The impact of season and vegetation on the survival and development of *Oesophagostomum dentatum* larvae in pasture plots

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SUMMARY

Pats of pig faeces containing known numbers of *Oesophagostomum dentatum* eggs were placed on plots with bare soil, short or tall herbage on 8 occasions during 1 year. The number of eggs and larvae and the relative distribution of larvae in faeces, soil and herbage was monitored for 1 year after deposition. On 2 occasions soil from 8 selected plots was given to pigs, which were later slaughtered and examined for the presence of adult *O. dentatum*. Less than 1% of the deposited eggs could be recovered as infective larvae. The highest recoveries were generally made on tall herbage plots. The majority of infective larvae was found within the faecal pats, which indicates that infective *O. dentatum* larvae, to a large extent, do not disperse onto the herbage or into soil. The infective larval stage was reached only when the mean temperature in the weeks post-deposition was above 10 °C. This stage was reached within 1 week when the mean weekly temperature was above 13 °C. After the winter period no infective larvae could be recovered from any plots and no parasitic worms could be isolated from pigs fed soil from 8 selected plots.

Key words: *Oesophagostomum dentatum*, pre-parasitic stages, season, herbage, pasture.

INTRODUCTION

In Denmark industrialized indoor systems have completely dominated pig breeding during the last decades. In such systems nematode parasites can be controlled efficiently by treatment of the pigs with anthelmintics and by disinfection of the housing facilities. Recently, consumers have demanded higher welfare for domestic animals and husbandry systems with a minimum use of drugs and chemicals. This has led to an increasing number of free-range pigs, and in the case of organic herds to the abandonment of non-curative use of drugs. In such 'new' production systems knowledge of the ecology of pre-parasitic stages of pig helminths within the pasture environment is needed to design an effective parasite control strategy.

Moisture and temperature have previously been shown to be abiotic factors that directly influence survival and development of the free-living stages of *Oesophagostomum dentatum* (e.g. Alicata, 1935; Spindler, 1936; Rose & Small, 1980). Herbage can reduce the detrimental impact of the climate on larval survival, because faecal pats deposited below the herbage canopy experience diminished temperature fluctuations and evaporation. With time the herbage cover is modified by pig behaviour. Thus, tall herbage may cover a pasture when pigs are first introduced to the pasture or when stocking rate is low. Areas of shorter herbage will appear due to the grazing behaviour of nose-ringed pigs, while areas of bare soil will appear where rooting has occurred or when the stocking rate is high.

The present study was carried out to investigate the survival, development and spatial distribution of *O. dentatum* larvae, when eggs were deposited at different seasons of the year on plots imitating 3 pasture environments: bare soil, short herbage and tall herbage.

MATERIALS AND METHODS

Experimental principle

On each of 8 occasions from April 1996 to February 1997, portions of fresh pig faeces with known numbers of *O. dentatum* eggs were deposited on plots with bare soil (BS), short (SH) and tall herbage (TH). Faeces, herbage and soil were separately sampled and analysed for the numbers of free-living stages of *O. dentatum* at 1, 4 and 16 weeks post-deposition (p.d.), in the autumn 1996 and in the spring and autumn of 1997. On the 2 latter occasions groups of pigs were fed with soil from selected plots to bioassay the infectivity of the soil.

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Preparation of plots and faeces for deposition

A 11 × 11 m area situated adjacent to pig pastures at a research station of the Royal Veterinary and Agricultural University, 20 km west of Copenhagen, was used for the experiment. The soil of this area had a high organic content (approximately 70 % of dry weight). Eighty cm wide plywood sheets were placed vertically reaching from 60 cm below to 20 cm above ground level along the entire border line of the experimental area. This was done to protect the plots from burrowing mammals. A nylon net (mesh-width 5 cm), placed 2 m above ground level and at the sides, protected the whole area from birds while leaving the deposited faeces exposed to invertebrates and weather. Within this area 24 plots of 1 × 1 m were separated by 1 m wide paths of short herbage. One week before the first deposition, the vegetation and the top 5 cm of soil which contained a dense root-mat was removed from 8 randomly chosen plots (BS plots). Emerging seedlings were removed from the BS plots at 2–3 week intervals during the growth seasons. The herbage of the remaining plots was cut and kept by cutting to a height of 5 cm (SH plots) or 20 cm (TH plots) at intervals as above. Cut herbage was left on the herbage plots.

