Model	Parameter estimate†	Log-likelihood	No parameters‡	χ^2 test for model improvement (P-values)	Parameter estimate	Log-likelihood	No parameters	χ^2 test for model improvement (P-values)
(1) Single NB*	$k=0{\cdot}53$	−532·24	1	—	$k=0{\cdot}46$	−539·82	1	—
(2) NB for each experiment	$k_S=0{\cdot}59$ $k_L=0{\cdot}48$	−531·92	2	0·4237	$k_S=0{\cdot}37$ $k_L=0{\cdot}59$	−538·13	2	0·0664
(3) NB for each treatment	$k_C=0{\cdot}31$ $k_E=0{\cdot}97$	−522·86	2	0·0000	$k_C=0{\cdot}29$ $k_E=0{\cdot}63$	−535·73	2	0·0042
(4) NB for each treatment/ expt. combination	$k_{CS}=0{\cdot}4$ $k_{CL}=0{\cdot}22$ $k_{ES}=0{\cdot}62$ $k_{EL}=1{\cdot}42$	−519·15	4	0·0000	$k_{CS}=0{\cdot}4$ $k_{CL}=0{\cdot}21$ $k_{ES}=0{\cdot}35$ $k_{EL}=1{\cdot}43$	−526·04	4	0·0000
(5) NB for control groups and separate for each exposure	**$k_C=0{\cdot}32$** **$k_{ES}=0{\cdot}38$** **$k_{EL}=1{\cdot}42$**	**−520·31**	**3**	**0·1294**	$k_C=0{\cdot}3$ $k_{ES}=0{\cdot}35$ $k_{EL}=1{\cdot}43$	−527·12	3	0·1426
(6) NB separate for long-term exposure versus 3 groups combined (common slope)	$k_{EL}=1{\cdot}42$ $k_{COM}=0{\cdot}38$	−522·49	2	0·0354	**$k_{EL}=1{\cdot}42$** **$k_{COM}=0{\cdot}32$**	**−527·26**	**2**	**0·2952**

* NB, negative binomial.
† k refers to the estimate of the negative binomial parameter, subscripts C, E, S and L refer to control, exposed, short-term and long-term exposure, respectively.
‡ Additional to the means estimated for each litter.

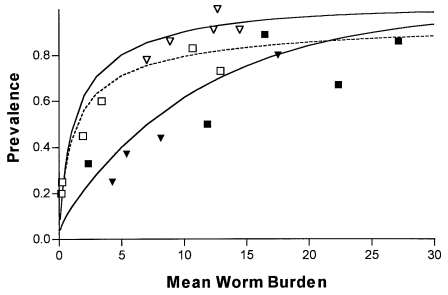

Fig. 3. Relationships between prevalence and the mean burden of *Ascaris suum* infection in piglets from exposed and parasite naive sows. The solid lines are the maximum likelihood fits for the negative binomial distribution with a linear k ($k=a_x+bm$; $a_c=0{\cdot}026$, $a_E=0{\cdot}72$ and a common slope $b=0{\cdot}023$). Each data point corresponds to a litter, with solid points control and open points exposed for each of the long-term (▼) and short-term (■) exposures. The dotted line is with constant k for comparison ($k=0{\cdot}53$).

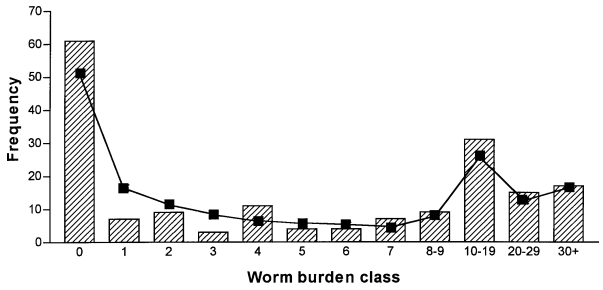

Fig. 4. Distribution of *Ascaris suum* in experimentally infected piglets (all experiments and treatments combined). The bars indicate the overall observed worm burdens. The line shows the expected distribution derived from the model with a linear relationship between k and mean burden in each litter as a weighted sum of the expected distributions for each litter.

It appears that the more parsimonious models with acceptable fit to the data are those that differentiate between treatments rather than experiments (models 3 and 4 in Table 4) for both sets of data. Analysis of the different treatment/experiment combinations by recipient mother shows that the model with the best fit is when the worm burdens of both control groups are combined ($k_c=0{\cdot}32$), their distribution and degree of overdispersion being significantly different from those of piglets suckling short-term exposed sows ($k_{ES}=0{\cdot}38$) and long-term exposed sows ($k_{EL}=1{\cdot}42$) (model 5 in Table 4). Analysing the different treatment/experiment combinations by biological mother, the piglets from control and short-term exposed sows could be combined into one k value (0·32), versus the worm counts of piglets from long-term exposed sows ($k=1{\cdot}42$) (model 6 in Table 4), i.e. in the optimal model the worm burden distribution and degree of overdispersion in piglets from long-term exposed sows are significantly different from the other distributions.

