# What is the effect of dung beetles on gastrointestinal nematodes of stock?

**Prepared for the Technical Advisory Group meeting, 18th September 2012, by Simon V. Fowler, Landcare Research, New Zealand**

## 1. Summary

1/ This review was undertaken because parasitologists have expressed concerns that dung beetles may increase levels of gastrointestinal nematodes in stock in New Zealand because; i) dung burial may increase survival of nematodes compared to dung remaining on the pasture surface; ii) large numbers of infective larvae could then migrate back to the surface in damp soils; iii) overseas studies cannot be used to inform risk in New Zealand because of differences, particularly in climate.

2/ The review found that most overseas studies comparing dung in the presence or absence of dung beetles reported substantial reductions in the number of infective 3rd instar larvae of gastrointestinal nematodes at the soil surface or on pasture foliage.

3/ The break-up of dung on the surface by dung beetles can result in death of the desiccation-intolerant 1st /2nd instar nematode larvae, although greater access to oxygen in broken-up dung can lead to a higher proportion of nematode eggs hatching compared to dung not exposed to beetles.

4/ In most cases, burial of dung by dung beetles appeared to destroy a high proportion of nematodes, presumably by the processing of dung by the adult beetles. One study showed that recently buried dung brood balls contained 99.5% less nematode larvae than undisturbed dung pats remaining on the surface.

5/ Nematode larvae are capable, given moist enough conditions, of migrating back to the surface from dung buried to 10 cm or sometimes deeper. However, even shallow burial is likely to provide a hurdle for larval migration when soils do not have high moisture contents, and this hurdle effectively increases with increased burial depth. Experiments where dung was buried near to artificial barriers often reported larval migration from depths of 15–20 cm. However, the barriers may be providing continuous films of moisture encouraging larval migration so need to be interpreted with caution.

6/ The concern that burial of dung by dung beetles could lead to a ‘time bomb’ effect where later migration to the surface could increase infection of grazing stock, compared with dung remaining intact on the surface, is a possibility that was not supported by the majority of studies.

7/ Many overseas field studies of the effect of dung beetle activities were conducted in warmer, more tropical environments than are found in New Zealand, however several key studies do include periods when the climate match to summers in warmer parts of New Zealand was reasonable. Other trials were laboratory based and were operated at temperatures that were not excessive compared to conditions in New Zealand. A few burial trials were conducted outside in temperate areas that were more directly comparable to most of New Zealand, but none used dung beetles. Overall, the effect of climate on the interaction between dung beetles, gastrointestinal nematodes and stock re-infection rates are complex, but some overseas studies can be used to predict the likely effect of dung beetles in at least the warmer regions/seasons in New Zealand.

8/ More research could be carried out to provide additional information for assessment. However this is highly unlikely to alter the overall conclusion that in most weather conditions/regions of New Zealand dung beetle activity will either reduce (or make little difference to) infective larval nematode numbers available to re-infect stock. It is thus highly unlikely that the action of dung beetles in New Zealand will increase infection rates of gastrointestinal nematodes of stock.

**2. Introduction**

The global prevailing view is that dung beetle activity reduces survival of eggs and free-living larvae of parasitic nematodes, and hence reduces the re-infection rate of stock. However the following quotes from Vlassoff et al (2001) sum up the issues raised by parasitologists in New Zealand:

*"results of studies in New Zealand and overseas have been variable; some beetles reduced larval numbers while others actually increased them."*

*"a risk of dung burial is that remaining viable eggs and larvae may be protected from climatic extremes and ultraviolet radiation which could enhance their survival"*

*"if large numbers of L3 are able to survive in soil during periods of drought ...* (then this could be) *a potential explanation for the rapid increase in pasture larval counts observed after the first significant rains in late summer/autumn in some years"*

Also in the same review series, Hein et al (2001) commented that:

"*Dung burial may not always lead to a reduction in pasture larval burdens. Persistence of soil moisture in the vicinity of faecal pats may cancel out the reductions achieved in surface larval numbers and actually encourage later larval migration onto herbage (Bryan 1973).*"

The same concerns were raised in Australia before dung beetles were introduced, and were later considered as minor (Durie in Hughes et al 1975). Durie also gives a useful account of how nematodes develop in dung and how dung beetle activity could affect this (Hughes et al 1975).

This review has attempted to access all the literature on the effects of dung beetles on the nematodes that can infect stock as free-living 3$^{rd}$ instar larvae on pasture. Where relevant, studies that artificially buried dung and measured the effects of this on nematodes have also been included. Studies are summarised in Table 1 and, where necessary, discussed in more details in the supplementary material in Appendix 1. This review then addresses the concern that the results of overseas studies cannot be extrapolated to New Zealand because of differences in climate.

This review does not cover effects of drenches on the activities of dung beetles. These have been extensively researched, and clearly can have an impact on the possible benefits to be gained from dung beetle activity in pastoral grazing systems (http://dungbeetle.org.nz/management-practices/).

