The Effect of Fasting on *Ascaris suum* and *Oesophagostomum* spp. in Growing Pigs

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Abstract—Petkevičius S., Nansen P. & Stephenson L. 1997. The effect of fasting on *Ascaris suum* and *Oesophagostomum* spp. in growing pigs. International Journal for Parasitology 27: 431–437. Experiments were conducted to study the possible influence of fasting on *Ascaris suum* and *Oesophagostomum* spp. in growing pigs. Forty young crossbred pigs naturally infected with *A. suum* and *Oesophagostomum* spp. were used. In one experiment 10 pigs were fasted and offered water ad libitum for 6 days, in another experiment for 10 days. Subsequently, these pigs, together with 10 non-fasted control pigs per experiment were slaughtered, and worm numbers, worm location, sex, developmental stage and female worm fecundity were determined. Pigs fasted for 10 but not for 6 days had decreased numbers of *A. suum* and *Oesophagostomum* spp. at slaughter vs controls, and worms were found in more distal locations in the gastrointestinal tract. Fasting for both 6 and 10 days significantly lowered the fecundity of both worm species. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

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INTRODUCTION

Over the past years many investigations have studied the effect of host nutrition on the host's capability to resist parasite infection or to withstand or compensate for losses brought about by the parasites (cf. Stephenson, 1987). However, diet or nutrition may exert more direct effects on growth, development and pathogenicity of gastrointestinal parasites, simply because these are dependent on their hosts for their nutrient supply and habitat requirements (Crompton, 1991; Solomons & Scott, 1994; Petkevičius et al., 1995).

Fasting or starvation is an extreme nutritional situation which leads to general and profound physiological changes in the host, including radical changes in the gut habitat. Starvation often occurs naturally in populations of wild animals during spells of drought, in severe winters or in injured animals. In domestic animals, starvation may occur because of sudden shortages of feedstuffs, extreme transport conditions (Mayes et al., 1988) or when animals suffer anorexia due to severe disease. In human populations, food deprivation and starvation are unfortunately common, especially in developing countries, e.g. when crops fail, or in association with wartime, civil disturbances and mass migrations.

Helminth infections have a widespread occurrence and significant impact on human and animal hosts worldwide. In humans, these infections cause health problems primarily in tropical and subtropical areas, where food shortages and famine are most likely to occur. Apart from studies on naturally infected horses which expelled strongyle nematodes in connection with starvation (Dvojnos G. M. & Timoshenko O. N., unpublished, Abstracts of Eighth International Congress of Parasitology, Izmir, Turkey, 1994), little
is known about how fasting or starvation may influence gastrointestinal helminths and severity of disease they may produce (Reisel, 1982; Shetty & Shetty, 1993).

The present investigation on pigs was designed to examine, under controlled conditions, the influence of fasting on persistence, location and fecundity of two commonly occurring nematodes, namely *Ascaris suum* and *Oesophagostomum* spp. There are close similarities between these two parasite species and their related species in man, and in addition, the two hosts have many points of resemblance including gastrointestinal anatomy and physiology. We expect that information obtained in a pig model may be of relevance to man, in which, for obvious reasons, this type of experimentation cannot be performed.

**MATERIALS AND METHODS**

**Experimental design.** In two separate experiments (I and II), pigs were fasted for 6 and 10 days, respectively, and compared with control pigs given a standard diet. The pigs used originated from a traditional Danish pig farm and were Danish Landrace/Yorkshire/Duroc crosses of both sexes. After transportation to an experimental animal facility pigs to be fasted were placed individually in small separate pens, whereas pigs fed a standard diet were kept in larger pens, 5 animals in each. The pens had solid concrete floors and were separated by partition walls, approximately 1.5 m high. No bedding was offered since previous observations on fasted pigs have shown that these ingest considerable amounts of bedding. Also, any bedding would have made recovery of expelled worms from the floor very difficult. Before fasting started there was an adaptation period of 4 days. The diet of the control pigs, which consisted of a ground barley plus a protein supplement, was similar to that given in the herd of origin (Petkevičius et al., 1995).

