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The Origin and Overwintering Survival of the Free Living Stages of Cattle Parasites in Sweden

By *S. O. Dimander*[1], *J. Höglund*[1] and *P. J. Waller*[2]

[1]Department of Parasitology (SWEPAR), Swedish University of Agricultural Sciences, and [2]National Veterinary Institute, Uppsala, Sweden.

Dimander SO, Höglund J, Waller PJ: The origin and overwintering survival of the free living stages of cattle parasites in Sweden. Acta vet. Scand. 1999, 40, 221-230. – During the 1997 Swedish grazing season, faeces were collected every 3 weeks on 7 occasions from young grazing cattle with moderate nematode parasite infections. From this source 12, 400 g dung pats were set up on each sampling occasion on a specially designated area of pasture. Half of these pats were placed on pasture where it was aimed to prevent snow cover during the subsequent winter. During the grazing season, herbage growth was kept at reasonably uniform height by clipping and the dung pats were protected from destruction by animals and birds. At the time of animal turn-out the following year (7th April 1998), it was observed that all dung pats had disappeared. Assessments of the survival of infective larvae, both on pasture and in soil, were made in a circular area encompassing the location of each pat. These sampling procedures were completed within a 3 week period. All faecal deposits yielded infective larvae at turn-out the following year, with proportionally greater numbers developing from nematode eggs deposited in cattle dung during the mid third of the previous grazing season. The surface layer of soil was found to be an important reservoir for infective larvae, with numbers recovered being approximately half those found in the overlying pasture samples. No significant differences were found between the normal pasture and snow excluded pasture in the number of infective larvae recovered from both pasture and soil samples. The epidemiological consequences of these findings are discussed.

Ecology; cattle nematodes; infective larvae; pasture; soil; Cooperia oncophora.

Introduction

There is considerable evidence to suggest that under European conditions, a direct relationship exists between the severity of winter and the survival of the free-living stages of cattle parasites on pasture. In other words, the more severe the winter, in terms of amount and persistence of snow cover, then the more successful is the survival of the free-living stages. Early studies on the ecology of the free-living stages of cattle parasites in the United Kingdom (*Rose* 1961, 1962) showed that only small numbers survived on pasture over winter. However, these larvae were important in providing a source of a new generation of worms in young cattle, whose faecal egg counts were, in turn, responsible for the seasonal peak in larval numbers on pasture around mid-late summer. Subsequent studies by a number of workers in the United Kingdom indicated that this was invariably the pattern, which led *Michel* (1969) to develop the evasive worm control strategies for cattle that are still widely recommended today throughout most of Europe (*Barger* 1997, *Eysker et al.* 1998).

Although the same phenomena seem to prevail in Denmark (*Nielsen & Nansen* 1970, *Nansen et al.* 1987, 1988), it was found many years ago that there were important differences to this ecological pattern of overwinter larval survival of cattle nematodes in the more northerly Scandinavian countries. Rather than low overwinter survival, the numbers of larvae on pasture in early spring could induce clinical parasitic disease and even mortality in young cattle within the first month following turn-out. This was first reported by *Tharaldsen* (1970) in Norway. Comparison between the occurrence of such clinical episodes and the prevailing meteorological data over several years led her to conclude that high larval numbers at the commencement of the grazing season correlated with early presence and continuous, long-term persistence of snow cover on pasture (*Tharaldsen* 1976, *Helle & Tharaldsen* 1976). The protective effect of snow was also attributed to the high levels of overwintered larval survival in Sweden (*Nilsson & Sorelius* 1973) and Canada (*Smith & Archibald* 1969).

The origin of overwintered bovine infective larvae in Scandinavia is open to some speculation. The pattern of faecal egg counts of young cattle often show that these rise dramatically in the middle, but fall towards the end of the grazing season (*Nilsson & Sorelius* 1973, *Tharaldsen* 1976). However, there does not seem to be a consistent correlation between levels of pasture contamination in late summer/early autumn and the number of infective larvae present at turn-out during the following spring (*Tharaldsen & Helle* 1984). The only ecological investigation on the dynamics of the free-living stages of cattle parasites in Scandinavia was conducted in Sweden by *Persson* (1974). In his work, he deposited relatively large amounts of faeces, derived from artificially infected pen reared calves, onto small pasture plots each month for 2 years. Subsequently, he made sequential estimates of the number of viable eggs and larvae in the remaining faeces and on pasture. One of the most important findings of his work was the prolonged survival of infective larvae which were derived from dung deposited some 12-18 months previously, albeit in very small numbers. He reported that snow fell during winter in both years of his study, but this was not quantified and no estimates of the numbers of parasitic larvae in soil were made.