There are two activities of dung beetles relevant to the potential suppression (or otherwise) of nematode re-infection rates in stock: firstly the feeding/processing of dung on the surface and the resulting break-up and possible desiccation of the fresh pat; secondly the burial of dung by beetles.

| Author | Animal dung used | Nematode numbers after dung burial/dispersal | Evidence larval migration from buried dung (+/- increased longevity of larvae)? | Location, pasture moisture or rainfall information | Comments |
|---|---|---|---|---|---|
| Bergstrom et al 1976 | Cattle & sheep | 10–20 *Aphodius* and *Canthon* spp beetles exposed to dung for 1–5 days reduced nematode eggs by 24–90% | n/a | Lab expt on moist filter paper. 20°C. | |
| Bergstrom 1983 | Elk | 1–2 *Aphodius* beetles per 1g of faeces over 4–36h reduced larval numbers by 77–92% | n/a | Lab expt: temp 4–24 C (night – day) on moist filter paper | Low numbers of beetles to simulate numbers seen in the field in the Tetons, USA |
| Bryan 1973 | Cattle | 48–93% less on foliage | Yes, but later peak small | SE Queensland, trial partly irrigated. Apr–Jun temperatures similar to northern NZ summer. | See detailed discussion in Appendix 1 |
| Bryan 1976 | Cattle | 40–74% less on foliage, massively less in dung. 99.5% reduction in larval numbers in brood balls cf surface dung unattacked by beetles. | Yes, but later peak small | SE Queensland, soil moisture high at start then declining. Temperature as for Bryan 1973 | See detailed discussion in Appendix 1. Brood ball data is unique but the massive reduction in larval numbers was not pointed out by the author. |
| Bryan & Kerr 1989 | Cattle | Infective larvae on grass reduced by 57% (with minimal beetle activity), 90% (moderate beetle activity) and 94% (intensive beetle activity) compared with intact pats | No | Central Queensland. 780mm rainfall/year. Warmer than NZ. | Study ran from 1975 to 1979 |
| Chirico et al 2003 | Cattle | Dung dwelling beetles initially increased L3 numbers in artificial pats (90% RH) over 12 days, but after another 12 days numbers were much higher in control pats. Overall, L3 numbers were 2.4x higher in dung without beetles. | n/a | Indoors – 90% RH and 21°C | Increase after 12 days attributed to increased aeration. Decrease after 24 days could be due to beetle feeding, dung desiccation or competition with saprophytic nematodes (arriving on beetles), but either way beetles overall decreased L3 numbers |
| Coldham 2011 | Cattle | Expts carried out in buckets and misted daily with 5 ml of water. Artificial dung burial resulted in 21x the numbers of nematodes in surface soil cf dung on surface. In contrast, beetle burial appeared to destroy nematodes. Where surface dung was kept wet then nematode numbers were similar from buried and surface dung treatments. Where surface dung was not subjected to artificial rain, nematode numbers were reduced by 86%. | Yes – for artificially buried dung (15 cm deep). No for dung buried by beetles (as no nematodes appeared to survive). | Indoors parts of trials at 25°C. Northern Tablelands, NSW. Annual rainfall 650–1200+ mm (mostly in summer). Cool summers (rarely > 32°C) .Winter snowfalls & frequent frosts (to -10°C) | Burial rates were low: *Onthophagus australis* (50%), *O. granulatus* (20%), *O. gazella* (30%) Artificially buried dung given optimal conditions for nematode larvae to migrate back to surface (pots stood in water to guarantee soil was wet). Sampling by extracting from top 2 cm of soil (no foliage in pots) |
| English 1979 | Horse | 60% reductions in strongylids on grass clippings from *O. gazella* in summer period, but beetle activity curtailed by heavy rain or cooler temperatures. | No sign of effect in data (no later peak in dung with beetles). However, author assumed migration was occurring as soil moisture levels were high for entire period. | Southern Queensland. Rainfall (av. 1158 mm/yr - described as regular and prolonged for much of study plus some periods of heavy rain noted (e.g. 288 mm in Feb1976) | *Onthophagus granulatus* (native to Australia) in large numbers but failed to disperse dung. Introduced *O. gazella* much more effective but only in summer. Most nematode infection of horses occurs in spring/auturm, so additional species needed if dung beetles to be effective. |