Experimental and control pigs were offered water ad libitum. In the pens harbouring fasting pigs, faeces were quantitatively collected at frequent (4–5 times per day) intervals during the entire experiment to minimize coprophagy and to determine the faecal volume excreted daily. From control pigs faeces excreted were collected for a 24-hour period prior to slaughter. The clinical condition of the pigs was observed daily by a veterinarian and all pigs were weighed at the start of the experiment, and again at slaughter on days 6 and 10. To determine parasite egg concentrations faecal samples obtained directly from the rectum were collected daily for experiment I and every second day for experiment II.

**Experiment I.** Twenty pigs, 24–26 weeks old, with a mean body weight of 78 kg (range 71–90 kg) collected on the basis of faecal egg counts of both helminth species were randomly divided into 2 comparable groups of 10 pigs each. The experimental group was fasted for 6 days. On day 6 all pigs were killed for postmortem worm determinations.

**Experiment II.** Twenty pigs 10–11 weeks old, with a mean body weight of 32 kg (range 28–37 kg) were collected and randomly divided into 2 groups and examined as above, the experimental group being fasted for 10 days.

**Parasitological techniques.** The number of eggs per gram of faeces (epg) was estimated with a modified McMaster technique (Roepstorff & Nansen, in press). On days 6 and 10, all animals initially euthanised by CO2 were exsanguinated. The entire small and large intestines were immediately removed and separated from the mesenteries. The small intestine was divided into 4 sections of approximately equal length (designated sections 1, 2, 3, 4 from the anterior end). The contents of each section were collected by pressing luke warm water through the intact section twice followed by washing over a sieve with a mesh size of 212 μm. The large intestine was divided into 5 sections, designated as follows, starting from the anterior end: (1) caecum; (2) 0–20% of the total length of the colon; (3) 21–40%; (4) 41–60% and (5) 61–100%. The sections were opened with scissors and the contents washed off the mucosal surface. All intestinal contents were examined from fasted pigs, and from control pigs representative subsamples of 10% were examined. Specimens of *A. suum* were weighed, their length measured with a ruler and the sex and developmental stage were noted. *Oesophagostomum* spp. worms were collected with an agar-gel method described by Slotved et al. (1996). The worms released from the agar gels were transferred to screw-capped plastic tubes, fixed and stored in iodine solution (6.75% iodine + 31.25% potassium iodide + 62.5% distilled water). Subsequently the samples were decolorised with 3% thiosulphate solution and the worms counted with a microscope. Species and developmental stage were determined by random collection and microscopical examination of 100 worms from each sample. All worms were examined when there was less than 100 worms per sample. The morphological criteria of Goodey (1926) and Haupt (1966) for differentiation of *O. dentatum* and *O. quadrispinulatum* were used. The sex of adults of both species was noted.

**Statistical analysis.** The experimental data were analysed using analysis of variance (ANOVA) to determine the effect of 6 and 10 days of fasting on worm faecal *egg* counts, numbers, location, and fecundity of female worms. As an indication of the average position of the worms along the small and large intestine of each pig, the “location” was calculated by multiplying the number of worms in each section of each pig by the section number of intestines, divided by the total number of worms in all sections.

\[
\text{Location} = \frac{\sum_{i=1}^{5} i \times \text{number of worms in section } i}{\text{Total number of worms in all sections}}
\]

Female worm fecundity was estimated by multiplying epg by total amount of faeces excreted per day, divided by the adult female worm burden for the relevant worm species. Log-transformed fecundity was examined by analysis of variance to test for the effect of starvation.

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\text{Fecundity} = \frac{\text{epg \times weight of faeces per day}}{\text{Total number of adult females in all sections}}
\]

**Ethical considerations.** The experiments were approved by the Danish Animal Ethical Committee (animal experiment permission 1994-101-115). Meetings were held with the agricultural and laboratory technicians to explain the purpose of the experiments and what was required from the persons handling the pigs.

