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Establishment of *Ascaris suum* in the Pig: Development of Immunity Following a Single Primary Infection

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Helwigh AB, Nansen P: Establishment of *Ascaris suum* in the pig: development of immunity following a single primary infection. Acta vet. scand. 1999, 40, 121-132. – Development of immunity after a single primary infection of *Ascaris suum* in pigs was investigated with regard to the worm population dynamics of a superimposed *A. suum* infection, host immune response and gross liver pathological changes. Group A was given a primary infection of 60 000 infective *A. suum* eggs and group B was left uninfected. Four weeks later both groups A and B were inoculated with 1 000 *A. suum* eggs, and subgroups were slaughtered 7, 14 and 21 days post challenge infection (p.c.i.). An uninfected control group C was slaughtered on day 21 p.c.i. The challenge worm recovery in group A was reduced compared to group B by 12%, 50% and 75% on day 7, 14 and 21 days p.c.i., respectively. In both groups was the expulsion of worms initiated between day 14 and 21 p.c.i. However, in group A the worms were recovered more posteriorly in the small intestine and 21 days p.c.i. the mean worm length was significantly shorter than in group B ($p = 0.01$). The results above were associated with significantly higher ($p<0.05$) antibody response and higher eosinophil counts in group A compared to group B. The present results suggest that the larval growth and survival of a challenge infection are decreased, probably due to higher antibody and eosinophil attack during the migratory phase.

challenge infection; worm kinetics; immune response.

Introduction

Ascaris suum is one of the most common parasites in the pig production (*Murrell* 1986, *Ajayi et al.* 1988, *Roepstorff & Jorsal* 1989). *Stewart* (1996) estimated the economic loss caused by this parasite to be $ 174.3 million in 1994 in the United States alone. Since it is impossible to eradicate the parasite, the main economic importance to the swine industry is to develop effective control programs (*Stewart* 1996). This requires insight into the host-parasite relationship and the population dynamics of *A. suum.* Many aspects of the interactions between *A. suum* and the pig host have already been investigated. The population dynamics of *A. suum* during a single primary infection have been studied by *Jørgensen et al.* (1975), *Eriksen et al.* (1992a) and *Roepstorff et al.* (1997). Resistance to *A. suum* has been evaluated following experimental trickle infections or natural infections (*Taffs* 1964, *Urban et al.* 1988, *Eriksen et al.* 1992a, *Eriksen et al.* 1992b), following vaccination with ultraviolet-irradiated eggs (*Urban & Tromba* 1984) and following immunization with

Table 1. Experimental design. The primary infection was 4 weeks old before the time of the challenge infection. All infections were given by oral inoculation.

Group	Total no. of pigs	n[a]	Primary infection	Challenge infection	Slaughter (days p.c.i.[b])
PC[c]	6	6	60,000 *A.s.*	0	
A	30	10	60,000 *A.s.*	1,000 *A.s.*	7, 14, 21
B	18	6	-	1,000 *A.s.*	7, 14, 21
C	3	3	-	-	21

[a] Number of pigs per slaughter. [b]Post challenge infection. [c] Primary control group.

parasite derived products (*Urban & Romanowski* 1985).

Little is known about the onset of acquired resistance after a primary infection and the possible influence of this resistance on the worm population dynamics. Few authors have investigated the effect of a single A. suum infection on a homologous challenge infection (*Ronéus* 1966, *Eriksen et al.* 1980). These studies have only one pig in each group and have not taken into account the large variation seen in the establishment of *A. suum*. The present experiment, comprising 6 or 10 animals in each group, was designed to examine the effect of a single primary *A. suum* infection in pigs on the migration and establishment of a challenge infection given 4 weeks later. The objective was to investigate the location and number of larvae established following a challenge infection and to correlate parasite recovery rates with gross liver pathology, antibody levels, eosinophil numbers and weight of the pigs.