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|---|---|---|---|---|---|
| Fincher 1973 | Cattle | 23–54% less in cattle; 75–93% less on foliage (comparing natural dung beetle levels or enhanced dung beetle levels with a dung beetle exclusion treatment) | If present, not enough to interfere with reductions in larval numbers in presence of beetles | Coastal Georgia, USA. 1174mm/yr - spread evenly. Summer mean temperature warm (July mean max 32°C) | No replication |
| Fincher 1975 | Cattle | 55–89% less in cattle (treatments as above) | As above | As above | No replication |
| Fincher & Stewart 1979 | Cattle | Variable numbers of L3 on grass with dung burial 2.5–10 cm (but no surface check, 1 rep/depth) Replicated glass tube expt – L3: 89% (2.5cm) and 95% (10cm) reductions cf surface dung. | Yes. In tube expt, numbers from surface dung peaked at 3–4 wks, much lower numbers from buried dung peaked at 3–8 wks. | As above. 37 cm rainfall during the 16 week experiment. Glass tube expt indoors at 27°C, 95% RH. | Comments that glass tubes may have facilitated migration of larvae from relatively deeply buried dung because there would be a continuous film of moisture on the surface of the glass (hence why some larvae could migrate from depths of 10–15 cm). |
| GrØnvold 1987 | Cattle | Earthworms (not dung beetles) excluded from pats. 50% decrease in *Cooperia* L3 over late summer to late spring. | Perhaps a very minor peak the following spring, but still about 60% lower than controls. | Denmark. Substantial rainfall in all months. | |
| GrØnvold et al 1992 | Cattle | With dung beetles: 88% less larvae in cow pats ($P<0.01$); 28% less in 0–6 cm soil (NS); 70% higher in soil 6–12 cm (NS). 70-90% less by rain splash. | No (controls without beetles had same no. larvae in soil as treatments where beetles buried dung – duration 33 days) | Zimbabwe, >800mm/yr. Warmer than NZ: average monthly temperature from 13°C (June) to 20°C (Nov) | |
| Houston et al 1984a | Horse | Mixed results over weekly sampling (4 dates). Treatments with beetles (22 or 44 pairs) usually higher than controls (no beetles) but differences not statistically significant. | They claim emergence from buried dung but I don't think this is supported by the data to any great extent. | Texas. Very low rainfall until 40 mm in one day between sampling periods 3 and 4 (by which time ¾ of control dung had been removed!). 5 mm water added after 2$^{nd}$ sample but not likely to be enough to penetrate *intact* pats. Warmer and drier than NZ. | Foliage and dung samples mixed. Authors thought more L3 might have emerged from controls if trial had run longer (but all dung sampled by then!). Slightly odd expt design as control treatment ended up with ¾ of dung removed by sampling period 3–4, whereas in beetle treatments the dung was dispersed/buried, so less of it would have been removed for sampling. Makes data hard to interpret. |
| Houston et al 1984b | Horse | Very few L3 migrated onto foliage from burial depths >5cm (although 1 L3 did emerge from dung buried to 20 cm) | Emergence from buried dung, but not really any later peak from more deeply buried dung. | Watered to saturation once dung buried. Then sufficient water provided for plant growth. College Station, Texas. As above. | |
| Hutchinson et al 1989 | Horse | More larvae in faeces protected from beetles (numbers per gram – see comments on same trial in Mfitilodze & Hutchinson 1988). Larval yields on grass samples were higher with protected faeces, but differences often not large and varied by year. | | Tropical Queensland. Warmer than NZ. Well-defined summer wet season. | Larval migration to grass only occurred in summer after rains had started (Jan–May). |
| Krecek & Murrell 1988 | Cattle | n/a | *Ostertagia* appeared capable of migrating 15 cm into soil and then returning to surface | Beltsville (nr Washington), USA. Most of trial in Oct/Nov (mean max 21°C, mean min 10°C). Rainfall 200mm in Oct/Nov. | 30 cm diam. steel cylinders to 5-15 cm: provided an easy pathway for larvae as suggested by Fincher & Stewart (1979) for glass tubes? High rainfall fits with last high no. larvae outside 15 cm cylinder. |