**RESULTS**

**Experiment I (6 day fast)**

Faecal egg counts. *Ascaris suum* and *Oesophagostomum* spp. egg counts are given in Figs 1 and 2, and
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Fig. 1. Geometric mean *Ascaris suum* egg counts in pigs fasted for 6 days (□), fed for 6 days (■), fasted for 10 days (■) and fed for 10 days (●).

Fig. 2. Geometric mean *Oesophagostomum* spp. egg counts in pigs fasted for 6 days (□), fed for 6 days (○), fasted for 10 days (■) and fed for 10 days (●).

Fig. 3. Distribution of *Ascaris suum* in pigs fasted or fed for 6 days. Sections of intestine: 1—0–25%; 2—26–50%; 3—51–75%; 4—76–100% of small intestine; 5—Large intestine; 6—Expelled; 7—Total.

Fig. 4. Distribution of *Ascaris suum* in pigs fasted or fed for 10 days. Sections of intestine: 1—0–25%; 2—26–50%; 3—51–75%; 4—76–100% of small intestine; 5—Large intestine; 6—Expelled; 7—Total.

Fig. 3. Distribution of *Ascaris suum* in pigs fasted or fed for 6 days. Sections of intestine: 1—0–25%; 2—26–50%; 3—51–75%; 4—76–100% of small intestine; 5—Large intestine; 6—Expelled; 7—Total.

Worm burdens

*Ascaris suum* burdens and distribution are given in Fig. 3. There were no statistically significant differences in total worm burden between the groups ($P>0.05$), but worm location was significantly affected by fasting ($P=0.04$). In the fasted pigs, 27% of the total worm burden was found in the proximal parts of the large intestine. The back-transformed mean (S.D.) of *A. suum* fecundity was 155,794 (77,791) eggs per female worm per day in experimental pigs and 396,259 (184,420) eggs per female worm per day in control pigs, and was reduced 60.7% by fasting ($P=0.01$).

*Oesophagostomum* spp. burdens and distribution are shown in Fig. 5. The total number of *Oesophagostomum* spp. was not significantly affected by fasting ($P>0.05$).

*O. dentatum* constituted on the average 92% and 95% of the total *Oesophagostomum* worm burden in experimental and control pigs, respectively. The remaining worms were identified as *O. quadrispinulatum*. The mean (S.D.) location of *O. dentatum* was sections 2.5 (1.0) and 2.2 (1.2), of *O. quadrispinulatum* sections 1.9 (0.4) and 1.5 (1.0) of the large intestine in experimental and control pigs, respectively. *O. quadrispinulatum* showed a more proximal location in the large intestine, but did not differ significantly from the location of *O. dentatum*. In the experimental group, 35% of *O. dentatum* and 22% of *O. quadrispinulatum* were found in the posterior part...
(section 5) of the large intestine, in which no worms were found in the controls. All Oesophagostomum examined were adults and for both species in both groups there was an equal distribution of males and females. The back-transformed mean (S.D.) of Oesophagostomum spp. fecundity was 865 (1348) eggs per female worm per day in fasted pigs and 2927 (2577) eggs per female worm per day in fed pigs, showing a reduction of 70.4% (P = 0.03).

Experiment II (10 day fast)

Faecal egg counts. Mean egg counts of A. suum and Oesophagostomum spp. are depicted in Figs 1 and 2, and did not differ significantly by species at the outset. There was a gradual rise in Oesophagostomum spp. and A. suum counts in experimental pigs up to day 6 and 8, respectively. For A. suum, the difference between experimental and control pigs was statistically significant (P = 0.03) on days 8 and 10; it was significant for Oesophagostomum spp. on day 6 (P = 0.04). Towards the end of the period, however, there were marked reductions in egg counts for both species which were more pronounced for Oesophagostomum spp. where counts approached zero.