Materials and methods

Experimental animals, housing and feeding

Fifty-seven helminth-naïve castrated male Danish Landrace/Yorkshire/Duroc cross-breed pigs were allocated randomly into 3 groups according to litter and weight. At the beginning of the experiment, all pigs were 8-10 weeks old and weighed 22.1 ± 0.7 (SD) kg. All pigs were housed in the same building and penned in groups of 9 in separate boxes according to infection. Precautions were taken to prevent cross-contamination between groups by having the staff to change tools and boots for the daily handling of each group. The pigs had free access to water and were fed twice daily with a standard diet consisting of ground barley plus protein (*Petkevicius et al.* 1995) and all pigs were offered the same amount of feed per kg body weight.

Parasite isolate

The *A. suum* strain (designated CEP, batch nr. A12-95) was originally isolated in 1993 (*Roepstorff & Murrell* 1997) and has since been maintained by repeated passages in helminth-naïve pigs. The eggs were isolated from fresh faeces and embryonated in 0.1 N sulphuric acid in the dark at room temperature for 3 months. This embryonation procedure is known to ensure high infectivity of eggs (*Oksanen et al.* 1990).

Experimental design

The design of the experiment is shown in Table 1. Infections were given as oral inoculations, a group A was initially inoculated with 60,000 eggs and challenge inoculated with 1,000 eggs. Group B was kept parasite-free until challenge and served as a challenge control group inoculated with 1,000 eggs. Group C was left uninfected through out the experiment and served as a negative control for eosinophil

counts, antibody response and pig weight. Six pigs (group PC) were inoculated with 60,000 eggs at the same time as group A and slaughtered at the time when the challenge infection was given, in order to establish the number and measure the length of worms from the primary infection. This was necessary because worm length was the criterion used to separate worms from the 2 infections at the slaughters 7, 14 and 21 days post challenge infection (p.c.i.). Ten pigs from group A and 6 pigs from group B were randomly chosen and slaughtered at each slaughter day. Group C was slaughtered at the termination of the experiment (day 21 p.c.i.).

The pigs were weighed 3 times; at the start of the experiment, at the time of challenge infection and on the day of slaughter. Faecal samples for egg counting were obtained at the same time. For the measurements of eosinophil counts and antibody response, blood samples were taken once a week from the beginning of the experiment until termination day 21 p.c.i..

Parasitological techniques

A concentration McMaster technique was used to estimate faecal egg counts (*Roepstorff & Nansen* 1998). This method has a sensitivity of 20 eggs per gram faeces (EPG).

The pigs were killed with a captive pistol, exsanguinated and eviscerated immediately after. The granulation-tissue type white spots (GT-WS) and lymphonodular white spots (LN-WS) on the liver surface were recorded as described by *Roneus* (1966). The liver and lungs were cut into pieces of approximately 5 mm using a kitchen blender. The larvae from subsamples of the liver (25% of the total volume) and the lungs (50% of the total volume) were isolated using a modified agar-gel method (*Slotved et al.* 1995). In short, the sample was mixed with agar and poured over cloths and placed vertically in a bucket containing 0.9% saline for 3 hours. Thereafter, the cloths were removed and the sample left for sedimentation for at least one hour. The most supernatant was removed and the remaining fluid and sediment were poured into champagne glasses and left for one hour. The supernatant was removed and the pellet containing the larvae was fixed in an iodine solution (6.25% iodine, 31.25% potassium and 62.5% distilled water).

The small intestine was divided into 4 sections of equal length and cut open, and large *A. suum* worms were collected immediately. The intestinal wall was washed with 0.9% saline and the content plus washing were embedded in agar to isolate ascarid larvae with the modified agar-gel method (*Slotved et al.* 1997) using the same modifications as described for liver and lungs, except for one step, i.e. the samples were left in the bucket for migration of the larvae for 2 hours only. All samples were fixed and stored in the iodine solution described above. The samples were decolorized using a 3% thio-sulphate solution immediately before counting. If possible, 25 worms were randomly selected from each of the liver, lungs and the 4 sections of the small intestine and their body lengths measured using a digital image analysis system (Microvision®, DTI, Denmark).