In the study outlined below, estimates were made of the relative contribution to the number of infective larvae present at the time of turn-out, derived from faeces of young grazing cattle and deposited at regular intervals throughout the entire grazing period of the previous year. Also, the importance of soil as a reservoir for infective larvae was estimated. An attempt was also made to ascertain the effect of snow cover on infective larval survival in residual faeces, pasture and soil.

Materials and methods

Source of Contamination

Faeces were obtained from 20, parasitised young cattle grazing on native pasture at the Kungsängen research farms of the Swedish University of Agricultural Sciences, Uppsala, during the grazing season of 1997. To ensure a reasonable faecal egg count in these animals, they were each given a priming dose of approximately 12 000 infective larvae (predominately *Cooperia oncophora*) 3 weeks prior to turn-out. These larvae were derived from cultured faeces which were obtained from young cattle raised on an organic farm in the Uppsala region, in February/March 1997. Animals were gathered and individual faecal samples were collected from the rectum every 3 weeks on 7 occasions, from 5th June until 7th October 1997. On each occasion, faeces from the donor calves were pooled, thoroughly mixed and then 4 estimates

of the number of nematode eggs/g faeces (epg) were made. Twelve, 400 g artificial dung pats were then prepared in aluminium pie dishes.

Experimental Site

An area of improved pasture, approximately 12 m×6 m, which had not been grazed by cattle the previous year was fenced and netted to exclude all vertebrate animals and birds. The pasture was cut to a height of approximately 5 cm prior to the first faecal pat deposition and all cuttings were removed. A 2 m buffer zone between areas designated as either "snow excluded" or "normal" pasture and a 1 m buffer zone around the entire periphery of the experimental area was created by clipping the pasture down to almost soil level. Six artificial dung pats were then evenly placed, 1 m apart and in 2 rows, on each of the 2 pasture types. All pats were marked with a garden stake placed immediately adjacent and to the north of each pat for the purposes of identifying their location because of their expected disappearance. Depositions were made from 5th June and every 3 weeks until 7th October 1997. During the year the grass was periodically clipped and on areas around where faecal depositions were made the clippings were allowed to remain, whereas they were removed from the areas which had not been used. The buffer zones were also mown at the same time. Hourly temperatures were continuously recorded at both soil surface and at 1 m above the pasture sward, from the time of initial pat deposition until the time of pasture/soil sampling in April 1998. Rainfall was recorded from an adjacent site and also a record of the occasions on which snow fell and remained present on the ground was made. On 1st November, before any falls of snow were recorded, an "A" frame covering made from horticultural shade cloth (mesh dimensions 5 mm) was erected over the series of dung pats that were designated as "snow excluded".

Sampling Procedure

At the commencement of sampling, it was observed that all pats had disappeared, except for a very small amount of desiccated faecal material (<10 g), derived from some of the pats deposited on the last contamination period (7th October 1997). From 7th April 1998, sampling of the soil and pasture commenced. On each occasion, soil and pasture were sampled from 6 deposition areas where dung pats were placed, comprising one of the six replicates from three different deposition occasions for both the "snow excluded" and the "normal" pasture. This procedure commenced with the area where the pats were last deposited in 1997, working towards the area where the first deposits were made. The whole sampling procedure was completed within 3 weeks. A 28 cm metal ring was placed immediately over the area where the pats were deposited, and all herbage was cut and collected in individually marked plastic bags. After this procedure, soil to a depth of approximately 1 cm was scraped from the same area and placed into separate plastic bags. Wet weights of herbage were recorded and larval recovery was achieved by thorough washing, sieving and sedimentation, which generally accorded to the procedures outlined by *Persson* (1974). Soil samples were weighed, 10 g sub-samples were taken for dry matter estimations and the remainder (generally 200-250 g) was processed according to *Yeates et al.* (1997) for the recovery of nematodes. The recovered nematodes from pasture and soil samples were concentrated into 10 and 20 ml volumes of water, respectively. From each sample, replicate 1 ml sub-samples were taken and stained with iodine before examination under a compound microscope to identify and differentiate between parasitic and saprophytic nematode larvae.