Worm burdens. The total worm burdens and distribution of A. suum are shown in Fig. 4. As expected large individual variations in A. suum burdens were observed between pigs within experimental and control groups, but the mean total A. suum burden of the experimental pigs was significantly lower as compared with the control pigs (P = 0.001). The mean (S.D.) location of A. suum in the experimental and control pigs was sections 2.4 (1.3) and 1.4 (0.4) of the small intestine, respectively, and an average of 0.9 (2.8) worms were found in the large intestine and 1.2 (2.5) were expelled. The back-transformed mean (S.D.) of A. suum fecundity was 68,502 (72,791) eggs per female worm per day in the experimental pigs and 461,369 (194,184) eggs per female worm per day in the control pigs, showing a 85.2% reduction due to fasting (P = 0.005).

Oesophagostomum dentatum and O. quadrispinulatum burdens and distributions are shown in Fig. 6. The total number of worms was not significantly affected by 10 days of fasting (P > 0.05). O. dentatum constituted on the average 94% and 92% of the total Oesophagostomum worm burdens in experimental and control pigs, respectively. The remaining worms were identified as O. quadrispinulatum. The mean (S.D.) location of O. dentatum was sections 2.8 (0.6) and 2.5 (0.6), of O. quadrispinulatum sections 1.6 (0.9) and 1.2 (1.0) of the large intestine in experimental and control pigs, respectively. O. quadrispinulatum showed a more proximal location in the large intestine and differed significantly from the location of O. dentatum (P = 0.04). All Oesophagostomum worms recovered were adults and for both species in both groups there was an equal distribution of females and males. The back-transformed mean (S.D.) of Oesophagostomum spp. fecundity was 184 (256) eggs per female worm per day in experimental pigs and 3390 (2608) eggs per female worm per day in control pigs, showing a 94.6% reduction (P = 0.0005).

Clinical and other observations

The behaviour of fasting pigs in both experiments differed from fed pigs but not significantly. During the first days the fasted pigs were restless and nervous, but later they became less active and were lying/sleeping most of the time. Some cases of coprophagy were observed in the experimental animals. The mean live weight of fasted pigs had decreased 1.8 kg per day after 6 days of fasting and 1.0 kg per day after 10 days, while mean live weight of fed control pigs had
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As expected the experimental pigs after fasting for 6 and 10 days were significantly lighter than control animals \((P = 0.001)\). The faeces of the fasted pigs gradually developed a hard consistency and after 6 days of fasting faeces appeared in “pelleted” form.

The mean (S.D.) weight of excreted faeces in the fasted pigs decreased from 892 (819) g at the start of the experiment to 669 (169) g to 39 (18) g at the 10th day of fasting, respectively. At slaughter intestinal contents in the guts of fasted pigs were sparse and seen only in the large intestines, primarily in the most distal parts.

**DISCUSSION**

The results of these experiments show a significant effect of fasting on parasitism. Fasting of pigs for 6 and 10 days influenced worm numbers, fecundity and location in the gut. A. suum and Oesophagostomum spp. epg in the experimental pigs started to increase from the first day of fasting, and high levels were observed until day 6 for Oesophagostomum spp. and day 8 for A. suum, after which marked decreases occurred (Experiment II). The initial increase may obviously be explained by the significant reduction in the volume of faeces excreted, while the drop in epg towards the end of the study might be a consequence of worm expulsion or lowered fecundity of remaining females, or both. Both possibilities were confirmed by determinations of worm numbers and worm fecundity at slaughter. On day 6, numbers of A. suum and Oesophagostomum spp. were not affected by fasting compared with fed controls, but fecundity was significantly reduced, especially for Oesophagostomum. On day 10 the fecundity of both A. suum and Oesophagostomum spp. was even lower, and at this time numbers of A. suum, but not Oesophagostomum spp., were significantly reduced in the fasted pigs as well.