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| Lucker 1936 | Horse | Percentage recoveries in surface soil of buried larvae ranged from 0.02–21% (at 2.5cm), 0–3.9% (at 5–6cm), 0.004–1.9% (at 7.5cm) 0–1.2% (at 10–15cm). Some unquantified recovery from 20 cm burial of horse dung in coarse sand. A range of soil types used, with reduced recovery from heavy clay soils. | Yes, but substantial migration to surface only with shallow burial | "Precipitation invariably occurred" in field trials. In laboratory/covered trials soil was kept moist. Beltsville (nr Washington, DC). Mean monthly max 5–31°C, min -5–19, avg annual rain 1110mm. More continental climate than NZ. | |
| Lucker 1938 | Horse | Percentage recoveries in surface soil from dung burial in sandy clay loam, sandy loam or coarse sand were 0.07–8.9% (7–9cm), 0.0009–3.5% (15–16 cm), 0.0009–0.9% (20–21 cm), 0.0–0.4% (25–27 cm). Zero recoveries from clay soil. | Yes. Evidence of ~week delay in larval recoveries with deep dung, but recoveries nearly always higher for shallow burial at all sample dates. | Laboratory study. "Soils were kept moist at practically all times … more conducive to the migration of the infective larvae than would occur ordinarily in a similar period in the field" | Both Lucker 1936 and Lucker 1938 indicate that "lighter soils, such as fine sandy loam, are most favourable for vertical migration of horse strongyle larvae." Dung beetles typically will bury to the deep end of their depth burial range in light soils. |
| Mfitilodze & Hutchinson 1988 | Horse | Dung exposed to beetles for 7 days had 20–80% less infective larvae compared with beetle excluded dung. Very variable across seasons so concluded that not epidemiologically significant. | Larvae measured in surface dung not on grass/soil | See Hutchinson et al 1989 | Hutchinson et al 1989 more important as it uses the same experiment over longer time frame and includes grass samples. L3 numbers in the dung turns out to not really matter. However, using larvae/gram ignores probable large reduction in total wet weight of beetle affected pats in 7 days ("faecal pellets were usually completely broken down to a flattened mass of fibrous debris in 24h"). |
| Popay & Marshall 1996 | Sheep | *Onthophagus posticus* and *O. granulatus* mostly reduced larval numbers in dung. (although 1 result showing significant reverse effect). | No: *O. posticus* increased larval no.s in soil, but no significant increase on foliage | NZ. Ruakura. | Little dung buried/removed. Not clear why *O. granulatus* did so little burial (nb this species ineffective even at high numbers in English (1979). Foliage sampling was only done on single dates for each trial, so no tracking through time. |
| Reinecke 1960 | Cattle | Dung beetle activity encouraged nematode eggs to hatch but larvae desiccated. Dung beetles reduced nematode larvae in pats by 99%, and no increase in larvae in the soil beneath pads. In wet trials, beetles reduced larvae on foliage by 87%. In dry trials no significant difference +/- beetles. | | NW Cape, South Africa "semi-arid". Cool dry winters and hot wet summers. 'Wet trials' – mean max 24–31°C, mean min 11–17°C, mean daily rainfall 2–15mm (annual equivalent = 1750mm) | The quantitative results presented here uses the original data from Reinecke (1960) but re-analyses it (details in Appendix 1) |
| Waghorn et al 2002 | Sheep | Buried dung increased nematode numbers on foliage in trial 2 (by 88%) but no increase in trial 1. Greater total numbers in pots from buried dung in both trials. | Yes, but burial only 5cm (no evidence on longevity) | NZ. Rainfall 90 mm (over 28 days), 103 mm (over 34 days) | Manual dung burial to 5 cm, so no dung beetle activity (breaking up/processing dung). Sampling only on one date so no tracking through time. Note contrast to Coldham (2011) result above where artificial burial led to survival and migration of larvae whereas burial by dung beetles didn't. |
| Waterhouse 1974 | Cattle | 48–93% reduction in larvae on pasture | Not mentioned | Not mentioned | Just summarising studies by Bryan (1973, 1976) etc |

Table 1. Summarised information from studies of dung beetle activity or dung burial and nematodes used in this review.

## 3. Break-up of dung on the surface and processing/feeding by dung beetles

### *3.1 Break-up and desiccation of dung*

Dung beetles break up the dung on the surface in the process of feeding or creating brood balls. Six studies show substantial reductions in nematode levels in dung exposed to beetles compared with dung unexposed to beetles (Bergstrom et al 1976; Bergstrom 1983; Bryan 1976; Chirico et al 2003; Grønvold et al 1992; Popay & Marshall 1996; Reinecke 1960). However if the broken-up dung on the pasture surface remains wet enough, and/or conditions are warm enough to result in very rapid development of the desiccation-resistant 3rd instar larvae, then surface dung could maintain high levels of nematodes despite some activity by beetles (Coldham 2011; Hutchinson et al 1989). One study of dung-dwelling beetles (i.e. species that don't bury dung) showed that the beetles improved the conditions in a dung pat for development of the infective 3rd instar nematode larvae in the first 12 days, but that larger numbers of larvae were present in the pats without beetles after 24 days – with overall numbers of 3rd instar larvae being 2.4x higher (Chirico et al 2003). Popay and Marshall (1996) also had one trial where nematode numbers were significantly higher in surface dung in the presence of dung beetles. A possible explanation could be that increased levels of oxygen in dung broken up by beetles led to a higher proportion of nematode eggs hatching (Durie in Hughes 1975; Reinecke 1960). Greater aeration due to the activity of dung-dwelling beetles was also thought to explain the higher numbers of 3rd instar larvae after 12 days in the presence of beetles by Chirico et al (2003). In this study, larval numbers were much higher in control pats after 24 days, giving an overall decrease in total larval numbers in the presence of beetles of 2.4×.

To summarise, most studies show that dung beetles breaking up dung on the surface decreases survival of nematodes to the infective L3 stage. However, if conditions are wet enough then insufficient desiccation could occur and development to the L3 stage might be relatively unaffected by the surface activities of dung beetles. An increased proportion of nematode eggs could hatch in dung beetle affected dung because of increased levels of oxygen (required for eggs to hatch) compared with intact dung pats. In wet conditions it is therefore possible that numbers of eggs developing to the L3 stage could be increased by the action of dung beetles on surface dung, although such an effect appears to occur very infrequently. In addition, when such damp conditions occur, intact dung pats (in the absence of dung beetles) would already be a major source of L3 nematodes, while dung beetles will typically be rapidly burying dung resulting in a proportion of dung (sometimes high) ending up buried before nematodes can develop to the L3 stage.