Statistical Analysis

Excel 5.0 (Microsoft) was used in data summary and descriptive analysis of the results. The effect of snow cover on larval numbers recovered from pasture samples for the different deposition dates was assessed by the Mann-Whitney U test for pair wise comparison. The Kruskal-Wallis test was applied to evaluate the differences in pooled data for pasture larval recovery with respect to date of deposition in relation to faecal egg counts. Statistical analysis was performed using StatView® 4.5 (Abacus Concepts) and differences considered significant at the $p<0.05$ level.

Results

Meteorological Data

Mean monthly precipitation and temperatures recorded throughout the experimental period are shown in Fig. 1, in comparison with the 30-year long-term averages (LTA).

Apart from the higher rainfall than the LTA for May and June 1997, the precipitation throughout the experimental period was remarkably similar to the LTA. Snow was first recorded on 24th November 1997, and there was an average snow cover on pasture of approximately 6 cm for the following 2 weeks. During this time, snow was successfully prevented from covering the "snow excluded" dung pats. Heaviest falls of snow were recorded in January 1998, which were responsible for the high level of precipitation recorded during this month. Blizzards occurred on the 24th and 30th January, causing deep snow drifts over the experimental site and total inundation even of the snow exclusion area. More than 25 cm snow covered the entire experimental site for more than one week and persisted to a lesser depth for a further week. Therefore it should be noted that from 24th January, all dung pats were essentially exposed to the same environmental conditions under snow.

Air temperatures during the experimental period were warmer during mid–summer than for the LTA and warmer conditions also were recorded during January/February 1998, the period when the greatest amount of snow fell. However, it should be noted that all dung pats were covered by a substantial layer of snow during this latter period.

Nematode Egg Counts of Faecal Deposits

The faecal egg counts of each source of faeces were not constant, which is the typical seasonal pattern for young grazing cattle. The estimated pooled faecal collections for each 3-weekly sampling occasion were 13, 288, 225, 138, 50, 113, 213 epg in chronological sequence. Cultures of faeces made on each occasion showed that the majority (>80%) of infective larvae were *C. oncophora.*

Infective Larval Recovery – Pasture

Comparisons between the number of infective larvae recovered from pasture, where attempts were made to exclude snow, and from normal pasture are shown in Table 1.

These showed no consistent significant differences, so the larval recovery data from pasture samples were pooled for both pasture types. The pasture larval recovery for each time of faecal deposition (n = 12) was expressed in terms of area, herbage wet weight and as a percentage of the egg count of the faecal deposits. These are shown in Table 2.

The numbers of larvae recovered on an area basis were found to be proportionally similar to the numbers expressed on a herbage wet weight basis, indicating that the pasture was of similar height and density over the entire experimental area. Larvae were recovered from all periods of dung pat deposition. The highest absolute numbers were derived from pasture on which faecal pats were deposited on 15th July the previous year. The highest number of larvae recovered as