Fasting of pigs for both 6 and 10 days led to a more wide distribution of both species along the gastrointestinal tract. The most striking feature was the recovery of worms from gut sections where they are normally not found, i.e. for A. suum in the large intestine; and for Oesophagostomum spp. in the very distal part of the large intestine. These were the regions where small amounts of gut contents were still found. In both experiments O. dentatum constituted 92–95% of the Oesophagostomum species.

O. quadrispinulatum had a more proximal location in the large intestine than O. dentatum, which is in agreement with findings of Roepstorff & Nansen (in press) and Christensen et al. (in press).

Few studies have been designed to analyse the influence of fasting on helminth parasites. In the faeces of domestic horses starved for ten days, the number of strongylid eggs decreased, and expulsion of adults and larvae of the genera Delafondia and Alfondia was observed (Dvojnos & Timoshenko, unpublished, Abstracts of Eighth International Congress of Parasitology, Izmir, Turkey, 1994). Under natural winter conditions in Mongolia wild horses had great difficulty finding food and showed a significant expulsion of adult strongylids (Dvojnos & Timoshenko, unpublished, Abstracts of 2nd European Congress of Mammalogy, Southampton, U.K., 1995).

The results of our experiments show that young, healthy pigs can sustain withdrawal of feed for 6 and 10 days without effects on health and behaviour, providing that they are adequately housed and allowed access to water ad libitum. There was a higher level of general behavioural activity of the fasting pigs than in control animals during the first few days of fasting in both experiments, before lethargy set in. Similar
observations on the behaviour and health status of pigs fasted for 10 days were made by Anderson (1988).

The mechanisms by which fasting decreases the survival and fecundity of *A. suum* and *Oesophagostomum* spp. in the gastrointestinal tract of pigs are unknown. One likely explanation is that the reduction in intestinal contents in the small and large intestine during fasting deprives the worms of their normal nutrient supply or otherwise creates unfavourable physiological or biochemical conditions. Wills (1985) states that if fasting in rats is prolonged for more than 24-48 h, metabolic adjustments are made to conserve body protein. This is accomplished by reduced glucose production and utilization, and increased concentration of ketones as a result of increased fatty acid oxidation. The most important nutrient for energy production in helminths is glucose, whereas fatty acids and monoglycerides cannot be metabolized to yield metabolic energy (Castro, 1991). Another possibility is, perhaps, coprophagy which may affect the composition of bacteria in the gut and the consequent microbial activity (Barnes & Fiala, 1957). It is not surprising that when nutritional deficiencies change normal physiological processes of the host, the parasite may find itself in an abnormal environment and often is unable to survive (Solomons & Scott, 1994). Our results agree with previous findings suggesting that, when some nutrients, e.g. proteins (Kambara et al., 1993) and carbohydrates (Crompton, 1991) are not present in adequate amounts in the host, because of deficient dietary intake, parasite survival is affected adversely, but they also show that both species did survive 10 full days of fasting.

The practical implications of these findings of decreases in worm burden and fecundity during host (porcine) fasting make evolutionary sense. Since parasites depend on their hosts for survival, parasites are much more likely to live to reproduce if their numbers decrease and their drain on their hosts' reserves decreases when their hosts are threatened by starvation.

However, fasting of tapeworm infected humans is said to result in rapid expulsion of large parts of the worms. Unfortunately, the scolex remains, since tapeworms, unlike nematodes, possess no alimentary tract and must absorb glucose as their energy source directly from the surrounding medium through their integument (Von Brand, 1979). A combination of fasting and purgatives is now used in traditional medicine in East Africa as a cure for *A. lumbricoides* infection, and probably for other intestinal worms in other areas as well. It is possible that brief fasts may play a therapeutic role in otherwise healthy and well-nourished but worm-infected older children and adults. But even if they do not, the further study of the effects of host malnutrition on gastrointestinal helminths is justified for what we can learn about the complex ways in which parasites and hosts co-exist, even in unusually adverse situations.

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