# The effect of concurrent or sequential *Oesophagostomum dentatum* and *O. quadrispinulatum* infections on the worm burdens of the two species in pigs

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SUMMARY

The interaction between the 2 nodular worm species in the pig, *Oesophagostomum dentatum* (*O.d.*) and *Oesophagostomum quadrispinulatum* (*O.q.*), was studied by comparing the development and distribution of the species following single or mixed infections. The faecal egg excretion levels were assessed at regular intervals from week 3 post-inoculation, and indicated a strong negative impact of the introduction of *O.q.* on the continued egg excretion of *O.d.* All pigs were killed 9 weeks after the first inoculations to determine the composition and location of the worm burdens in the large intestine. *O.q.* was found more anteriorly located in the intestine than *O.d.*, thus confirming previous descriptions. When both species were present, the distribution of *O.d.* was moved further posteriorly and was more spread out than in single-species infections. There appeared to be no adverse effect of *O.d.* on the establishment and fecundity of *O.q.* However, the worm recoveries corroborated the egg excretion observations, namely reduced worm burdens of *O.d.* if *O.q.* was introduced, or if *O.q.* was already present. It is uncertain whether this effect is caused by differences in host reaction against the two species, or whether a more specific competition occurs between the two nodular worm species in pigs.

Key words: *Oesophagostomum dentatum*, *Oesophagostomum quadrispinulatum*, interaction, population dynamics, pig.

## INTRODUCTION

Two species of nodular worm, *Oesophagostomum quadrispinulatum* (Marcone, 1901 – in Alicata, 1935) and *Oesophagostomum dentatum* (Rudolphi, 1803), are common in pigs in many parts of the world (Urquhart *et al*. 1987), and are usually found to coexist in the same host individual, frequently with a predominance of *O. dentatum* (Nickel & Haupt, 1964; Roepstorff, 1986). Previous studies have demonstrated that *O. quadrispinulatum* is generally found more proximally in the large intestine than is *O. dentatum* (Nickel & Haupt, 1964; Jacobs, 1967; Kendall, Small & Phipps, 1977; Roepstorff, 1986). However, it is not known how the location of one species is affected by the other, when inoculations are simultaneous or when one species is introduced after the other is already present and well established in the intestine.

In sheep, the 2 species of *Oesophagostomum* commonly found, *O. columbianum* and *O. venulosum*, have been observed to interact such that the establishment of *O. venulosum* was reduced by up to 90% in sheep previously infected with *O. columbianum* (Dash, 1981). In addition, the distribution of *O. venulosum* was extended throughout the intestine when *O. columbianum* was present, whereas it was tightly distributed in the caecum and first part of the colon if present as a homologous infection.

For other genera of gastrointestinal helminths there have also been observed various forms of interactions, either on the distribution and size of the worms (e.g. negative effect of *Moniliformis dubius* on *Hymenolepis diminuta*, Holmes (1961)); or on the mutual reduction of worm burdens (e.g. adverse effects of either *Ostertagia ostertagi* or *Cooperia oncophora* on the establishment of the other species, Kloosterman, Albers & van den Brink, (1984)). The causes of such interactions might be based on immunological reactions (Kloosterman *et al*. 1984; Dobson & Barnes, 1995), or be related to physiological changes in the gastrointestinal environment (Dobson & Barnes, 1995), or be due to some other undefined factor (Christensen *et al*. 1987).

The present experiment was designed to describe the consequences of concurrent or sequential infections with the 2 *Oesophagostomum* spp. in pigs on the distribution and numbers of the worms in the large intestine.

## MATERIALS AND METHODS

### *Experimental animals*

Thirty-five cross-bred Danish Landrace/Yorkshire/Duroc pigs were allocated into 7 similar

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Table 1. Protocol for inoculation with infective 3rd-stage larvae ($L_3$) of either *Oesophagostomum dentatum* (*Od*) or *O. quadrispinulatum* (*Oq*)

(Each group consists of 5 pigs.)

