**Experimental infection of pigs with three dose levels of *Trichuris suis***

PEDERSEN S.* & SAEED I.*

Summary:
The objective of the study was to follow the course of *Trichuris suis* infection in pigs given infective eggs at low (400 eggs), medium (4,000 eggs) and high inoculation dose (40,000 eggs), respectively. Interestingly, despite a 100-fold difference in dose level no significant difference was found in either blood parameters, total faecal egg excretion, fecundity or worm burdens at necropsy 12 weeks post inoculation. The highest and lowest median faecal egg output was found in the medium and high dose group, respectively. With increasing dose level, worm size, establishment and prevalence of *T. suis* positive pigs decreased while worms were dislocated aborally. In addition there was a highly significant correlation between female worm burden and faecal egg excretion.

KEY WORDS: *Trichuris suis*, whipworm, pig, single infection, dose.

**INTRODUCTION**

The whipworm *Trichuris suis* (Schrank, 1788) is a widely occurring intestinal nematode parasite of pigs (Powers *et al*., 1959; Jacobs & Dunn, 1969; Pattison *et al*., 1980). Adult worms are found in the caecum and colon, either in the intestinal contents or with part of their front ends embedded superficially in the mucosa (Jenkins, 1970). The prepatent period is 41 to 45 days and the parasite has a life span seldom exceeding four to five months (*Powers*, 41 to 45 days and the parasite has a life span or with part of their front ends embedded superficially in the mucosa (Jenkins, 1970). The prepatent period is 41 to 45 days and the parasite has a life span seldom exceeding four to five months (*Powers*, 41 to 45 days and the parasite has a life span). *Trichuris suis* infection in pigs is considered to be a good model for direct comparison with the equivalent infection in humans, which affects 500 million people worldwide (*Stephenson*, 1987).

Hitherto, most experimental studies have employed high inoculation doses, i.e. 15,000–400,000 infective eggs (*Powers* *et al*., 1960; *Beer* *et al*., 1971; *Batte* & *Moncol*, 1972; *Beer* & *Lean*, 1973; *Beer* *et al*., 1973; *Batte* *et al*., 1977; *Hale* & *Stewart*, 1979). However, under natural conditions ingestion of smaller infective doses is more likely to occur, partly causing less intense activation of the host immune response, partly modulating the population dynamics of *Trichuris*. Eventually, a more prolonged and chronic infestation could result as documented in the clinical syndrome of children (*Holland*, 1987). Few experimental studies have been performed to investigate the course of infection after patency following inoculation at different dose levels. In the present study, three inoculation doses were used: 1) a low dose imitating a natural low level of infection; 2) a medium dose not expected...
to produce clinical signs or crowded worm populations (Johansen et al., 1997) and 3) a high dose known to be able to provoke clinical symptoms (Powers et al., 1960). Single doses given to parasite-naïve pigs were chosen, which made it possible to investigate the result of the intricate interaction between the initial immune response and the entire development from larvae to adults of one age-group of parasites, without introducing an interfering effect of acquired resistance associated with trickle infections.

The aim of our study was to follow the course of T. suis infection in pigs given infective eggs at low (400 eggs), medium (4,000 eggs) and high inoculation doses (40,000 eggs), respectively. During the course of infection clinical, haematological and coprological parameters were monitored, and T. suis burdens were determined at autopsy 12 weeks post inoculation (p.i.).

MATERIALS AND METHODS

ANIMALS AND FEEDING

Twenty-nine specific-pathogen-free cross-bred Danish Landrace/ Yorkshire/ Duroc pigs of both sexes weighing 77 ± 15.5 kg (mean ± S.D.) were purchased from a helminth-free experimental farm and housed together in a stable (5 x 25 m²) with straw bedding and access to pasture via a soiled area. The pigs had free access to water via drinking nipples. The pigs were weighed every second week.

A standard diet consisting of ground barley plus a commercial premix (16 % crude protein) was fed to the pigs.

EXPERIMENTAL DESIGN

The pigs were randomly divided into four groups; an uninoculated control group (four females and two males), a low dose group (400 eggs per pig; six females and two males), a medium dose group (4,000 eggs per pig; five females and three males) and a high dose group (40,000 eggs per pig; four females and three males).