Pigs experimentally infected with *O. dentatum* (EH-strain, maintained by serial passage in helminth naive pigs (Roepstorff, Bjørn & Nansen, 1987)) were kept on a pasture close to the experimental area. These pigs were fed a diet of ground barley plus protein supplement, which is favourable for the establishment of *O. dentatum* in pigs (Petkevičius *et al*. 1996) as well as for larval development in faecal cultures (Petkevičius *et al*. 1998). Four days before each deposition the donor pigs were moved to individual cages at the nearby farm.These cages were constructed so that urine drained off to minimize faecal contamination. Faeces were collected twice daily and kept at 8 °C until the day of deposition, when 12 kg of the faeces containing the highest concentration of parasite eggs were mixed thoroughly. Portions of 50 g faeces with a length and diameter of approximately 7 cm and 3 cm, respectively, were prepared. In total, 72 portions were placed separately on 10 × 10 cm mini-plots located within a 80 × 90 cm central area of each of 3 plot types. Deposition of faeces took place on 23 April (D1), 28 May (D2), 2 July (D3), 30 July (D4), 27 August (D5), 24 September (D6) and 15 October 1996 (D7) and on 11 February of 1997 (D8).

Sampling

With the exception of 2 samplings being cancelled (D7, 16 weeks p.d. and D8, 1 week p.d.) and 1 being collected 2 weeks later than scheduled (D7, 6 weeks p.d.), samples were collected from 3 randomly selected mini-plots (replicates) of each plot type 1, 4 and 16 weeks after deposition. Furthermore, all plots on which depositions had been made were sampled on 22 October 1996, 22 April 1997 and 4 November 1997. To reduce any net loss of parasites due to horizontal transport, only samples from mini-plots that had unsampled mini-plots at all 4 sides were collected for analysis. Further, each mini-plot was only sampled once. Faeces, herbage and soil from each mini-plot were sampled separately. The herbage was cut 1 cm above the soil surface to avoid mixing with soil. Soil was sampled by pounding a 10 × 10 cm steel frame 10 cm into the ground and transferring the contents of the frame to a bucket. Soil was then mixed thoroughly and stones bigger than 0·5 cm were removed before the soil was weighed. A subsample of approximately 250 g of soil was retained for analysis while the remainder was poured back into the hole.

Laboratory procedures

The faecal portions were individually weighed and manually mixed thoroughly. A subsample of faeces was dried (105 °C for 24 h) to estimate the water content. For nematode egg counts, 4 g of faeces (or less if the total was < 5 g) were examined by a concentration McMaster flotation technique (Roepstorff & Nansen, 1999). This technique was further modified by soaking the sample in tap water overnight and by repeating the post-soaking procedure on the faecal matter retained during the filtration step of the original method. The technique has a lower detection level of 20 eggs per gramme (e.p.g.). At low egg counts larger volumes of the faecal suspension were counted, thus yielding a detection level of 3 e.p.g.

Larvae were isolated from faeces and herbage by a modification of the agar technique described by Mwegoha & Jørgensen (1977). A suspension of 5 g faeces in 30 ml of water (room temperature) was mixed with 30 ml of 2 % agar solution (40 °C) and spread onto half of a cotton cloth (Johnson and Johnson cloth, Code Nr. P45282). After the agar–faeces mixture had stiffened, the cloth was incubated in tap water in a glass sedimentation tube (diameter 5 cm) overnight at room temperature. The sediment was then transferred to test tubes and kept at 5 °C until microscopical identification and counting of *O. dentatum* larvae took place.

Herbage samples were weighed and cut into 1 cm pieces. Sixty ml of water was used for rinsing the sample bag and the water was then mixed with the herbage. If more than 10 g of herbage was present in the sample, a 10 g representative subsample was mixed with an amount of rinse water corresponding to the proportion of the subsample and tap water was added to 60 ml. This mixture was then mixed with 60 ml of 2 % agar solution (40 °C) and spread out on a cotton cloth. When the agar–herbage mixture had

set, the cloth was cut in two and each half was suspended in a separate sedimentation tube and handled as described above.