| Group | $L_3$ dose/spp. at week 0 | $L_3$ dose/spp. at week 4 |
|---|---|---|
| A | 20000/*Oq* | 0 |
| B | 20000/*Oq* | 20000/*Od* |
| C | 0 | 20000/*Od* |
| D | 20000/*Od* | 0 |
| E | 20000/*Od* | 20000/*Oq* |
| F | 0 | 20000/*Oq* |
| G | 0 | 10000/*Oq* +10000/*Od* |

groups of 5 animals according to weight and sex. The pigs were derived from a farm established as helminth-free through repeated monitoring. The pigs were approximately 27 kg body weight at the start of the experiment. Throughout the study they were fed a diet of ground barley with protein supplement (Petkevicius *et al*. 1995) according to the Danish standard norms and had free access to water. The experiment was conducted at the experimental and breeding farm unit, Sjælland III, managed by the Federation of Danish Pig Producers and Slaughterhouses.

### *Parasites*

The strain of *O. dentatum* used was originally isolated in 1983 (Roepstorff, Bjørn & Nansen, 1987), and the strain of *O. quadrispinulatum* in 1992 (Dangolla, 1994). The infective 3rd-stage larvae ($L_3$) of both species were isolated by Baermannization from faecal cultures after cultivation for 14 days at room temperature, and subsequently stored at 10 °C at least 2–3 weeks for maturation.

All pigs were inoculated with $L_3$ by stomach tube.

### *Experimental design*

The 7 groups were infected according to the protocol listed in Table 1. In essence, the pigs were initially inoculated with 20000 $L_3$ of 1 of the species. Some of them served as controls for the establishment of that infection, while others were inoculated 4 weeks later with 20000 $L_3$ of the opposite species, and some others served as controls for the second infection. In 1 group (group G) the pigs were given a total dose of 20000 $L_3$ of both species (10000 $L_3$ of each) to assess the effect of simultaneous infection. Faecal samples were collected 1 week prior to inoculation, at inoculation, and at weekly intervals of the experiment. A modified McMaster technique was used with a sensitivity of 20 eggs/gram faeces. Eggs of the two species are morphologically indistinguishable (Haupt, 1966). Also 3rd-stage larvae are practically indistinguishable, therefore faecal larval cultures were not established.

All pigs were killed at an abattoir by bolt pistol and exsanguinated 9 weeks after the start of the experiment to determine worm numbers and location, and to perform species, stage and sex differentiation. The large intestine was divided into 8 parts (i.e. from the anterior end: caecum, colon 1 (10 % of the total length of colon), colon 2 (10 %), colon 3 (10 %), colon 4 (10 %), colon 5 (10 %), colon 6 (20 %), and colon 7 (30 %)) in order to study the location of the worm populations. Worms were isolated from 10 % of the intestinal contents by the agar-gel migration technique (Slotved *et al*. 1996), and preserved in 80 % ethanol until processed. Samples were then rehydrated before fixation, clearing in 85 % lactic acid, and subsequent differentiation of all the adults according to morphological characteristics (Haupt, 1966).

The $L_4$ larvae could not be distinguished readily according to species, and thus were counted into 1 category.

### *Statistical methods*

The location of worms was calculated as described by Petkevicius *et al*. (1995). The location in single-infected groups (A, C, D, and F) was tested by analysis of variance to determine the effect of worm species, day of inoculation and their interaction. The location, the proportion of female worms and the worm burdens were compared between groups using the Wilcoxon non-parametric test. All statistical analyses were performed using SAS Release 6.04 (Statistical Analysis Systems, 1990).

## RESULTS

The geometric mean faecal egg excretion patterns are illustrated in Fig. 1. It should be noted that egg excretion by *O. quadrispinulatum* was not detected until week 5–6 p.i. (group A–Fig. 1A) while eggs were detected in the faeces from most *O. dentatum*-infected pigs by week 3 p.i. (groups C, D, and E – Fig. 1B and C).