Immunological techniques

The number of eosinophil leucocytes in the peripheral blood was determined as described by *Astrup et al.* (1959). All the sera were tested in duplicate against *A. suum* excretory/secretory antigens by use of an ELISA technique specific for IgG antibodies (*Jungersen* 1998). Antigens were obtained from in vitro-cultivated 2nd and 3rd stage larvae (L_2/L_3-ES) and kindly provided by Dr. Jungersen (Danish Centre for Experimental Parasitology). Dilution series of *A. suum*-positive sera and negative controls (*Oesophagostomum denta-*

Table 2. Weight of the pigs (mean ± SD); at the start of the experiment, at challenge infection (day 28 post primary infection) and termination of the experiment. For group designations see Table 1.

Group	Experimental start	Challenge infection	Termination
A	22.3 ± 3.4	36.2 ± 7.4	41.6 ± 6.0*
B	22.6 ± 3.3	37.1 ± 8.1	46.3 ± 5.5
C	22.7 ± 1.8	37.0 ± 2.7	51.8 ± 4.6

* Significantly lower than group C ($p<0.05$)

tum-positive sera) were applied to all plates and the optical density values (OD) were corrected for plate-to-plate variation. The ELISA technique used is known to be highly sensitive in both single infections (*Roepstorff et al.* 1997) and repeated infections (*Lind et al.* 1993).

Calculations and statistical analyses

Statistical analyses are based on data recovered from the challenge infection. All means presented are calculated as arithmetic means. The average length of the worms in each pig was calculated as the average of the mean lengths of worms from each of the liver, lungs and 4 sections of the small intestine of the pig. The degree of aggregation of the total worm numbers in the host population was measured by calculating the parameter k of the negative binomial distribution, where $k = mean^2/(variance-mean)$. When $k<1$ the aggregation follows the negative binomial distribution. The difference in worm numbers on day 7, 14 and 21 (log y+1 transformed) were analysed using Students t-test, and the difference in length of *A. suum* and in number of white spots on the liver surface were analysed by slaughter day p.c.i. using Student's t-test. Differences in the total weights of pigs, eosinophil counts and ELISA OD-values were compared between samples with the Mann-Whitney U-test. The proportions of the total *A. suum* worm numbers in the liver, lungs and different sections of the small intestine were compared by slaughter day p.c.i. using a repeated-measures analysis of variance to test for the effect of primary infection. To analyse changes in worm numbers (log y+1 transformed) between slaughter day 7, 14 and 21 p.c.i., analysis of variance (ANOVA) was performed to test for effects of infection, slaughter day and their interaction. Pearson's correlation coefficient was used to analyse linear relationships between worm numbers, white spots, pig weight at slaughter, antibody response at challenge infection and eosinophil counts at challenge infection.

Results

All pigs in group A coughed and developed fevers (39.8-41.3 °C) about one week after the primary infection. The pigs also suffered from anorexia and were apathic. In order to avoid eventual secondary infections of the lungs, all pigs including the uninfected ones were treated with the antibiotic Ampivet® (250 mg/ml, 20 ml per kg body weight) for 3 days. The rectal temperature was recorded once every day for 4 days and it had returned to normal after 3 days of treatment, the coughing had also stopped and all pigs were active and eating again. No other clinical complications occurred during the experiment.

The body weights at the beginning of the experiment, at challenge infection and at termination of the experiment are shown in Table 2. There were no significant differences between groups at the beginning of the experiment ($p = 0.1$) or at challenge infection ($p = 0.3$), but at the termination of the experiment the weight of group A was 10.2 kg lower than that of the uninfected group C ($p = 0.028$).

Primary infection

On the day of challenge (i.e. 4 weeks after primary infection) 6 primary infected pigs were

Table 3. Recovery and length of *A. suum* and number of white spots. Average number ± SD of white spots on the liver surface and number of *Ascaris suum* larvae from the different locations of the host. Average length of the worms with the range in brackets and *k*-value (measure for the degree of aggregation of the total worm numbers). For group designations see Table 1.