A modification of the method described by Baermann (1917) was used for enumeration of larvae in soil. Subsamples of 25 g of soil were spread onto a single layer of Kleenex tissue on a plastic sieve (diameter 15·5 cm, mesh-width 3 mm). The sieve with soil was placed in a plastic box and tap water was added so that it just reached the soil. The box was then sealed with a lid. After 24 h at room temperature the sieve with soil was removed. The water in the box was poured into a sedimentation tube and left for 18 h, before the sediment was collected in a plastic tube and stored at 5 °C until microscopical examination.

Infectivity of soil

On the sampling occasions of 22 April 1997 and 4 November 1997 the total $10 \times 10 \times 10$ cm soil volumes from the BS and TH mini-plots of depositions D1, D4, D7 and D8 were brought to the laboratory. The soil was mixed thoroughly and a subsample was put aside for enumeration of larvae as described above. Equal amounts of soil from each of the 3 replicate mini-plots were then combined, mixed thoroughly and sieved (mesh-width 3 mm) to remove large particles. On each of 2 successive days portions of 75 g of soil from 1 of the selected plots were administered to each of 4 helminth-naive pigs (2 males and 2 females of cross-bred Landrace × Yorkshire × Duroc of 20–30 kg body weight). The soil was suspended in lukewarm tap water and administered to the pigs by a stomach tube. The pigs (64 in total) were stabled in helminth-free pens and fed the above mentioned diet. They were slaughtered 39–44 days after administration of soil. At slaughter the large intestine was opened, washed and a representative 20% subsample of the intestinal contents was sieved (212 μm mesh-width).The collected matter on the sieve was fixed with iodine (80 g iodine, 400 g potassium iodine, 800 ml of distilled water) and later analysed on a light board for the presence of *O. dentatum*.

Climate

Data of mean daily soil temperature at 10 cm depth and mean maximum and minimum daily air temperatures 2 m above soil surface, as well as precipitation data were recorded at the experimental farm approximately 500 m from the plot area.

Calculations and statistics

A non-parametric Kruskal–Wallis analysis was used to compare larval recoveries between plot types. A Scheirer–Ray–Hare extended Kruskal–Wallis analysis was used to compare the water contents of faeces between plot types and deposition date. Non-parametric tests with only 3 replicate observations in each of 3 treatments as in the present study do not allow detailed pairwise comparisons, except in the case of the treatments with the highest and the lowest sum of ranks (Fowler & Cohen, 1992).

RESULTS

The temperature and precipitation of the experimental period are represented in Fig. 2A and B. Precipitation in June–October 1996 was only 53% of the normal. The average temperatures in December–January were 2·4 °C below normal. The mean temperatures of August 1996 and 1997 were 2·4 and 4·0 °C above the average temperature of that month, respectively (Danmarks Statistik, 1997).

Plot type and date of sampling had a highly significant ($P < 0{\cdot}001$) influence on the water content of the soil. Bare soil plots had the lowest water content on 12 out of 22 sampling occasions with SH and TH plots having the lowest water content at 5 occasions each. The lowest water content registered during the entire period was found in a BS plot. Similarly, plot type ($P < 0{\cdot}05$) as well as deposition date ($P < 0{\cdot}01$) had a significant influence on the water content of the faecal pats 1, 4 and 16 weeks after deposition. In general the faeces on the bare soil plots had the lowest water content (Fig. 2).

No eggs were recovered later than 1 week post-deposit (p.d.) from any plot type of the depositions made in April (D1) and from the end of July (D4–D8) or in the bare soil (BS) and short herbage (SH) plot of the May deposition (D2). Eggs were recovered 4 weeks p.d. in the tall herbage (TH) plot of May and in all plot types of the early July deposition (D3).

No infective larvae were recovered from any plots after the winter 1996–1997 (Fig. 1) and no infective larvae were recovered at all from D1, D7 and D8, i.e. when faeces were deposited in April, October and February (Fig. 1). With the exception of 6 larvae found 16 weeks p.d. in the faecal subsamples from 1 SH mini-plot of D2 (deposited late May), infective larvae were only found in the TH plot of D2. More eggs and pre-infective larvae were present in the TH plot than in the BS plot 1 week p.d. ($P < 0{\cdot}01$) of this deposition.