A decline in egg excretion levels was obvious in group E (inoculated with *O. dentatum* week 0 and *O. quadrispinulatum* week 4) by week 6 p.i., reaching zero by the end of the experiment (Fig. 1C). In contrast, faecal egg counts remained elevated in the group which received a single *O. dentatum* infection (group D–Fig. 1B). Egg excretions seen in group B (inoculated with *O. quadrispinulatum* week 0 and *O. dentatum* week 4) were very erratic, for each sampling date representing only 1 animal, which was different between samplings. In contrast, egg excretions

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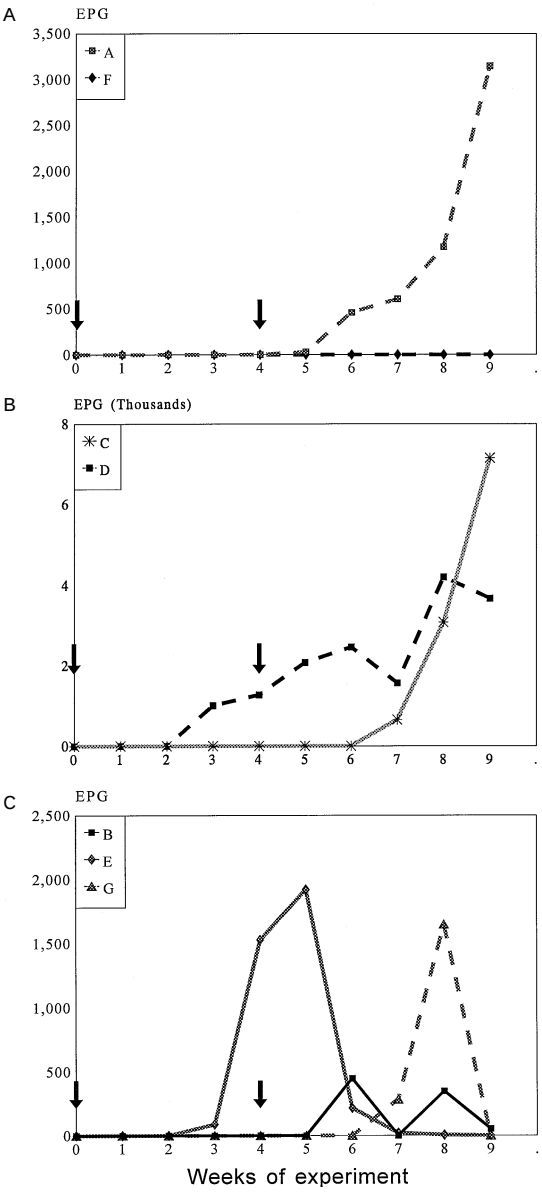


Fig. 1. Geometric mean faecal egg counts of pigs infected with *Oesophagostomum quadrispinulatum* (A), *O. dentatum* (B) or both species (C). See Table 1 for group designations and infection protocols. (↓) Indicates time of inoculations.

remained elevated in group A, which received an inoculation with *O. quadrispinulatum* on day 0 (Fig 1A and C). Finally, in group G the initial egg production may be due to *O. dentatum* with a possible subsequent negative effect of *O. quadrispinulatum*. Due to the rather late start of *O. quadrispinulatum* egg production (see group A and F) it is difficult to assess any negative effect of *O. dentatum* on *O. quadrispinulatum* in this regard.

At post-mortem examinations of the pigs, it was observed that *O. quadrispinulatum* had caused more severe damage (for descriptions compare, for example, Stockdale (1970)) and larger nodules in the caecal and colonic mucosa than *O. dentatum* when given at the same dosage level (no data shown).

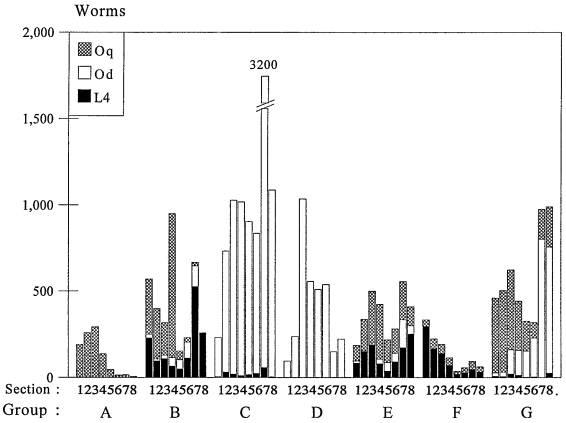


Fig. 2. Group arithmetic mean worm burdens for 8 sections of the large intestine. See Table 1 for group designations and inoculation protocols. Section 1: caecum, 2: colon (10 % of the length of the colon), 3: colon (10 %), 4: colon (10 %), 5: colon (10 %), 6: colon (10 %), 7: colon (20 %), and 8: colon (30 %). Oq = *Oesophagostomum quadrispinulatum*; Od = *O. dentatum*; L4: 4th-stage larvae of either species.