### *3.2 Direct feeding and processing of dung into brood balls*

It has been suggested that the feeding action of dung beetles, and their processing of dung into brood balls for their larvae, could directly reduce the survival of nematode eggs. In particular, Miller et al (1961) suggested that the grinding action of dung beetle mandibles destroyed nematode eggs. However, Holter (2002) showed dung beetles filter small particles out of the dung and consume these rather than doing any chewing. Note that dung beetle larvae do have chewing mouthparts, but the larvae will only hatch after 3rd instar nematode larvae have matured and possibly left the brood ball. The Miller et al (1961) result that showed that dung beetle guts did not contain many intact nematode eggs is still significant in that the beetles themselves would not be capable of transporting nematode eggs or larvae to any great extent. Despite the critique of Holter (2002) it is still worth pointing out that in a parallel study with *Cryptosporidium* oocytes, Mathison & Ditrich (1999) showed that only small numbers of oocytes survived passage through the beetle guts. Like Miller (1961) they concluded that the grinding action of the beetle mouthparts destroyed most of the oocytes. Again this appears to be unlikely given Holter's (2002) findings. However, Ryan et al (2011) then go on to show that dung buried by dung beetles had far fewer oocytes than dung remaining on the surface. So beetle processing of dung is reducing the survival of *Cryptosporidium* oocytes by some means even if the beetles do not chew or grind the dung. Note that Cryptosporidium oocytes are only 4-6μm in diameter so will be consumed by all the beetle species tested by Holter (2002). As Nichols et al (2008) points out, we don't know the mechanism for the destruction of the small *Cryptosporidium* spores. It's quite possible that some of the same destruction could happen with nematode eggs. Indeed, there is direct evidence of this: Bryan (1976) dug out brood balls three days after dung beetles had been introduced to fresh dung pats on the ground above. The control dung pats (no beetles) had approximately 100,000 nematode larvae per 100 g fresh weight of dung. In contrast, the brood balls had only about 800 nematode larvae per 100 g fresh weight of dung, a 99.5% reduction. Given the three day sampling period, all the nematode larvae would have been 1$^{st}$ or 2$^{nd}$ instar, so would not yet have migrated from the surface pat or the brood balls. The most obvious conclusion is that processing of the dung to create the brood ball resulted in the death of the vast majority of nematode eggs and/or larvae. This is clearly very relevant to the discussion of the effects of dung beetle burial versus artificial burial experiments (section 4.1, below).

### *3.3 Studies that don't distinguish effects of surface activities of dung beetles from dung burial*

Several studies report reductions in nematode numbers due to dung beetle activity by measuring larval numbers on foliage/surface soil (Bryan 1973; Bryan & Kerr 1989; English 1979; Hutchinson et al 1989; Reinecke 1960; Waterhouse 1974), but cannot distinguish the direct effects of beetles on dung on the surface with the effects of dung burial. Similarly, Fincher (1973, 1975) show reduction in infection rates of cattle from beetle activity that could be due to increased surface desiccation of dung and/or dung burial. Another study also shows a similar reduction in larval nematode numbers on foliage from earthworm burial of dung (Grønvold 1987).

One study showed dung beetles having no significant effect on nematode numbers on pasture adjacent to horse dung (Houston et al 1984a), although the number of infective larvae was, at times, higher in the treatments with beetles than controls. Reinecke (1960) reported that the removal of small pieces of dung from pats by dung beetles could, under conditions of low rainfall, lead to low numbers of larval nematodes on grass (whereas the same low levels of rainfall were insufficient to allow migration of larvae out of intact pats). Hence, under these particular conditions there were occasions when larval numbers appeared higher in the presence versus the absence of beetles. However, an analysis of Reinecke's (1960) data failed to show that this was a statistically significant effect (Appendix 1). The larval numbers on the grass in these low rainfall trials were extremely small compared to the larval counts on grass in trials that were conducted in higher rainfall conditions (Reinecke 1960; data analysed in Appendix 1). In another study (Popay & Marshall 1996), one trial resulted in an increase in nematode larvae on foliage with dung beetles present compared with controls, which approached statistical significance (P=0.07). However in the same study, most trials did not result in an increase in nematodes on foliage in the presence of dung beetles. Finally, a few studies tracked larval nematode numbers over time and, although there were usually higher numbers from dung in the absence of beetles at first, there were occasions when a lesser, later peak occurred in the presence of beetles which was assumed to be due to larval migration from buried dung. In two of these studies (Bryan 1973, 1976) there were examples where, towards the end of the experiment, there were more larval nematodes on the pasture foliage in the presence of dung beetles than in their absence. However, the levels were always much lower than the earlier peak numbers from dung in the absence of beetles. These important findings are discussed in more detail in the next section on migration of larvae from buried dung.

In summary, most studies using dung beetles show a substantial reduction in nematode larval numbers in the surface soil or on pasture foliage. Several studies show no statistically significant effects, and a few show that dung beetle activity can result in a delayed effect where nematode larvae are assumed to have migrated from buried dung and can give rise to occasions when larval numbers are higher in the presence rather than the absence of beetles. However, in these examples, this later peak in larval numbers is always small compared to the numbers of nematode larvae migrating from dung in the absence of beetles, and there are other issues of data interpretation discussed in more detail below.