Infective larvae appeared 1 week p.d. in all plot types of D3, D4 and D5, i.e. in the early and late July and late August depositions, when the weekly mean temperature was above 13 °C. The recoveries of infective larvae 4 weeks p.d. from D3 plots (deposited early July) were higher than from any other sampling or deposition (0·6, 0·5 and 0·4% of the deposited number of eggs in the TH, SH and BS plots, respectively). The sum of eggs, pre-infective and infective larvae was higher on the TH plot than

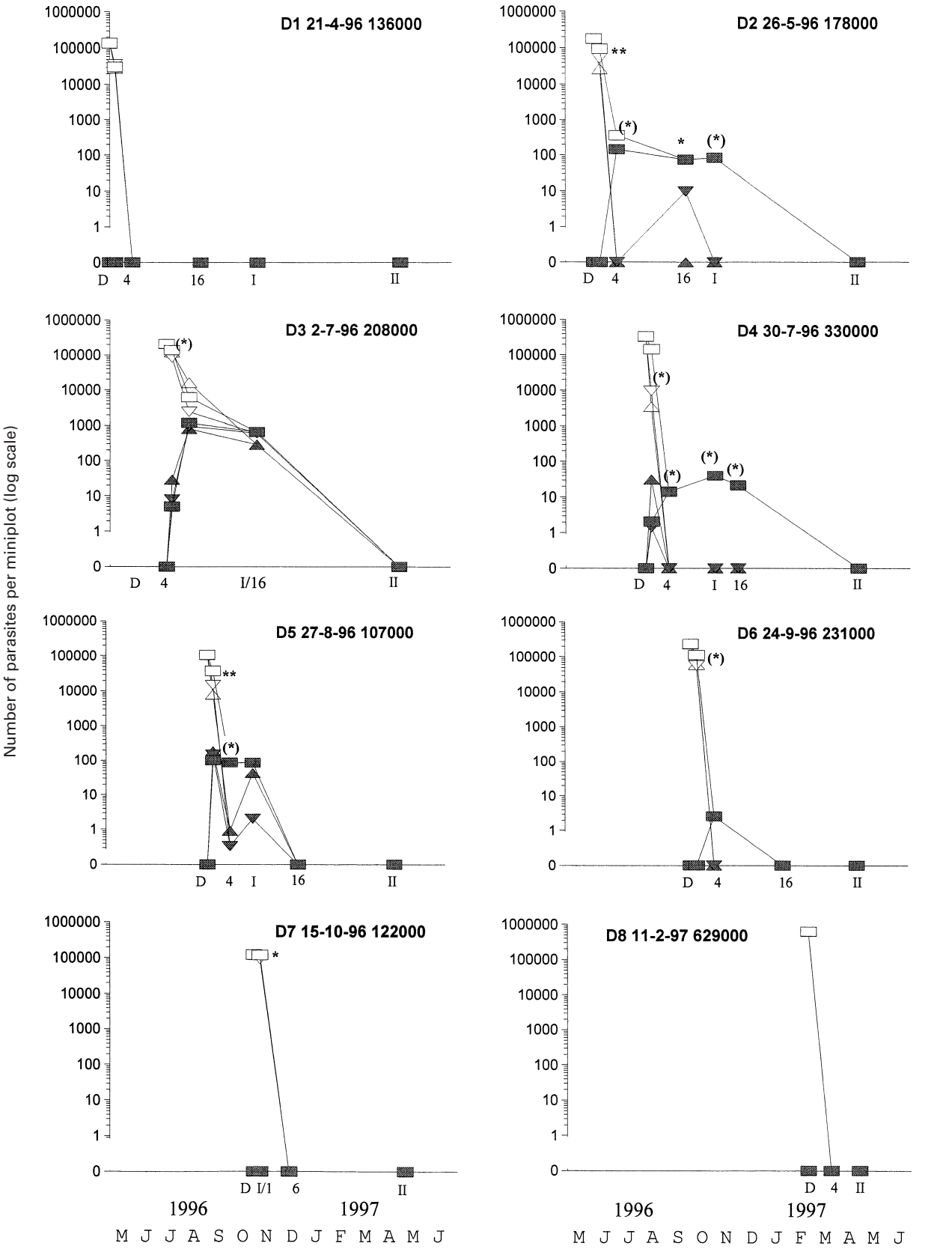

Fig. 1. Survival and development of *Oesophagostomum dentatum* free-living stages in plots with bare soil (▲), short herbage (▼) and tall herbage (■). Open symbols connected by thin lines represent the mean total number of eggs, pre-infective and infective larvae, shaded symbols and thick lines represent the mean total number of infective larvae. Time runs from April 1996 to June 1997 with samplings indicated by D (date of deposition), 4 (4 weeks p.d.), 16 (16