Group	Slaughter (day p.c.i.[1])	White spots	Liver	Lung	SI 1	SI II	SI III	SI IV	Total	Worm length cm (range)	k-value
			Number of worms								
A	7	158 ± 66	0.4±1.2	42.4±24.2	0	0.1±0.3	0.3±0.5	0	43.2±25.3	0.1 (0.06-0.21)	3.12
B	7	215±69	0.2±0.4	55.0±15.8	0	0	0	0	55.2±17.4	0.1 (0.07-0.12)	12.18
A	14	119±79	0	1.6±2.6	0.4±0.7	57.9±113.5	192.7±95.7	25.9±38.0	288.0±213.6	2.6 (0.66-5.99)	1.35
B	14	175±89	0	1.3±1.9	2.8±5.1	280.0±361.2	194.5±148.3	83.2±117.5	561.8±289.0	2.9 (0.72-5.17)	3.86
A	21	93±149	0	0	0.1±0.3	1.2±3.6	35.3±62.2	39.1±41.0	75.7±90.8	4.8 (2.11-8.90)*	0.70
B	21	49±96	0	0	0	151.8±275.2	84.2±91.1	60.5±119.5	295.5±309.4	6.7 (1.65-10.51)	0.92
C	21	-	-	-	-	-	-	-	0	-	-

[1] Post challenge infection, SI I-IV: Section I-IV of the small intestine
* Significantly shorter than group B

slaughtered and examined in order to determine the number and length of worms at challenge. Five out of 6 pigs harboured *A. suum* worms with a mean of 19.5 ± 23.0 and the worm length ranged from 4.2 cm to 25.0 cm. No worms were recovered from the liver and lungs. The livers were fibrotic with a total average of 20.7 ± 19.8 lymphonodular white spots (LN-WS). On the 3 slaughter days following the challenge infection, worms from the primary infection were recovered from 6 of the 10 pigs on day 7 and 21 p.c.i. and 5 of the 10 pigs on day 14 p.c.i.. The worms were easily distinguished from the worms of the challenge infection, based on the difference in length.

Challenge infection

Total number and distribution of *A. suum* larvae in the liver, lungs and 4 sections of the small intestine on the 3 slaughter days are shown in Table 3. Seven days after challenge, the mean recoveries of larvae were 43 and 55 in group A and group B, respectively, and there was no significant difference between the groups ($p = 0.3$). The mean recoveries of larvae on day 14 p.c.i. were 5-10 times higher than on day 7 (288 and 562 in group A and group B, respectively). On day 21 p.c.i. the recoveries of larvae were larger than on day 7 but lower than day 14 (76 and 296 in group A and group B, respectively). In group A the larvae numbers was reduced with 12%, 50% and 75% compared to group B on day 7, 14 and 21 p.c.i., respectively. However, due to large variations within each group, the differences between groups A and B on day 14 and 21 p.c.i. was on the borderline of being statistically significant ($p = 0.054$ on both days). The degree of aggregation of the total worm numbers, described by the k-value, within the groups became more aggregated with time (Table 3). In both groups A and B, the k-value decreased with time and at day 21 p.c.i. the distribution of total number of worms in

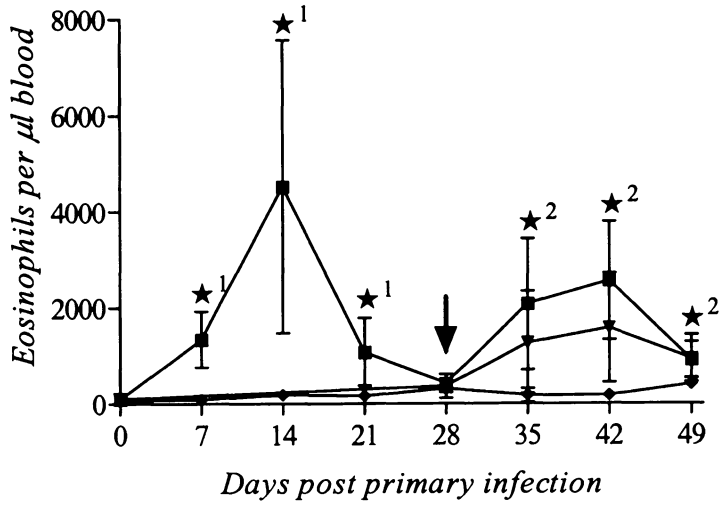

Figure 1. Number of eosinophils per µl blood. Samples are taken weekly throughout the experiment. →: challenge infection with 1,000 infective *A. suum* eggs. —■— = group A, —▼— = group B, —◆— = group C, ★[1]: group A significantly higher than group B and C ($p<0.05$), ★[2]: group A and B significantly higher than group C ($p<0.05$). For group designations see Table 1.