In Fig. 2 are illustrated the location and distribution of the different worm species according to their developmental stage ($L_4$ or adult worms). Several features should be noted: *O. quadrispinulatum* was positioned primarily in sections 1 through 4, whereas *O. dentatum* was located throughout the intestinal sections when present alone (groups C and D), but predominantly in the posterior sections when present together with *O. quadrispinulatum* (groups B, E, and G). *O. quadrispinulatum*, on the other hand, seemed unaffected by the presence of *O. dentatum*. These observations do not take into account the $L_4$ larvae, which were not differentiated according to species. It is noteworthy that following a mono-infection with *O. quadrispinulatum* there are approximately 72 % $L_4$ and 28 % adults (group F), whereas in group G, the simultaneous inoculation with both species gave rise to worm populations consisting of approximately 2 % $L_4$ and 98 % adults.

In no case did the challenge infection significantly affect the mean location of the primary infection, nor did the primary infection affect the mean location of the challenge, but in all cases the within-group variance was higher in the double-infected groups than in the single-infected group.

The day of inoculation influenced the location of the worms in the single-infected groups such that adult worms from an 'older' infection (week 0; *Od* – group D, *Oq* – group A) were more anteriorly located than those from a 'younger' infection (week 4; *Od* – group C, *Oq* – group F) ($P = 0{\cdot}0005$).

Most notably, the numbers of *O. dentatum* were significantly reduced in the groups which had received both species, but at different times (B versus C, and D versus E) ($P = 0{\cdot}02$). In contrast, the worm burdens of group G (which received 10000

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Table 2. Mean worm burdens

(See Table 1 for group designations and infection protocols. *Od* = *Oesophagostomum dentatum*; *Oq* = *O. quadrispinulatum*; M = males; F = females; M/F = male–female ratios; $L_4$ = 4th-stage larvae of either species. Numbers in parentheses are the standard deviations.)

| Group | $L_4$ | *Od* M | *Od* F | *Od* M/F | *Oq* M | *Oq* F | *Oq* M/F |
|---|---|---|---|---|---|---|---|
| A | 0 | 0·2 (0·4) | 0 | 0 | 448 (296) | 510 (342) | 0·9 |
| B | 1440 (931) | 194 (138) | 172 (96) | 1·1 | 432 (315) | 654 (468) | 0·7 |
| C | 174 (246) | 4512 (2311) | 4506 (2728) | 1·0 | 0 | 0 | 0 |
| D | 0 | 1964 (1710) | 1360 (1076) | 1·4 | 0 | 0 | 0 |
| E | 1058 (1391) | 178 (321) | 174 (295) | 1·0 | 674 (481) | 828 (592) | 0·8 |
| F | 802 (749) | 0 | 0 | 0 | 150 (95) | 156 (106) | 1·0 |
| G | 82 (105) | 996 (1850) | 1080 (2088) | 0·9 | 1012 (553) | 1264 (746) | 0·8 |

$L_3$ of each *O. dentatum* and *O. quadrispinulatum* at week 4) were equally represented by both species – and at a higher recovery rate than that seen in either group B or E. There did not appear to be any effect of *O. dentatum* on the presence of *O. quadrispinulatum*.

There were no significant differences between the groups in the proportions of female worms for each species, hence these are not depicted, but are listed in Table 2.

## DISCUSSION

In the present experiment, an impact of *O. quadrispinulatum* on the populations of *O. dentatum* was demonstrated when the two species were given to the same pigs at different times. This not only resulted in an influence on location but also on the overall intestinal worm burdens at the time of slaughter and the egg production in the case of group E. It is uncertain whether this could be attributed to a higher degree of host tissue reaction against *O. quadrispinulatum* (Schwartz, 1925; Spindler, 1933; Scheuermann, 1985). Equal dose levels were elected for both parasites, but it might have been better to use lower levels of *O. quadrispinulatum* than *O. dentatum*, considering the possible difference in pathogenicity observed between the two species. The overall recovery of *O. quadrispinulatum* was lower than that of *O. dentatum*. This phenomenon has previously been observed with the parasite strains presently employed (H. Bjørn, personal communication). Whether some of the inoculated larvae were harboured in the host intestinal wall tissue as immature stages was not examined. However, it is known from previous studies of single infections with *O. dentatum* that the number of tissue-dwelling $L_3$ may be related to the number of immature worms/$L_4$ present in the gut lumen (Christensen *et al.* 1995).