A linear relationship between k and the mean worm burden by treatment, i.e. control versus exposed – using recipient mother data – gave the optimal fit to a negative binomial distribution ($a_c=0{\cdot}026$, $a_E=0{\cdot}72$ and common slope $b=0{\cdot}023$). Figure 3 shows the derived relationships between mean worm burden and prevalence by litter for piglets from control and exposed sows (the cross-suckled piglets are grouped by recipient mother). Prevalence and mean worm burden have an approximately linear relationship in litters from exposed sows when between 20 and 70% are infected; for litters from control sows this linear relationship is observed in a more narrow prevalence range: 20–60%.

Figure 4 shows the overall distribution of worms in all piglets and the expected distribution calculated as a weighted combination (where weights are litter sizes) of different negative binomials using estimated means and linearly-related k values for each litter (Billingsley *et al*. 1994). The overall fit is good ($\chi^2=18{\cdot}20$, D.F.$=11$, $P=0{\cdot}077$), although there is some discrepancy for zero and single worm infections, but the use of the negative binomial is justified. Figure 4 demonstrates that the negative binomial with k varying between experimental groups and with mean worm burden offers a good empirical description of the distribution of worms between hosts.

DISCUSSION

The results of our study indicate that in the case of *A. suum* infection in piglets there is a measurable effect of maternal exposure. However, this effect may not be directly observable by comparison of mean worm burdens, but operates more subtly through alteration of the distribution of parasites. Duration of maternal exposure appears to be an important influence. The results of the cross-suckling further suggest that the route for transfer of maternal factors is via colostrum, as proposed by Kelley & Nayak (1965).

Parasitological aspects

The mean number of white spots was low and the majority were of the lymphonodular type, which indicates that they were the result of the inoculations and not of uncontrolled infection (Roneus, 1966; Roepstorff *et al*. 1997). In the long-term experiment the only significant observation regarding white spots was an increased variability in numbers in litters from exposed sows. In the short-term experiments, the control litters had unusually low numbers of white spots, suggesting either reduced numbers of larvae reaching the liver (perhaps due to gastro-enteritis), or reduced immunological response in this group.

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All piglets in the short-term exposure and cross-suckling studies experienced diarrhoea shortly after birth due to *E. coli* infection. As it recently was shown that *A. suum* larvae penetrate the wall of the caecum and upper large intestine (Murrell *et al.* 1997) there was some concern about the effect of *E. coli* on the migratory activity of the larvae in the colon. However, the effect of *E. coli* and migrating *Ascaris* in pigs has been reported to be synergistic (Adedeji, Ogunba & Dipeolu, 1989) rather than one infection limiting the other. Furthermore, the *A. suum* worm burden distribution in control piglets that experienced diarrhoea was similar to that in piglets in the unaffected control group, indicating that the *E. coli* infection was of minor influence.

Recent results for *Schistosoma japonicum* indicate that the possibility of pre-natal parasite transmission in pigs should be investigated further (Willingham *et al.* 1999). However, in the present study there is no parasitological evidence of transplacental transmission – assuming that the pre-patent period would not be altered – as *Ascaris* eggs were only observed in the faeces of piglets from week 8 after inoculation. This is in agreement with early studies reporting that prenatal infection with *A. suum* is highly improbable in pigs (Van der Wall, 1958; Alicata, 1961; Olson & Gaafar, 1963).

Epidemiological aspects

The prevalence of infection was similar in both control groups. In the short-term exposure group the prevalence was not significantly different from the control groups. However, when exposure of the sows occurred for a longer period (i.e. 6 months) both before and during gestation, the prevalence of infection in their piglets increased significantly to nearly 90%.

The worm burden distributions in both control and exposed groups are overdispersed, and the distributions in the 2 control groups were very similar. In the short-term exposure group, although a prevalence was found similar to that observed in the controls, the distribution and the degree of overdispersion changed due to a higher number of lightly infected animals. Long-term exposure resulted in an even more pronounced change in worm burden distribution and overdispersion with the majority of animals harbouring light to moderate infections. These observations are shown to be statistically significant. Apparently, the distribution of *A. suum* in piglets from exposed sows differs from that in piglets of helminth naive sows.