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on the SH plots 1 week p.d. of D3 ($P < 0{\cdot}1$). The total number of free-living stages recovered was also higher for the TH plot of D4 ($P < 0{\cdot}1$) and D5 ($P < 0{\cdot}01$) than for the respective BS plots 1 week p.d. More infective larvae were recovered from the TH plot than from the other plots of D4 at 4, 12 and 16 weeks p.d. ($P < 0{\cdot}1$). Also more infective larvae were recovered from the TH plot than from the SH plot 4 weeks p.d. of D5 ($P < 0{\cdot}1$). Only 1 infective larva (in faeces of one mini-plot 4 weeks p.d.) was found in the D6 plots (deposited in September). More eggs ($P < 0{\cdot}1$) were recovered from the TH plot compared to the SH plot 1 week p.d. in D6. One week p.d. more eggs were recovered from the faeces of the BS plot than from the SH plot of D7 ($P < 0{\cdot}05$).

Pre-infective larvae were only found in the faecal subsamples at 1 week p.d. of D2–D5 (deposited May–August), and more pre-infective larvae were present in the TH plot of D4 and SH plot of D5 than in other plots of the respective depositions (Table 1). More infective larvae were in general recovered from faeces than from soil or herbage (Table 1). In total, infective larvae were encountered in 68 mini-plots (or 53 when bare soil mini-plots are excluded). These were found in faeces of 64, in soil of 11 and in herbage of 9 mini-plots. In only 2 cases were more than 5% of the total number of infective larvae within a mini-plot found on the herbage. In 1 of these 2 cases (D5, SH, 4 weeks p.d.) the single specimen present within the mini-plot was found on the herbage, in the other (D5, TH, 1 week p.d.) 15 infective larvae were recovered from herbage while only pre-infective larvae were present in the faeces.

No *O. dentatum* worms were recovered from any pigs that had been given soil from the spring sampling 1997, while 1 specimen was recovered from the large intestine of each of 2 pigs that had been administered soil from the BS plot of D1 in the autumn sampling 1997.

DISCUSSION

In conclusion, tall herbage plots had the highest recoveries of infective *O. dentatum* larvae, the recoveries never exceeded 0·6%, the majority of infective larvae was found to stay in the pig faeces and that neither eggs nor larvae seemed to survive a normal Danish winter.

Development of *O. dentatum* does not take place at 5 °C (Roepstorff, 1986) and low temperatures ($\leqslant 4$ °C) have been shown to be lethal to eggs and pre-infective larvae (Rose & Small, 1980). At 10 °C the development to the infective stage has been observed to take at least 28 days (Rose & Small, 1980). Thus, the seasonal differences in larval development found in the present study were expected, since the daily mean maximum temperatures of the winter months, November–March, were below 7 °C in Denmark (Danmarks Statistik, 1997). However, larvae did not develop when faeces was deposited in April (D1), even though daily maximum temperatures exceeded 10 °C. Thus, the low minimum temperatures following deposition D1 may have been responsible for the complete elimination of the parasite from these plots.

Dessication has previously been shown to destroy eggs (Spindler, 1936) and pre-infective larvae (Rose & Small, 1980) of *O. dentatum*. With regards to the infective larvae the results are somewhat inconclusive (Goodey, 1924; Alicata, 1935; Spindler, 1936; Rose & Small, 1980). Rose & Small (1981) ascribed higher recoveries of *O. dentatum* from long herbage plots compared to short herbage plots to better protection against dessication by long herbage. The faeces in TH plots were generally more moist than in the other plot types, suggesting that the better survival of *O. dentatum* in the TH plots was related to moisture. The shadow provided by tall herbage may also have increased survival by keeping the temperatures in the faeces at sublethal levels. A temperature of more than 50 °C has been recorded in faeces exposed to the sunlight on a Danish pasture with short grass (Larsen & Roepstorff, 1999). It has been found that more than 99% of *Oesophagostomum* spp. ova were killed within 1 h of exposure to 55 °C (Burden & Ginnivan, 1978). The higher maximum temperatures which may be reached in sun-exposed faeces on BS and SH plots may on the other hand be responsible for a faster development and thus the higher numbers of infective larvae recovered 1 week p.d. on the BS plots of the summer depositions D3, D4 and D5 and on the SH plots of D3 and D5 compared to the TH plots.