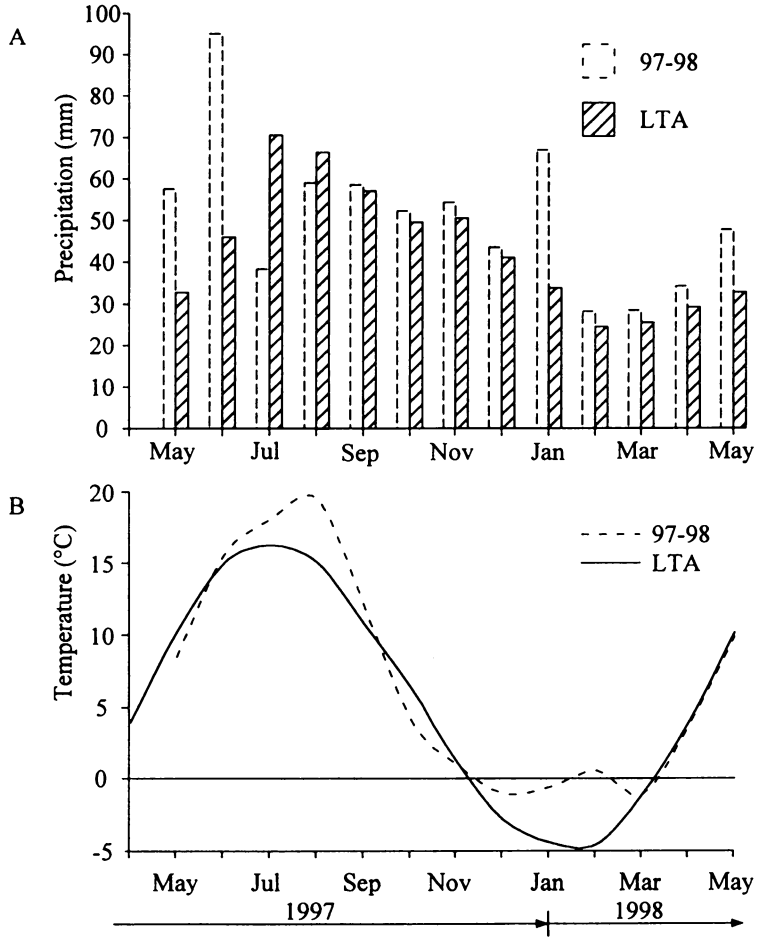

Figure 1. Meteorological data of experimental site. Comparison between 1997-1998 and Long Term Average (LTA 30 year). A. LTA precipitation (solid bars) compared with precipitation during the trial period (broken bars). B. LTA temperature (solid line) compared with temperature during the trial period (broken line).

a percentage of the egg count of faeces ($p<0.0001$), was from dung deposited on 3 occasions which spanned the summer period (15th July–26th August). However, a surprisingly high percentage of infective larvae in relation to faecal egg count (0.08%) was also recovered from pasture samples collected from sites where dung pats were first deposited on the 5th June in the previous season.

Infective Larval Recovery – Soil

Similar to pasture samples, infective nematode larvae were recovered from all soil samples, which were taken directly beneath the same area from where the pasture samples were collected (see Table 3).

The partitioning of infective nematode distribution between pasture and soil showed that less larvae were recovered in the soil. The number

Table 1. Infective trichostrongylid larvae of cattle recovered from herbage samples in early spring 1998 derived from faecal deposits (n = 6) placed on either pasture excluded from snow during the winter or normal pasture the previous season.

Faecal deposition	Herbage L_3/m^2				Pairwise comparison (snow excluded vs normal)
	Snow excluded		Normal		
1997	Mean	Range	Mean	Range	Probability
5th June	121	0-242	13	0-81	0.10
24th June	1062	81-1532	417	81-565	0.63
15thJuly	1761	806-2742	3360	968-3871	0.52
5th Aug	1223	565-2177	551	161-1210	0.47
26th Aug	1355	403-2823	1113	403-2258	0.06
16th Sept	65	0-161	215	0-565	0.16
7th Oct	16	0-81	16	0-81	0.76

Table 2. Infective trichostrongylid larvae of cattle recovered from pasture samples in early spring 1998, derived from uniform faecal pats (n = 12) regularly deposited the previous grazing season.

Faecal deposition 1997	Area L_3/m^2		Herbage L_3/kg		Recovery L_3/epg faeces (%)	
	Mean	Range	Mean	Range	Mean	Range
5th June	67	0-242	73	0-268	0.08	0.0-0.30
24th June	739	81-1532	1331	114-3493	0.04	0.0-0.08
15th July	2560	806-3871	6051	1543-20413	0.18	0.06-0.49
5th Aug	887	161-2177	2370	439-6338	0.10	0.02-0.25
26th Aug	1234	403-2823	2857	383-6434	0.38	0.13-0.88
16th Sept	140	0-565	313	0-1331	0.02	0.0-0.08
7th Oct	16	0-81	40	0-220	<0.01	0.0-0.01

Table 3. Infective trichostrongylid larvae of cattle recovered from soil samples in early spring 1998 obtained below uniform faecal pats (n = 12) deposited the previous grazing season.