 Infective T. suis eggs were originally isolated in 1996 from soil in a small organic farm and subsequently passaged in helminth-naïve pigs. The eggs used in the present experiment were isolated from faeces of pigs, embryonated in vermiculite according to the method described by Burden & Hammet (1976) and subsequently stored at 10°C for two years. The pigs were inoculated orally via a stomach tube. Faecal samples were collected from the rectum of each pig two, four and six weeks (wks) p.i. and weekly hereafter. Egg counts were evaluated using a concentration McMaster Technique as described by Roepstorff & Nansen (1999), with a detection limit of 20 eggs per gram (e.p.g.). Blood samples were taken biweekly from the truncus bijugularis of each pig and haemoglobin concentration, packed cell volume (PCV), serum protein concentration, serum albumin concentration, albumin/globulin (A/G) ratio and the peripheral eosinophil count were measured and analysed.

NECROPSY AND WORM RECOVERY

All pigs were slaughtered 12 wks p.i. Feed was withheld on the day of necropsy and pigs were euthanised by electrical stunning followed by exsanguination. Trichuris suis worms were recovered according to the method described by Roepstorff & Murrell (1997). The large intestine was divided into five sections, designated as follows, starting at the caecum: 1: caecum; 2: 0-20 % of the total length of colon; 3: 21-40 %; 4: 41-60 % and 5: 61-100 % including rectum. The sections were opened with scissors and the intestinal wall was gently washed to liberate T. suis. Representative aliquots of 10 % of the intestinal contents and intestinal wall washings were washed over a sieve of mesh size 212 μm. Retained samples were fixed in 15°C iodine (80 g iodine and 400 g potassium iodide in 800 ml of distilled water) for later isolation of T. suis. Samples were transferred to a Petri dish held over a light table and decolourized with a 30 % sodium thiosulphate solution. Worms visible with the naked eye were recovered, stored in 70 % ethanol and differentiated according to developmental stage and sex (Beer, 1973a). The intestinal location of T. suis in each pig was estimated by assuming that all worms in each section were localized in the middle of that particular section. The percentage of worms in each section of individual pigs was calculated, allowing relative distributions of T. suis in sections of the large intestine to be compared between groups. Body lengths of intact worms were measured by means of a stereo microscope and a digital image analysis system (Microvision®, DTI, Denmark). For those pigs harbouring more than 10 worms of each sex, 10 female and 10 male worms were selected as randomly as possible. The proportion of female worms was calculated by dividing the number of female worms by the total number of adult worms. Percentage establishment was defined as the percentage of the infective eggs recovered as worms. For those pigs harbouring female worms, fecundity was estimated by dividing e.p.g. at the day of slaughter by the number of females recovered.

STATISTICAL ANALYSIS

Worm numbers, intestinal location, worm distribution in sections, percentage establishment, proportion of female T. suis, worm length, faecal egg counts, worm
fecundity and body weight were compared using one-way analysis of variance (ANOVA). Where appropriate the non-parametric Kruskal-Wallis test was used. Univariate repeated measurements ANOVA was used for longitudinal analyses for body weight and blood parameters. Longitudinal analysis of faecal egg excretion was performed by estimating the area under the e.p.g. curve of individual pigs from six to 12 wks p.i. It was assumed that the counts obtained weekly represented mean daily counts for the whole week. Groups were then compared using the Kruskal-Wallis test.

For those pigs harbouring female T. suis at slaughter Pearson’s correlation coefficient was used to relate the female worm burden to faecal egg excretion. Before analysis worm numbers and e.p.g. were logarithmically transformed (log10(x + 10)). The prevalences of T. suis positive pigs at slaughter were compared between groups using Fisher’s Exact test. The parameter of the negative binomial distribution, k, was estimated as $k = \frac{\text{mean}^2}{\text{variance} - \text{mean}}$; $k$ decreases as overdispersion increases. Mean and variance were calculated on total worm burdens.