each group followed the negative binominal distribution ($k < 1$). The distribution of *A. suum* worms in the liver, lungs and 4 sections of the small intestine on the 3 slaughter days was significantly different in group A and B ($p = 0.048$). The larvae in group A were recovered from a more distal position than the larvae in group B on day 14 and 21 p.c.i.. On day 7 p.c.i., almost all of the larvae were recovered from the lungs in both groups. On day 14 and 21 p.c.i., approximately 50% of the larvae were recovered from the second quatre of the small intestine in group B. In group A, 76 and 99% of the larvae were found in third and fourth quatre of the small intestine on day 14 and 21 p.c.i., respectively. However, there was no significant difference in the changes in total number of larvae from day 7, 14 and 21 p.c.i. between groups A and B ($p = 0.27$).

The average larval length is also shown in Table 3. There were no significant differences in the length of the larvae between the groups on day 7 and 14 p.c.i. ($p = 0.9$ and $p=0.7$, respectively), however on day 21 p.c.i. the larvae were significantly shorter in group A compared with group B ($p = 0.01$).

Blood parameters

The eosinophil counts measured once a week during the experiment are shown in Fig. 1. All pigs had an average of 70 ± 30 eosinophils per ml at the beginning of the experiment. Seven, 14 and 21 days post primary infection (p.p.i.) the eosinophil counts were significantly higher in group A compared with group B and the uninfected control group C ($p<0.02$). Group A reached a maximum of 4,422 ± 2,620 eosinophils per µl 14 days p.p.i.. After challenge infection the eosinophil counts were significantly higher, on all sampling days, in both groups A and B compared to group C ($p<0.05$). The eosinophil counts in group A and

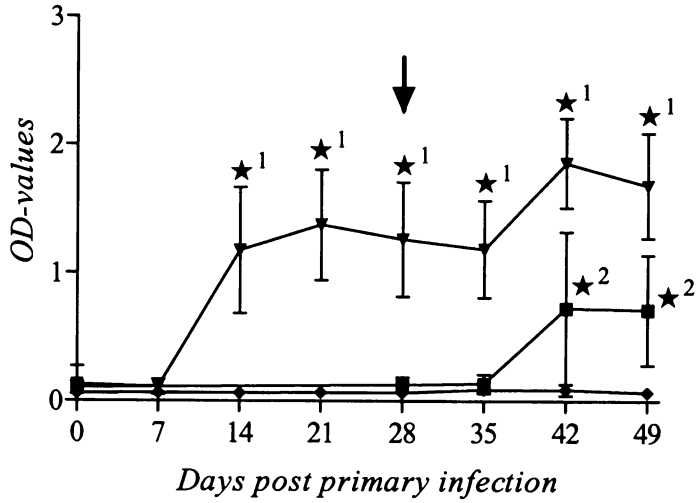

Figure 2. A. suum specific IgG antibody response against L2/L3 ES-antigen. OD- values: optical density values. Samples are taken weekly throughout the experiment. →: challenge infection with 1,000 infective A. suum eggs. —■— = group A, —▼— = group B, —◆— = group C, ★[1]: group A significantly higher than group B and C ($p<0.05$), ★[2]: group B significantly higher than group C ($p<0.05$). For group designations see Table 1.

B were comparable after the challenge infection. Both after primary and challenge infection were the maximum number of eosinophils observed 14 days after infection. The maximum number of eosinophils in groups A and B after challenge infection were significantly lower than the maximum number of eosinophils in group A after primary infection ($p<0.005$).

The corrected optical density values (OD) for the *A. suum* ES-antigen ELISA are shown in Fig. 2. Two weeks after the primary infection, the antibody response had increased to approximately 1 OD in group A, which in the uninfected groups B and C, the levels were only approximately 0.07. This low level was maintained in group B until one week after challenge infection when there was a significant increase ($p<0.05$). In contrast the uninfected group C maintained the same low level throughout the experiment. On all sampling days from day 14 p.p.i., group A levels were significantly higher than those of groups B and C ($p<0.02$). As it appears from Fig 2., the antibody response in group A and B reached a new maximum level 2 weeks after challenge infection. Group B had a significantly higher response than group C towards the end of the experiment ($p<0.03$).