Differences in duration of exposure, i.e. trickle infection of sows for 3 or 6 months, also influenced the distribution of *A. suum* burdens in piglets from exposed sows, even though the only contact these piglets had with maternal infection was via colostrum and, although less likely, transplacental. The mechanism for this phenomenon remains unknown, although immunity is the most likely candidate. Recently, Boes *et al.* (1998) suggested that differences in exposure result in different *A. suum* distributions in experimentally and naturally infected growing pigs: there was a distinct difference in worm distribution between pigs that initially were parasite naive and pigs that had been previously exposed to infection. The results of the present study seem to be in agreement with this, suggesting that previous exposure to parasites results in reducing overdispersion, in particular, increasing the frequency of pigs harbouring small parasite numbers and reducing the frequency of very high burdens. Furthermore, preliminary results indicate that overdispersed worm burdens differ significantly between pigs experimentally infected with a single dose of *A. suum* eggs and pigs exposed continuously by trickle or natural infection (S. Coates, unpublished results), and further work is being undertaken to attempt to untangle the effects of dose and duration of exposure.

An interesting aspect of *A. suum* infection in piglets in the current study is the relationship between prevalence of infection and mean worm burden. This relationship appeared different for piglets from exposed and control sows, suggesting that piglets from exposed sows will at a given prevalence harbour fewer worms than piglets from control sows, at all low and moderate prevalences. Alternatively, when mean worm burdens are similar, as was the case in the long-term exposure study, the prevalence of patent infection is much higher in piglets from exposed sows compared with piglets from parasite naive sows.

The relationship between prevalence and intensity of *Ascaris lumbricoides* infection in humans has been described previously (Guyatt *et al.* 1990; Bundy & Medley, 1992), and was found to be non-linear and well described by the negative binomial distribution using a linear relationship between k and the mean. A similar relationship was demonstrated for *A. suum* in the present study. Relationships of this type have been used previously to guide appropriate control strategies (Guyatt & Bundy, 1991; Lwambo *et al.* 1992).

Immunological aspects

In the present study it appears that exposure duration of the sows is an important factor that may influence not only their own immune response but also that of their offspring. Our results suggest that long-term exposure of sows may induce a form of tolerance to infection rather than support a primary immune response in their offspring.

Maternal factors (e.g. immunoglobulins, parasite circulating antigens, immune cells and cytokines) can interact with invading parasites or induce a modulation of the offspring's capacity to mount an

immune response to subsequent exposure to parasites by different mechanisms (reviewed by Carlier & Truyens, (1995)). For example, it is known that maternal antibody can suppress the synthesis of immunoglobulins by newborn piglets (Hoerlein, 1957; Tizard, 1977), while repeated infection with *A. suum* can induce immunosuppression in experimentally infected mice (Crandall & Crandall, 1976) and pigs (Barta *et al*. 1986; Stankiewicz & Froe, 1995). And furthermore, maternal immunity may also have a cellular component. T-cell hyporesponsiveness has been found in uninfected offspring of infected mothers (see Carlier & Truyens, 1995) and newborn piglets have been shown to absorb colostral lymphoid cells within a few hours after administration (Tuboly *et al*. 1988). If immunity is the mechanism through which exposure alters the pattern of heterogeneity, then exposure must enhance both tolerance and protection depending on the degree of parasitism.

The results of the cross-suckling experiment, although perhaps of limited value due to low numbers of animals included, indicate that protective immunity against patent *A. suum* infection can be transferred from sows to piglets by colostrum and not transplacentally. This corresponds with the observation that in naturally infected pig herds high amounts of *A. suum* specific IgG antibody are transferred from sows to offspring in colostrum during the first 6 weeks of life (Roepstorff, 1998).

It is not clear why the distribution of infection was so different between the piglets from the short-term and long-term exposed sows. Therefore, a detailed evaluation of the serological response to *A. suum* infection and its relationship with the resulting liver pathology and worm numbers is currently being carried out and may provide additional information.

Implications and perspectives

It is clear from these results and others (Boes *et al*. 1998) that there is a complex relationship between exposure to *Ascaris* parasites and the resulting distribution of parasites between hosts. At present it remains unclear to what extent hosts are regulating the number of parasites they harbour through the immune response, as opposed to infection and establishment being simply random processes (Medley, 1992). It is also possible that the relationship between exposure and immunological modulation of parasite burden will be different for different degrees of exposure (host behaviour and environmental suitability for the parasite) and durations of association between host population and parasites. For example, we do not know how the offspring of individuals born to exposed mothers react to parasite assault. We believe that the experimental systems currently being investigated will shed light on the relative roles of the many factors determining worm burdens at both individual and population level, which will lead to improved understanding and eventually more cost-effective control of parasite infection.

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