RESULTS

WORM RECOVERY

The number of T. suis worms recovered are presented in Table I. Two pigs from the control group picked up infection of 10 and 90 T. suis, respectively. Mean ($\pm$ S.E.M.) worm numbers were 93 ± 39, 399 ± 280 and 3,536 ± 2,291 in the low, medium and high dose group, respectively, representing a mean (maximum) individual establishment of 23 (65) %, 10 (57) % and 9 (33) %, respectively (Fig. 1). Neither the number of worms ($P = 0.57$) nor establishment of infection ($P = 0.17$) were significantly different between experimental groups. Six pigs (86 %) in the low dose group, five pigs (63 %) in the medium dose group and two pigs (29 %) in the

<table>
<thead>
<tr>
<th>Group</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>Total</th>
</tr>
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<tr>
<td>Control</td>
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<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>90</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
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<td>Low</td>
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<td>(n = 7)</td>
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<td>110</td>
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<td>220</td>
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<td>20</td>
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<td>60</td>
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<td>370</td>
<td>410</td>
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<tr>
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<td>1,860</td>
<td>4,790</td>
<td>4,130</td>
<td>700</td>
<td>13,300</td>
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<tr>
<td>(n = 7)</td>
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<td>3,150</td>
<td>2,070</td>
<td>3,440</td>
<td>1,200</td>
<td>11,450</td>
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Table I. - Number of Trichurus suis recovered from the large intestine of pigs inoculated with 400, 4,000 or 40,000 eggs, together with uninoculated control pigs. Only pigs harbouring T. suis at slaughter are included. S1: section 1 (caecum). S2: section 2 (0-20 % of the total length of colon). S3: section 3 (21-40 %). S4: section 4 (41-60 %). S5: section 5 (61-100 % including rectum). Total: all five sections.

Fig.1. - Mean ($\pm$ S.E.M.) number of T. suis recovered from five intestinal sections of the A) low, B) medium and C) high dose group, respectively. S1: caecum. S2: 0-20 % of the total length of colon. S3: 21-40 %. S4: 41-60 %. S5: 61-100 % including rectum. Only T. suis positive pigs are included.

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high dose group harboured worms but this was not significant \((P > 0.10)\). In the high dose group a significantly higher percentage of the worm burden was recovered from section 4 compared with the low dose group \((P = 0.026)\). Median intestinal location of the worm burden was 2.6, 2.8 and 3.0 for the low, medium and high dose group, respectively \((P = 0.47)\). The relative distributions of worms in the five sections of the large intestine for the three groups are shown in Fig. 2. Only adult \textit{T. suis} were recovered and they ranged from 16 to 48 mm in length. Total median lengths of males and females as well as the total median (min.-max.) worm lengths \(56.9\) (28.8-40.2), medium: 36.1 (28.3-40.9), high: 26.7 (25.8-27.5); \(P = 0.10\) did not differ significantly. The median proportion of female \textit{T. suis} was not significantly different between groups \((P = 0.90)\). The parameter \(k\) of the negative binomial distribution was found to be \(k < 1\) in all three experimental groups \((P = 0.83);\) medium: 0.25; high: 0.34\) indicating a high degree of aggregation of the adult \textit{T. suis} populations.

Eight wks p.i. one pig from the high dose group – which harboured worms at necropsy – excreted adult \textit{T. suis} worms in the faeces.

**FAecal EGG COUNTS**

Faecal egg excretion in some of the inoculated pigs started six wks p.i. There were one and four pigs in the low and high dose group, respectively, which did not have faecal egg counts above 20 e.p.g. at any occasion. The highest and lowest mean e.p.g. levels were observed in the the high and low dose group, respectively, and all groups peaked nine wks p.i. \((P = 1.026;\) medium: 5.58; high: 24.643 e.p.g.) \((P = 0.026)\). Figure 3B shows that between wk seven and 12 the median was 140-580 \((\text{max. wk 10})\), 10-3725 \((\text{max. wk 7-8})\) e.p.g. for the low, medium and high dose group, respectively, reflecting the decrease in number of pigs excreting eggs with increasing inoculation dose. In spite of these observations, longitudinal analysis showed that there was no significant difference in total median (min.-max.) egg excretion between experimental groups during the study i.e. low: 2740 (20-14,120), medium: 8670 (420-73,520) and high: 40 (20-289,680) e.p.g. \((P = 0.36)\). Faecal egg excretion did not differ between groups at any sampling time \((P > 0.13)\). Each female \textit{T. suis} excreted a median (min.-max.) of 9.5 (3.4-160), 0.4 (0-6) or 9.1 (8.6-9.5) e.p.g. in the low, medium and high dose group, respectively. Comparison of the median fecundity revealed no significant difference between groups \((P = 0.065)\).