Recovery of infective *O. dentatum* infective larvae was always less than 1% of the number of deposited eggs. The design employed in this study compensates for possible horizontal transport and with the sample depth of 10 cm it is unlikely that vertical transport could be responsible for the low recovery. It should be noted that surplus faeces from depositions D2–D8 stored in the laboratory showed normal development of *O. dentatum* eggs to the

weeks p.d.), I (22 October 1996), II (22 April 1997). Symbols placed on the x-axis indicate that no parasites were found on that day of collection. The deposition identity (D1–D8), the date of deposition (day-month-year) and the mean number of *O. dentatum* eggs deposited onto a mini-plot are shown in each diagram. Significant differences in total numbers of parasites between plot types at the respective days of collection are indicated with asterisks. (*)$P \leqslant 0{\cdot}1$; *$P \leqslant 0{\cdot}05$; **$P \leqslant 0{\cdot}01$.

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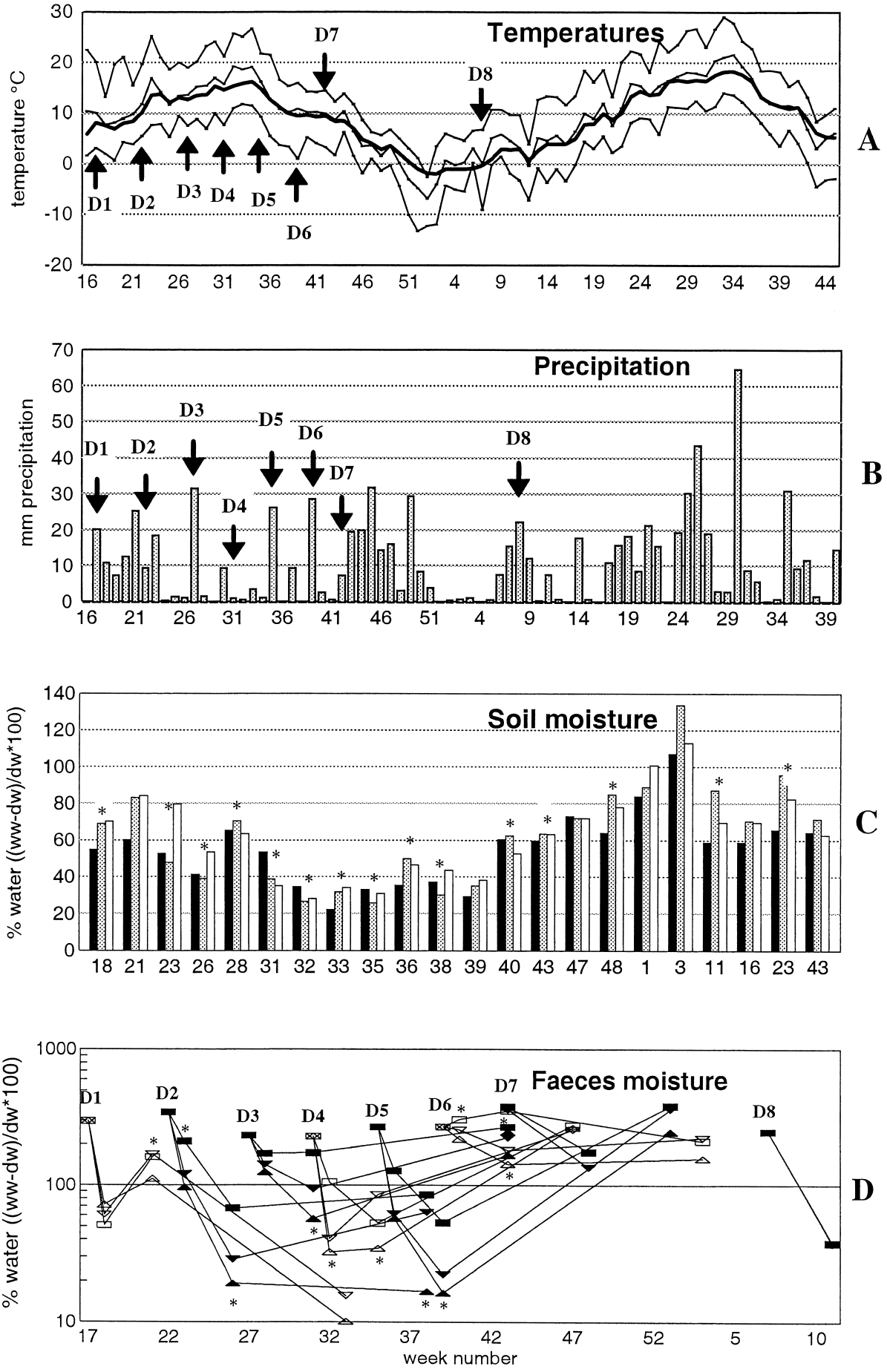