Gross liver pathology

The total number of visible white spots is shown in Table 3. The highest number of white spots was observed 7 days p.c.i. in both groups A and B and the spots were all of the GT-WS type. Livers from both groups were fibrotic and had a white appearance. On day 14 p.c.i. the white spots were in the process of healing, and were less white in colour, but still of the granulation-tissue type white spots (GT-WS). Livers were still fibrotic and had a whitish appearance on day 14 p.c.i.. On day 21 p.c.i.,

the livers in group A and B showed few lymphonodular white spots (LN-WS) and some weak GT-WS. Livers were less fibrotic, but were still whitish in appearance. Generally, group A livers were more fibrotic than group B livers. However, there was no difference in the number of white spots between groups A and B comparing them on each slaughter day ($p>0.1$).

Correlations between measures of infection

The relationships between worm numbers, white spots, pig weight at slaughter, antibody response at challenge infection and eosinophil counts at challenge infection were calculated. There was no correlation between the different parameters ($p>0.09$), except for a positive correlation between antibody response and worm numbers 14-days p.c.i. in group A ($p = 0.04$, $r = 0.45$) and between eosinophil counts and white spots 7 days p.c.i. in group B ($p = 0.03$, $r = 0.85$).

Discussion

A reduction in recovery of larvae was observed in the small intestine of the primary-challenge infected group by 12 %, 50 % and 75% on day 7, 14 and 21 p.c.i., respectively, compared to the challenge-control group. This suggest that pig acquire some degree of protection to challenge infection also after a single infection. The reduction in larval recovery was associated with a more distal location of the larvae in the small intestine on day 14 and 21 p.c.i. and reflects that larvae are being expelled faster in the primary-challenge infected group compared with the challenge control group. However, in both groups the expulsion of worms was initiated between day 14 and 21 p.c.i. This finding is in good accordance with several other investigations (*Eriksen et al.* 1980, *Urban et al.* 1988, *Yang et al.* 1990, *Roepstorff et al.* 1997). After the expulsion of worms are initiated the distribution of the remaining worms becomes overdispersed within the host population following the negative binominal distribution as described by *Roepstorff et al.* (1997) and *Boes et al.* (1998). Earlier experiments applying trickle infections have shown a higher and earlier reduction in larval recovery than observed in the present study (*Urban & Tromba* 1984, *Eriksen et al.* 1992b). *Urban & Tromba* (1984) observed a 73% reduction in recovery of larvae 7 days after a challenge infection superimposed 7 days after 3 inoculations with 1000 eggs, and *Kelley & Nayak* (1964) showed a 93% reduction in larvae recovered from a 7 day old challenge infection given 15 days after 3 inoculations with 10,000 eggs, and a 100% reduction day 7 following challenge infection when superimposed 15 days after 6 inoculations with the same doses. *Eriksen* (1981) infected pigs with 350,000 eggs and gave a challenge infection with 350,000 eggs after 3 weeks. An 80% reduction in lung larvae recovery 7 days after challenge infection and a 99% reduction in larvae recovery 14 and 21 days after challenge infection was observed. Both *Eriksen* (1981) and *Urban et al.* (1988) pointed out the possible existence of a prehepatic barrier stopping the migration of larvae before they reach the liver, since both authors observed significantly reduced liver pathology in challenge infections compared to controls. This was supported by investigations by *Urban* (1986) showing that naturally immunized pigs developed white spots when the infection was given as an injection directly into the mesenteric vein of hatched L_2 larvae, thereby bypassing a prehepatic barrier.