For pigs harbouring female \textit{T. suis} there was a significant correlation between female worms recovered at

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**Fig. 2.** Comparison of the relative distribution (\%) of \textit{T. suis} in the low, medium and high dose groups. S1: caecum. S2: 0-20 \% of the total length of colon. S3: 21-40 \%. S4: 41-60 \%. S5: 61-100 \% including rectum. The asterisk marks the significantly higher percentage of the worm burden in S4 found in the high compared to the low dose group \((P = 0.026)\).

**Fig. 3.** A) Mean and B) median \textit{T. suis} eggs per gram faeces (e.p.g.) in the low, medium and high dose groups from six to 12 weeks post inoculation.
necropsy and concurrent e.p.g. \((r = 0.80, n = 12, P = 0.002)\). Two pigs did not excrete eggs but harboured worms (false-negative egg counts) while three pigs showed false-positive faecal egg counts (20, 20 and 120 e.p.g.) at slaughter.

**HAEMATOLOGY & ANIMAL PERFORMANCE**

Longitudinal analysis of serum protein \((P = 0.43)\) and albumin levels \((P = 0.63)\), A/G ratio \((P = 0.63)\), haemoglobin \((P = 0.09)\), PCV \((P = 0.11)\) and peripheral eosinophil counts \((P = 0.84)\) did not reveal any significant differences between controls and/or inoculated groups. However, the high dose group experienced a significantly higher decrease in albumin levels between wk 2-6 p.i. \((P = 0.012)\).

During the study there were no significant differences in body weight gains between groups at any time. Overall, there was no difference between groups in weight gains with time \((P = 0.71)\). One female pig in the high dose group developed diarrhoea seven wks p.i. and became emaciated. Looseness of the faeces occasionally occurred in a number of infected pigs in the medium and high dose groups. During the study one female pig from the low dose group died from incarcerated umbilical hernia. Another pig in the low dose group had a diseased leg and was injected intra-muscularly with penicillin. At necropsy most female pigs were pregnant.

**DISCUSSION**

**PARASITOLOGY**

There was a tendency towards a decrease in prevalence of *T. suis* positive pigs with increasing inoculation dose. This could be due to a more vigorous stimulation of the immune system in those pigs receiving a high inoculation dose (Keymer, 1982). Likewise there was an indication of a decline in establishment associated with an increase in numbers of eggs given. This has formerly been described in single infections (Hale & Stewart, 1979) and trickle infections (Powers et al., 1960) and could reflect a self-regulation of the *T. suis* population size. A prevalence of 15-42 % *T. suis* positive pigs has been reported in naturally infected pigs which harboured a mean number of 6-29 worms (Jacobs & Dunn, 1969; Pattison et al., 1980).

In the low dose group worms were located mainly in the caecum and the upper colon. With increasing dose worms were also found in the more aboral parts of the colon, which was most pronounced in the high dose group. *Trichuris suis* is reported to have a predilection for the caecum and the upper third of the colon, but the entire colon is a potential habitat, with worms being found occasionally in the rectum (Powers et al., 1960). A similar phenomenon has been described in pigs inoculated with increasing doses of *Oesophagostomum* spp. larvae (Christensen et al., 1995).

The lengths of adult *T. suis* recorded in this study were less (27-36 mm) than those (48-50 mm) reported by Beer (1973b). This is likely to be due to differences in parasite strains and the fact that *T. suis* worms continue to grow after reaching the adult stage. However, preservation of worms in iodine may cause minor shrinkage. Worms recovered from pigs receiving a high inoculation dose were shorter than those from the low and medium dose groups, which has also been reported for pigs inoculated with different dose levels of *Oesophagostomum* larvae (Christensen et al., 1995). This suggests a crowding effect with suboptimal growth conditions, a view that is supported by the enlargement of the intestinal habitat. The observed proportion of female worms was quite constant in the three inoculated groups (i.e. 0.41-0.43) and probably represents a level characteristic of established adult *T. suis* populations in experimentally infected pigs, although it may reflect the sex proportions of the infective larvated eggs. For comparison Jacobs & Dunn (1969) found that the proportion of female *T. suis* was 0.55-0.66 in naturally infected pigs.