## 4. Burial of dung and potential migration of nematodes back to the soil surface

Dung beetles bury dung as brood balls in underground chambers for their offspring to feed on. As sexually immature adults they may also temporarily bury some dung which they feed on over a period of days. In these burial processes, some dung can end up on the insides of the burrow. The key question here is whether burial could increase the survival/longevity of nematode eggs and larvae compared to dung exposed to desiccation and sunlight on the surface, perhaps creating a 'time bomb' in the soil if nematode larvae could migrate back to the surface given sufficient moisture (Coldham 2011; Vlassoff et al 2001; Waghorn et al 2002). Here the evidence for increases in nematode larvae in soil at depth when dung is buried versus when dung remains on the surface is reviewed. The question is then asked whether burial depth influences the ability of L3 nematodes to migrate back to the soil surface and/or on to pasture foliage. Finally the possibility of a delayed emergence of nematode larvae from buried dung, the so-called 'time bomb' effect, is reviewed.

### *4.1 Does burial of dung increase numbers of nematodes buried in the soil?*

Two studies show that nematode numbers were higher in soil after dung beetles had buried dung compared to the numbers in soil when the dung remained on the surface (Bryan 1976; Popay & Marshall 1996). However Bryan (1976) also presented data suggesting that 99.5% of nematode eggs/larvae were destroyed by dung beetles in making brood balls (as described in section 3.2, above). Two other studies suggest that dung buried by dung beetles has highly reduced numbers of nematodes. Firstly, Coldham (2011) showed that in moist conditions the same number of nematodes returned to the soil surface from artificially buried dung compared to moist dung on the surface, but that burial of dung by dung beetles appeared to result in the destruction of nematodes. Secondly, Grønvold et al (1992) showed no increase in nematodes in the soil (0-6 cm, 6-12cm, 12-18 cm) when beetles buried an average of 62% of the dung on the surface (compared with controls i.e. dung on the surface with no dung beetles).

Although Reinecke (1960) commented that "where rainfall was inadequate to cause larval migration, the presence of larvae in the soil was probably due to the mechanical removal of dung by beetles" a statistical analysis of the data as part of this review shows no increase of larvae in soil where dung beetles had been active in either dry or damp conditions (Appendix 1).

One study showed increased nematodes in the soil from artificial burial of dung compared to dung on the soil surface (Waghorn et al 2002). Lucker (1938) and Persson (1974) record nematodes in soil after dung had been artificially buried but did not have a surface dung treatment to compare to the effect of dung burial.

To conclude, artificial burial may increase nematodes in the soil much more dramatically than burial by dung beetles. In some cases burial by beetles appeared to destroy a high proportion of the nematodes, presumably by their processing of the dung.

### *4.2 Effects of burial depth on nematode larval abilities to return to the surface*

It has been suggested that shallow buried dung may allow more nematodes to return to the surface compared with deeper burial. Two New Zealand studies have examples of nematodes returning to the surface from dung buried artificially to 5 cm (Waghorn et al 2002) and by beetles to generally <5 cm (Popay & Marshall 1996).

Studies that artificially buried dung to a variety of depths typically show a dramatic reduction in the numbers of nematode larvae migrating to the soil surface and/or foliage with increased burial depth (Fincher & Stewart 1979; Lucker 1936, 1938). Several studies also report that the presence of artificial surfaces that would have provided continuous films of water seemed to encourage migration of nematode larvae from dung buried quite deep e.g. 25 cm in glass tubes in Fincher & Stewart (1979), or buried wooden boxes in Lucker (1936). Although not mentioned by the authors this could explain the unusual result of Krecek & Murrell (1988) who had one result where large numbers of nematodes apparently migrated from surface dung to get past a 15 cm deep steel barrier and then managed to return to the surface outside the barrier. This issue is discussed further in Appendix 1. The same effect may also explain the examples of large numbers of 3$^{rd}$ instar larvae migrating from dung buried as deep as 20 cm by Persson (1974).

To conclude, it is clear that nematode larvae are capable, given moist enough conditions, of migrating from dung buried to <10 cm or sometimes deeper. However, even shallow burial is likely to provide a hurdle for larval migration when soils do not have high moisture contents, and this hurdle effectively increases with increased burial depth. Experiments using artificial barriers that could provide continuous films of moisture for larval movement need to be interpreted with caution.

### *4.3 Evidence of delayed migration of L3 from buried dung resulting in a 'time bomb' effect?*

As mentioned in section 3.3 above, with studies that have extended sampling over time we can ask if there is a first peak in larval numbers on the surface as expected from direct migration of infective larvae from surface dung, followed by a second peak in larval numbers from buried dung. This is particularly well illustrated by data from Bryan (1973). This data has been taken from a graph in the paper and then re-plotted here as non-cumulative totals per date (Fig 1).