Fig. 2. For legend see opposite.

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infective stage. Low recoveries (<1 %) of infective *O. dentatum* larvae have previously been found on grass plots in areas adjacent to those of the present study (Larsen, 1996), thus supporting our results.

Freezing tolerance and longevity are the most important characters potentially enabling *O. dentatum* to overwinter. Laboratory studies have shown that infective *O. dentatum* larvae are tolerant to temperatures below 0 °C (Alicata, 1935; Spindler, 1936), and infective larvae kept in laboratory cultures 'appeared normal' after more than 10 months (Alicata, 1935). Previous plot studies have shown that *O. dentatum* larvae may overwinter (Rose & Small, 1980, 1981; Ferber, 1988; Haupt, 1969). But, while the 2 former studies were performed in climates without long periods of frost, an insulating snow layer was reported in the latter. The absence of *O. dentatum* from all samples collected in 1997 and from the large intestine of all pigs that had been administered soil in the spring 1997 suggests that the parasite cannot survive on pasture during a Danish winter. Since *O. dentatum* worms had never previously been recovered from any sample of the D1 plots the finding of a total of 2 specimens in the pigs inoculated with the D1 soil from the autumn 1997 sampling i.e. 18 months after deposition, were most likely due to contamination. In a simultaneous study conducted at areas adjacent to the present plots, none of 50 helminth-free pigs excreted *O. dentatum* eggs 6 weeks post-turn-out (in May 1997) onto pastures that had been contaminated the previous summer and autumn (Mejer, Thomsen & Wendt, 1998). Similar observations have been made in Canada (Smith, 1979). In the present study frozen soil with a high ice content was observed to a depth of more than 10 cm during the winter, which may account for the mortality. In a study conducted in Denmark, Roepstorff & Murrell (1997) found few overwintering infective *O. dentatum* after a winter with temperatures comparable to those of the present study. The survival observed in the latter study may have been caused by the presence of habitats for survival such as deeper frost-free soil layers or uninsulated pig houses. Thus, although our results indicate that *O. dentatum* larvae die out during a winter with frozen soil, the range of environments investigated in this study may not cover all winter refuges of the parasite.

Movement of the infective larvae into the soil would not only offer protection against frost but also against dessication, since soil offers an environment of high relative humidity (> 95 %) even below the wilting point of plants (Brady & Weill, 1996). Such movement would not leave them out of the reach of rooting pigs. Goodey (1924) deduced positive geotropism from observed lack of negative geotropism; however, no support of such movement has been found elsewhere in the literature. No massive movement of *O. dentatum* towards the soil environment was observed in the present study. In fact fewer parasites were, in general, recovered from the soil than from faeces. Movement of parasites into the soil may be caused by agents not included in our plot study, e.g. by the rooting behaviour of pigs. To mimic this situation Larsen (1996) compared the development of *O. dentatum* in plots where faeces were deposited on top of and below the soil surface. Although more larvae were recovered from the subsurface plots than from the surface plots of that study no larvae were recovered following the first winter. This negative result may have been caused by the depth where faeces were placed (2 cm soil above faecal portion) being too shallow to avoid freezing.