Our study shows that *T. suis* has an aggregated distribution within pig populations which becomes more pronounced with increasing inoculation dose. This overdispersion, which implies that a few hosts harbour most worms while the majority of hosts have few or no worms, has also been documented for *T. trichiura* in human populations (Bundy & Cooper, 1989) and is of major importance in understanding the epidemiology and control of this parasite (Keymer, 1982).

The observed rise in mean egg production followed by a decline has been described to be typical of *T. suis* (Powers et al., 1960). In the low dose group faecal egg excretion was quite constant as reflected by the similar course and culmination of the median and mean e.p.g. curves. The medium dose group had consistently higher median and mean e.p.g. levels compared to the low dose group except for wk 12 p.i.; the somewhat earlier culmination of the median compared to the mean suggests a decrease in prevalence of egg-excreting pigs in the weeks following patency. In the high dose group the median e.p.g. was very low during the entire patent period possibly due to expulsion of larval infection in a number of pigs while the high mean e.p.g. level largely represents a few heavily infected pigs. In order to establish a moderate infection in a high proportion of experimentally infected pigs the medium dose seems to be preferable: except for wk 12 p.i. the “average” (median) pig had the highest egg excretion in the medium group followed by the low
dose group whereas the lowest value was found in the high dose group. This tendency has also been reported following trickle infections with oesophagostomes in pigs (Roepstorff et al., 1996). Assuming a daily faecal excretion amounting to two kg this would correspond to 19,000 (6,800-320,000), 800 (0-12,000) and 18,200 (17,200-19,000) eggs (median, (min.-max.)) produced per female per day in the low, medium and high dose group, respectively, indicating that *T. suis* is not necessarily such a non-prolific egg layer as suggested by Beer et al. (1971).

The unintentional infection of two control pigs is likely to be due to infective eggs passing unhatched through the intestinal tract of inoculated pigs and subsequently re-emerging to establish as adults on the mucosal surface of the intestine (Sansom et al., 1974; Batte et al., 1977). This is confirmed in the present study by the steeper fall in serum albumin levels in the high dose group from wk 2-6 p.i. compared to the other groups. Batte et al. (1977) observed that a marked decrease in the albumin fraction of the total serum protein occurred beginning seven to 14 days p.i. Anaemia was not observed in any group as deemed by PCV and haemoglobin levels. An average rate of loss of erythrocytes which was approximately proportional to the infective dose of eggs ranging from seven to 35 ml/day has been reported by Beer et al. (1974). The lack of peripheral eosinophilia due to *T. suis* infection seen in the present study has been reported formerly (Beer & Lean, 1973), although eosinophils have been described to be prominent in the colonic mucosa of infected pigs (Powers et al., 1960).

Clinical manifestations of experimental trichuriasis may follow from inoculation with 25,000 to 400,000 infective eggs (Powers et al., 1960; Batte & Moncol, 1972; Beer & Lean, 1973; Beer et al., 1973; Batte et al., 1977). On the other hand some authors registered negligible clinical signs and haematological changes following inoculation with 15,000 eggs (Beer et al., 1971; Beer et al., 1974). Apart from dose size the main determinant for the presence of clinical signs seems to be whether or not secondary microbial invasion occurs (Beer, 1973b; Mansfield & Urban, 1996) although there are most likely also differences in parasite strains. Symptoms seem to coincide with the emergence of the larvae from their histotrophic phase of development to form 3rd and 4th stage larvae (Batte et al., 1977). In this study body weights in the groups were comparable. However, pigs in this experiment were heavier at the time of inoculation, which makes direct comparison with other studies reporting decreases in weight gains difficult (Beer et al., 1973; Hale & Stewart, 1979). The results of the current study suggest that dose level influences the course of experimental *T. suis* infection in pigs in terms of parasitological parameters i.e. there exists a dynamic regulation of the adult *T. suis* burden, which could have important implications for the comparison of epidemiology and control of *T. trichiura* infection in humans. We observed an increase in worm aggregation with increasing dose, suggesting that persons with high worm burdens in a heavily contaminated environment might be especially important for the propagation and potential control measures of this parasitic infection.

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**REFERENCES**


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