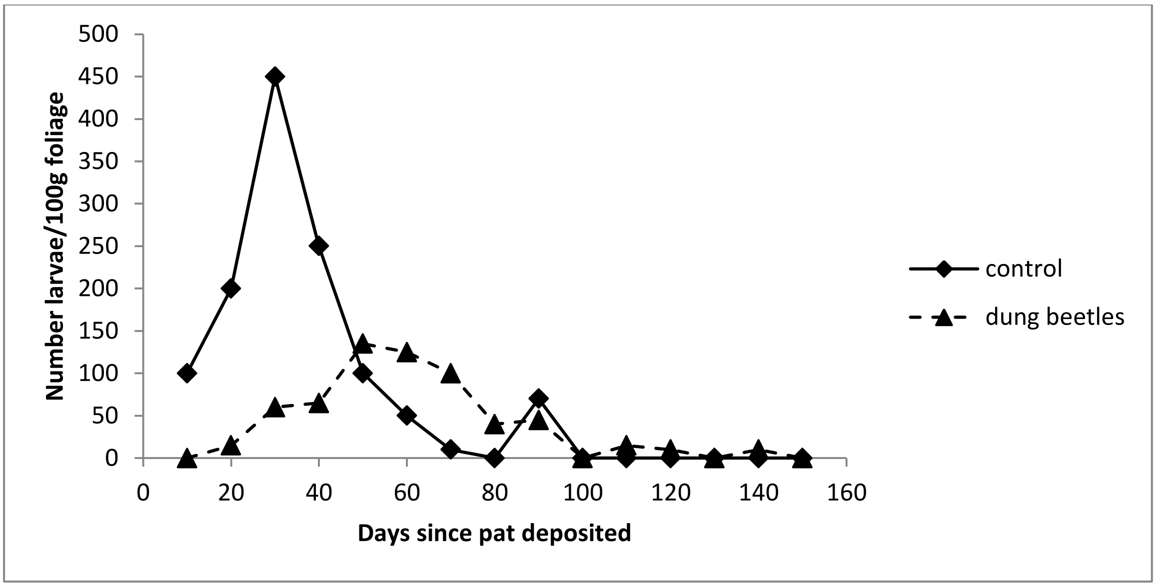


Figure 1. The number of 3rd instar larvae of nematodes recovered from pasture foliage adjacent to artificial dung pats infested with nematode eggs, either in the presence or absence of dung beetles. Data from cumulative figure in Bryan (1973).

There is a large first peak in larval nematode numbers on foliage (day 30) from direct larval emergence from surface dung in the absence of dung beetles. There is a second peak when dung beetles are present (days 50-70) which is thought to be caused by nematode larvae emerging from buried dung (in this case as a response to a substantial rain event on day 40). Note that the numbers of nematode larvae are much less than in the first peak, nevertheless between days 50-70 there are more nematodes on the grass in the dung beetle treatment than in the dung only treatment. However, this second peak needs to be interpreted with care as the sampling method used removed all foliage to ground level around each pat on each sampling date. Thus had the grass not been removed, the numbers of nematode larvae around the control plots would have remained high until the L3 larvae died (rather than until they were removed by sampling). Presumably this is why Bryan (1973) presented the data as cumulative numbers. Predicting L3 survival (had they not been removed by sampling) is not easy, but survival in warm, moist field conditions is typically suggested to be 10 weeks or more (e.g. Rattray 2003).

In his later study, Bryan (1976) carried out a more detailed experiment where dung pats, brood balls, soil etc were destructively sampled on four dates. Much of this has been reported above (sections 3.1 to 4.1), but for this section the important difference to Bryan (1973) is that foliage samples were different radial subsamples around each pat approximately every 10 days, so the earlier sampling was probably not reducing nematode numbers in the later sampling by overall removal. Thus this data can be examined non-cumulatively with more confidence. Interestingly, the numbers of nematode larvae recovered from foliage were nearly always greater around intact pats compared with pats with dung beetles (see Appendix 1 for details). There was a slightly higher peak at day 84, right at the end of the experiment, in the treatment with the lowest number of dung beetles. Bryan (1976) concluded that the small amount of rain that fell had wetted dung that had been shredded by beetles, and hence encourage nematode larval migration from the shredded dung. However, it was suggested that the rain was insufficient to wet the intact encrusted pats in the control treatment that had no dung beetles, so nematodes remained trapped in these larger pats. Bryan (1976) considered that the encrusted control pats would have continued to release larger numbers of nematodes had there been sufficient rain to wet the intact pat.

Another study shows a delayed, small peak in nematode numbers reaching the surface due to migration from buried dung, but the numbers were still lower than the numbers migrating on these dates from intact pats which had had no beetle activity (Fincher 1973). Grønvold (1987) showed the same pattern with earthworm-buried dung. Finally, Fincher & Stewart (1979) show later peaks of infective larvae in their glass tube experiment: by week 8, higher levels of infective larvae were being recorded on the soil surface from dung buried at 7.5 – 10 cm compared with the dung on the surface treatment (but total numbers emerging from buried dung at these depths for the whole duration of the trial were only 2.3% of those in the surface dung treatment).