Defecation areas of feeder pigs potentially get large inputs of parasite eggs (Mejer *et al.* 1998). However, even in the late summer no infective *O. dentatum* larvae were recorded within defecation areas of infected pigs (Roepstorff, unpublished results). Urine from the pigs may have acted as a lethal factor, as it has been shown to have an ovistatically or ovicidal effect on *Ascaris suum* (Nilsson, 1982).

The findings of this study suggest that the life-strategy of *O. dentatum* does not include a stay on the herbage as is the case with some ruminant parasites (e.g. *Cooperia* spp. (Anderson, 1992)). While such a strategy supposedly is a great advantage for infective larvae of helminths of grazing animals, this is not the case with pig parasites, since pigs display rooting behaviour.

In order to increase the utilization of nitrogen for crops and to reduce leaching into ground water, pasture rotation is likely to be included in future management of outdoor pigs. Our results indicate that an appropriate rotation scheme may be able to eliminate *O. dentatum* from contaminated pastures due to its poor ability to survive temperate winters. This is in marked contrast to the high survival rate of *Ascaris suum* and *Trichuris suis* eggs (Larsen &

Fig. 2. Temperature, precipitation and moisture during the experimental period. (A) Weekly mean, minimum and maximum temperatures 2 metres above the ground (thin lines) and 10 cm depth below the soil surface (thick line). (B) Weekly precipitation. (C) Water content of the soil at the days of collection (black bars: bare soil; grey bars: short herbage; white bars: tall herbage). (D) Water content of faecal pats at the days of collection. Symbols show the water content of the faeces on the respective plots (▲ or△ bare soil, ▼ or ▽ short herbage, ■ or □ tall herbage. Open and shaded symbols indicate different depositions). * Significant differences ($P \leqslant 0{\cdot}05$) in the moisture content of faeces or soil between plot types.

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Table 1. Spatial distribution of the free-living stages of *Oesophagostomum denatum* within miniplots, shown as mean total numbers of larvae present in faeces, soil or herbage of a miniplot

(Larvae were only recovered from the depositions D2–D6. Since only 1 L3 was recovered from faeces subsamples of D6 plots, data from this deposition are not shown. BS, bare soil; SH, short herbage; TH, tall herbage; wpd, weeks post-deposit. The date of deposition and the total number of eggs deposited on each miniplot is given in parentheses after the deposition identity. L1 and L2 larvae were only observed 1 week post-deposition and only in faecal pats.)

			D2 (26-5-96, 178000)				D3 (2-7-96, 208000)				D4 (30-7-96, 330000)				D5 (27-8-96, 107000)			
Sampling	Sample type	Parasite stage	Weeks p.d.	BS	SH	TH	Weeks p.d.	BS	SH	TH	Weeks p.d.	BS	SH	TH	Weeks p.d.	BS	SH	TH
1 week p.d.	Faeces	L1+2	1	570	330	320	1	41000	60000	36000	1	38	120	4400	1	4000	7200	3500
	Faeces	L3		0	0	0		420	210	120		1	1	2		190	150	95
	Soil	L3		0	0	0		14	0	0		30	0	0		0	0	0
	Herbage	L3		—	0	0		—	0	2		—	0	0		—	0	6
4 weeks p.d.	Faeces	L3	4	0	0	140	4	790	960	1200	4	0	0	14	4	1	0	77
	Soil	L3		0	0	0		13	0	0		0	0	0		0	0	9
	Herbage	L3		—	0	0		—	0	21		—	0	0		—	<1	0
16 weeks p.d.	Faeces	L3	16	0	10	63	16	270	600	600	16	0	0	21	16	0	0	0
	Soil	L3		0	0	9		23	0	59		0	0	0		0	0	0
	Herbage	L3		—	0	0		—	0	1		—	0	0		—	0	0
Autumn	Faeces	L3	21	0	0	82	16	270	600	600	12	0	0	40	8	29	2	10
	Soil	L3		0	0	0		23	0	59		0	0	0		14	0	76
	Herbage	L3		—	0	<1		—	0	1		—	0	<1		—	0	0
Spring	Faeces	L3	56	0	—	—	51	0	—	0	47	0	0	0	43	0	—	—
	Soil	L3		0	0	0		0	0	0		0	0	0		0	0	0
	Herbage	L3		—	0	0		—	0	0		—	0	0		—	0	0

Roepstorff, 1999). Practical studies of these and other management procedures are needed to provide insight, which future management can be based on.

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