Faecal deposition 1997	Area L_3/m^2		Herbage L_3/kg		Recovery L_3/epg faeces (%)	
	Mean	Range	Mean	Range	Mean	Range
5th June	54	0-161	28	0-88	0.07	0.0-0.30
24th June	215	0-806	112	0-396	0.01	0.0-0.04
15th July	914	161-2419	510	91-855	0.06	0.01-0.17
5th Aug	417	0-968	218	0-561	0.05	0.0-0.11
26th Aug	423	0-1371	227	0-665	0.13	0.0-0.43
16th Sept	87	0-242	53	0-121	0.01	0.0-0.03
7th Oct	10	0-645	70	0-545	0.01	0.0-0.05

of infective larvae recovered from soil samples was, on average, approximately half the number found on overlying pasture samples (for comparison see Tables 2 and 3). The only departure from this was the much larger proportion of infective larvae found in soil samples which originated from faecal deposits placed on pasture on 7th October 1997, being the last occasion for the year. However, it should be noted that the total larval recovery for this last faecal deposition occasion was relatively small.

Discussion

This study on the origin and the overwinter survival of the free-living stages of nematode parasites of cattle on pasture under Swedish conditions provides new information with important epidemiological consequences. The results show that faecal contamination throughout the entire grazing season may contribute to the infective larvae that are available on pasture around the time of turn-out of cattle in the following year. There is no indication that contamination in the latter part of the grazing season is relatively more important. In fact, our data suggests the opposite. Namely, that contamination in the first half of the season yields numbers of infective larvae that are as high, or even higher, than those derived from cattle grazing pasture after mid-summer.

Nematode faecal egg counts of cattle are dynamic, and the invariable pattern for young animals in northern Scandinavia is to have their highest faecal egg counts early in the grazing season, usually from mid–late summer (*Nilsson & Sorelius* 1973, *Helle & Tharaldsen* 1976). Our study shows that the transmission potential (development of eggs through to infective larvae), is greatest for pasture contamination during this time of the year. Therefore, in absolute terms, the majority of larvae available around the time of animal turn-out is likely to be derived from this early contamination of the previous year. The faecal egg count trajectories of donor calves used in our study follow the epidemiological pattern described above, which is in contrast to the pen reared donor calves used by *Persson* (1974), where he reported that egg counts remained virtually constant throughout his study. As a consequence, the larval availability patterns reported in his work would have little relevance to the epidemiological pattern of cattle parasite infections in Sweden, although they do indicate the capacity for prolonged survival of free-living stages on pasture.

Our study also indicates the importance of the upper layer of soil as a reservoir for infective larvae. Approximately half the number of larvae found in pasture samples was also found in the top 1 cm of soil, taken directly below the area which supported the pasture growth. This is a surprising finding, as it is generally believed that few infective larvae are found in soil, which is largely due to the early pronouncements to this effect (for example, see *Levine et al.* 1974). Few studies have been subsequently carried out to confirm, or deny, the ubiquity of this finding. However, this may be due to the difficulty and tedium of dealing with nematode recovery and differentiation from soil samples. To obtain efficient nematode recovery from soil samples, there has to be a departure from the normal Baermann procedures which are familiar to most veterinary parasitologists. We followed the technique described by *Yeates et al.* (1997), which was developed to obtain high recovery rates of soil nematodes. These, of course, yielded enormous numbers of soil nematodes ($\simeq 0.5 - 1.5 \times 10^6/m^2$), which had to be differentiated from infective larvae of parasites. However, with training and careful attention to detail, this did not prove to be a difficult task.

The presence of large numbers of infective larvae in the upper layer of soil in our study is, intuitively, not difficult to reconcile. The origin of

the infective larvae were the dung pats deposited during the previous season and all these pats had disappeared from the pasture sward/soil surface at the time of sampling. Although the translation of eggs to infective larvae and their subsequent migration from dung pats to pasture would have occurred more-or-less continuously throughout 1997, disappearance of the dung pats from pasture would also be going on at the same time. This would be attributed to physical breakdown of the dung by rainfall and also the biotic activity of microbes, arthropods and earthworms. In the cool, moist conditions that typify the grazing season in Scandinavia, earthworms play an important and often dominating role in the structural decomposition and disappearance of cattle dung (*Holter* 1979, 1983). Infective larvae of cattle parasites have been shown to survive passage through the gut of earthworms (*Grønvold* 1987) and almost certainly some of these would have been voided in worm casts in the upper layers of the soil. The importance of this soil reservoir of infective larvae needs to be resolved. Because of the essentially random movement of infective larvae in moisture films, it is certain that this soil reservoir would contribute to the number of larvae available on pasture during the normal grazing season when adequate rainfall occurs.