$L_4$ were present in 5-week-old (groups C and F) but not 9-week-old infections (groups A and D). It is conceivable that the $L_4$ present week 4 after infection could be prevented from developing further by a second infection, in which case $L_4$ in the mixed infection groups could be from either species.

O. quadrispinulatum was found to be more anteriorly located than *O. dentatum*, although with considerable overlap. This confirms previous observations (Nickel & Haupt, 1964; Jacobs, 1967; Roepstorff, 1986), which showed that in the caecum the number of *O. quadrispinulatum* was greater than that of *O. dentatum* – even when *O. dentatum* constituted a considerably higher proportion of the total worm population in the large intestine (Nickel & Haupt, 1964). It is possible that the rather substantial infection doses of 20000 $L_3$ caused the worm population locations to overlap to a high degree, and that the *O. dentatum* worms in the mono-specifically infected groups (C and D, inoculated week 4 and 0 p.i., respectively) would have been more clustered if they had been introduced at lower numbers, as observed by Christensen *et al.* (1995). The higher recovery of both species and the substantially increased proportion of adult worms in the group which received them simultaneously (group G) might be related to the halved dose level of each species, possibly below a threshold for the intensity of *O. quadrispinulatum* infections and their subsequent influence on the establishment of *O. dentatum*. However, egg production stopped in this group at the termination of the experiment, perhaps

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indicating even here a negative influence of *O. quadrispinulatum* on *O. dentatum*, as also suggested by the more posterior position (largely segment 7–8) of *O. dentatum*. The significantly lower proportion of $L_4$ in group G (simultaneous inoculation with both species) than in group F (mono-infection with *O. quadrispinulatum*) might, however, also be speculated to suggest that *O. dentatum* has a somewhat immunosuppressive effect on the host thus allowing the unhampered development of *O. quadrispinulatum*.

In comparison, the situation in sheep should be emphasized, where *O. columbianum* gave rise to such strong host reactions that *O.venulosum* was displaced and might be completely expelled from the common host in heterologous infections (Dash, 1981). However, any expulsion of *Oesophagostomum* spp. in pigs would probably be dependent on, for example, dose as well as frequency of infection, and it should be kept in mind that under natural conditions both *Oesophagostomum* species studied in this paper coexist in their host animals. It is well known from other host–parasite systems that a variety of interactions between two closely located helminth parasites may exist, spatial segregation being only one example (cf. Holmes, 1973).

In calves, a reciprocal negative interaction between *Ostertagia ostertagi* and *Cooperia oncophora* was found in sequential but not in concurrent infections (Kloosterman *et al*. 1984). The latter point might resemble the findings of the present experiment (group G) where the adult worm burden of the two species was markedly higher after concurrent infection than after sequential infections, especially *O. dentatum*. Also when studying the possible interaction between *O. circumcincta* and *Haemonchus contortus* in sheep, it was found that concurrent trickle infection with the two species caused only a small and insignificant reduction in the populations of each species by comparison with the single species equivalent (Dobson & Barnes, 1995). While a high level of immunity towards *H. contortus* following prolonged exposure afforded some cross-protection against *O. circumcincta*, it also appeared that the observed reduction of *H. contortus* establishment in the case of an existing *O. circumcincta* infection was due to a disrupted abomasal physiology rather than to actual immunity (Dobson & Barnes, 1995). As alluded to above, it is possible that a similar interaction exists between the two nodular worm species in the pig, and it has been suggested that most antagonistic interactions between parasites are induced by immunologically non-specific factors (Christensen *et al*. 1987).

It is not known what actually happens to incoming larvae when other parasites of the same or a closely related species are already present in the gut. Will the incoming larvae become established, and if so will they cause the pre-existing worms to be expelled? This was seen in calves infected daily with larvae of *O. ostertagi* (Michel, 1963, 1970); incoming larvae caused the displacement of older, established worms in favour of smaller worms, developing from the arrested $L_4$ larvae, with lesser reproductive capacity.

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