In the present experiment the length of larvae was the same in the 2 groups on day 7 and 14 p.c.i.. However, 21 days p.c.i. the larvae in the primary-challenge infected group were significantly shorter than those of the challenge-control group indicating that the larvae growth are stunted in the immunized pigs. *Kelley &*

Nayak (1964) found a small reduction in the worm length 7 days after a challenge infection with 100,000 *A. suum* eggs superimposed on 3 inoculations of 10,000 eggs. Taffs (1964) also observed some inhibition in the growth of larvae from challenge infections in immunized pigs. In both experiments, the growth inhibition was observed at an earlier stage than in the present experiment. However, both *Kelley & Nayak* (1964) and *Taffs* (1964) infected the pigs with higher doses and used repeated inoculations before challenge, thereby probably inducing a more pronounced immune response in the pigs.

In the present study the eosinophil counts were significantly higher from day 7 to 21 after both the primary and the challenge infections compared to the uninfected controls. This corresponds well with observations by *Jørgensen et al.* (1975), *Eriksen* (1981) and *Yoshihara et al.* (1983). In the present experiment there was a higher response following the primary infection with 60 000 eggs compared to an infection with 1 000 eggs (challenge control). This is expected as the eosinophil response to migrating larvae is an unspecific immune response, which depends on the actual number of larvae migrating.

In the present experiment the primary infected pigs showed a strong antibody reaction 14 days after infection, followed by a further increase 14 days after challenge infection. The challenge-control pigs also showed an increase in the antibody response following infection, though significantly lower than the response in the primary-challenge infected pigs. This correlates well with observations made by *Roepstorff et al.* (1997), who found that the antibody level increased until approximately day 14-21 after infection with either 1,000 or 10,000 *A. suum* eggs.

There were no differences between the groups in the number of white spots on the liver surface on the 3 slaughter days. On day 21 p.c.i. the white spots and fibrosis had started to heal in both groups, which corresponds well with the findings of *Roepstorff et al.* (1997). On the day of challenge infection, the livers of the primary infected pigs showed minimal fibrosis and on the following slaughter day the liver fibrosis in the primary-challenge infected group was much more severe than in the challenge-control group. These observations are in accordance with *Roneus* (1966), *Taffs* (1964), *Urban & Romanowski* (1985) and *Yoshihara et al.* (1987), who observed stronger liver reactions against a challenge infection.

At the termination of the experiment the weight gains of the primary-challenge infected pigs were significantly lower than for uninfected control pigs. This finding is in good agreement with other authors, as *A. suum* infections are known to depress the performance of the host even when sufficient feed is provided e.g. *Roepstorff & Jorsal* (1989), *Yang et al.* (1990) and *Andersen* (1976) .

After the first 2 slaughter day (day 7 and 14 after challenge) there was no significant indication of acquisition of immunity. However, on day 21 after challenge fewer and shorter worms were found and they were located at a more distal position in the small intestine in the primary infected pigs. This was associated with a higher eosinophil level and antibody response. The migrating L_2/L_3 larvae are exposed to a specific antibody attack, which probably results in decreased survival rates in the small intestine.

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Sammendrag

Ascaris suum i svin: Udviklingen af immunitet efter en primær infektion.

Effekten af en primær *A. suum* infektion, på en challenge infektion med *A. suum,* blev studeret med hensyn til etablering og udvikling af challenge infektionen samt svinets immun reaktion og patologiske forandringer i leveren. En gruppe svin blev podet med 60,000 *A. suum* og en gruppe var uinficeret. Fire uger senere blev begge grupper podet med 1,000 *A. suum* og delgrupper blev slagtet efter 7, 14 og 21 dage. Antallet af larver var reduceret med 12%, 50% og 75% på dag 7, 14 og 21 i de immuniserede svin. I begge grupper startede udskydelsen af larver mellem dag 14 og 21, men i de immuniserede dyr var larverne lokaliseret lidt længere tilbage i tyndtarmen og på dag 21 efter infektionen var larverne signifikant kortere på dag 21 end i kontrol dyrene. Disse resultater var associeret med en signifikant højere *A. suum* antistof reaktion og et højere niveau af eosinofile leucocyter i de immuniserede svin i forhold til kontrol svinene. Resultaterne viser, at væksten og overlevelsen af larverne fra challenge infektionen var lavere i de immuniserede grise end i kontrol grisene, sikkert som et resultat af højere antistof og eosinofil angreb under den migratoriske fase.

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