It could be argued that not all the infective larvae which originated from each discrete dung pat were recovered in this investigation. Our pasture and soil sampling was restricted to a 28 cm circular area, centred on the middle of where each dung pat was deposited. Many studies have been conducted on the extent of lateral migration of infective larvae from dung pats. Although larvae have been recovered as far as 40 cm from dung pats, the overwhelming number is found within 15 cm from the centre, or 5 cm from the edge, of artificial dung pats (for review, see *Stromberg* 1997). An additional physical factor influencing the dispersal of infective larvae is rain splash, which on exposed dung pats can disperse larvae considerable distances (*Grønvold* 1984, *Holter* 1977). However, in our study, pasture growth was rapid as evidenced by the need to regularly trim the sward and although the average normal rainfall occurred during the trial, there were never any excessively heavy showers. Dung pats in our study would soon have been shielded from such physical effects as heavy rain. Irrespective of the fact that some larvae would have been missed, the sampling procedure for both pasture and soil is internally consistent for this study, and our interests were in the comparative number of infective larvae found in both sites for faecal pats deposited over the entire grazing season.

Unfortunately, we were unable to ascertain the effect of extended snow cover on the survival of the free-living stages of nematode parasites of cattle during this trial. Apart from the blizzard in January that even inundated the snow exclusion barrier, snow falls were infrequent and persistence of snow cover was only transient throughout winter. Consequently there was little difference between the dung pats experiencing the pasture micro-environment on either the designated "snow excluded" area or the normal pasture. Intuitively, it would seem obvious that free-living stages of parasites could better withstand constant micro-environmental conditions, albeit cold, than repeated freezing and thawing. Alternatively, or in addition, it is possible that the northern Scandinavian parasites of cattle have evolved to withstand harsher environmental conditions of winter in the free-living stages than those parasite strains found in more southerly locations. These 2 factors will be the subject of our further investigations into the ecology of the free-living stages of cattle parasites in Sweden.

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Sammanfattning
Övervintringsförmågan hos pre-parasitära stadier av gastrointestinala trichostrongylida nematoder hos nötkreatur i Sverige.

Under betessäsongen 1997 insamlades träck vid 7 tillfällen med 3 veckors intervall från 20 förstagångsbetande nötkreatur. Samtliga kalvar infekterades före betessläppningen med ca 12 000 larver, huvudsakligen *Cooperia oncophora.* Vid varje provtagningstillfälle räknades antalet ägg per gram faeces och tolv

identiska konstgjorda komockor à 400 g preparerades. Dessa placerades sedan enligt ett förutbestämt mönster på betesmark, markerades med bambukäppar och skyddades mot fågelangrepp. Gräset klipptes regelbundet och hölls på en enhetlig höjd (5-10 cm). Under vinterperioden (november–mars) förhindrades snöfall på hälften av mockorna. Vid tidpunkten för betessläpp den 7:e april följande år noterades att samtliga träckhögar var fullständigt nedbrutna. Antalet överlevande infektiösa larver uppskattades från en cirkulär yta (diameter 28 cm) centrerad över platsen för den utplacerade träcken, i såväl gräs- som jordprover. Resultaten visar att all träck som deponerades under betessäsongen genererade infektiösa larver till tidpunkten för betessläppning efterföljande år. Det högsta antalet larver, uttryckt både i absoluta och relativa tal, härrörde från träck som deponerades under den inledande betesperioden i juli. Flest larver påträffades i gräs/förnaskiktet men en betydande andel återfanns i det översta jordskiktet. Inga statistiskt säkerställda skillnader noterades mellan antalet infektiösa larver, vare sig från gräs eller jordproverna från den normala respektive snöfria betesytan.

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Reprints may be obtained from: S. O. Dimander, Department of Parasitology (SWEPAR), Swedish University of Agricultural Sciences, Box 7073, SE-750 07 Uppsala, Sweden.