Coldham (2002) specifically set out to test for a 'time bomb' effect from dung buried by dung beetles, but concluded that dung beetle burial destroyed all nematodes. Gronvold et al (1992) showed a similar effect with dung beetle burial not increasing nematode numbers in soil.

In summary just one study (Bryan 1973), carried out under irrigation, shows that dung beetle burial can create a situation where higher numbers of nematode larvae are sampled on pasture foliage in the presence versus the absence of beetles. However, the support for a 'time bomb' effect is weak for two reasons. Firstly, the later peak was far smaller than the earlier peak caused by direct nematode migration from pats without beetles. Secondly, without the destructive sampling of all the foliage around the pats on each date it is likely that many larvae would have survived from the first large peak around the control pats. The sampling in this case was akin to the 'vacuuming' of nematode larvae that can be achieved by use of a different stock type – one of the potential recommendations for suppressing larval nematode numbers (e.g. Ratray  2003). It's quite possible then that in the absence of the sampling, larval nematode numbers would have remained higher in the controls than in the dung beetle affected pats. This is the pattern revealed in virtually all the other studies of the potential 'time bomb' effect, with the exception of one final, low-level sample in Bryan (1976). The lack of the 'time bomb' effect in most/all studies is probably due to one of more of the following reasons:

a) Dung beetle feeding/processing of dung destroyed many/all nematodes in buried dung;
b) Buried nematodes can be trapped underground by lack of sufficient moisture in surface soil;
c) Dung was buried too deep for many L3 to return to the surface;
d) Dry dung on the surface still provided enough of a reservoir of desiccation-resistant L3 nematodes to swamp any later emergence of L3's from buried dung.

Point (d) is contradictory to the quote from Vlassoff et al (2002) used in the Introduction: *"if large numbers of L3 are able to survive in soil during periods of drought ...* (then this could be) *a potential explanation for the rapid increase in pasture larval counts observed after the first significant rains in late summer/autumn in some years"*.

However, point (d) is consistent with an observation by Bryan & Kerr (1989): "*Larvae in faecal pats deposited from July onwards during the 1977 drought survived in large numbers in these pats until mid-November when rain fell. Then they migrated as a single wave and the resultant pasture contamination was 10x higher than the usual spring maximum.*" In this example, faecal pats deposited in unusual increasingly dry conditions that lasted about 100 days, contributed to the massive peak when the drought broke. It is also likely that as the ground became dry and hard there would be little burial activity by dung beetles because numbers of beetles typically decrease in drought conditions and any that were present would be unlikely to be able to bury dung in the hard, dry soil. Overall, it seems that the presence of dung beetles, either working through or burying dung, won't enhance the nematode reservoir problem in drought (rather they are likely to offer some mitigation).

**5. Comparing the climates for overseas studies with New Zealand conditions**

Where known, comments on the rainfall and temperatures under which overseas studies were undertaken are given in Table 1. Also noted is whether the study used irrigation. Data are from the papers where possible, otherwise a Google search was used to find climate data for the area where the trial was carried out.

Many of the studies in this review were carried out in warmer, more tropical regions than New Zealand e.g. Bryan (1973, 1976) and English (1979) were conducted in southern Queensland. However, Bryan (1973, 1976) conducted experiments in autumn (April – June) and it is clear from climate data that these months typically have temperatures and rainfall similar to parts of New Zealand over the summer period (Appendix 1). Thus the results of Bryan (1973, 1976) should at least be pertinent to parts of New Zealand in summer. The same is true for some of the large number of trials reported by Reinecke (1960), which although conducted in a semi-arid area has some spring and autumn periods when average temperatures were reasonably similar to the warmer parts of New Zealand in summer (see Appendix 1). However, several studies were clearly conducted in hotter weather than New Zealand normally experiences even in summer (Bryan & Kerr 1989; English 1979; Fincher 1973, 1976; Grønvold et al 1992; Houston et al 1984a, 1984b; Hutchinson et al 1989).

Other trials were laboratory based and were operated at temperatures that were similar to those in some season/regions of New Zealand (Bergstrom 1983; Bergstrom et al 1976; Coldham 2002; Lucker 1936, 1938). A few dung burial trials were conducted outside in temperate areas that were more directly comparable to most of New Zealand (Krecek & Murrell 1988; Lucker 1936), but none used dung beetles. Finally, the trial that tested dung burial by earthworms (Grønvold 1987) was located in Denmark, in a climate that would be cooler on average than New Zealand.

Regarding moisture levels/rainfall, many trials either had similar amounts of rainfall to areas in New Zealand in at least some periods (Lucker 1938; Fincher 1973, 1975; Fincher & Stewart 1979; Grønvold 1987; Grønvold et al 1992; Krecek & Murrell 1988), or supplemented lower rainfall with irrigation (Bryan 1973). Even Reinecke (1960) in a semi-arid zone only had very low rainfall in winter, with actually quite substantial rainfall in the warmer months (Appendix 1). Similarly, the study by Hutchinson et al (1989) was carried out in a dry tropical region of Queensland, but there is a pronounced summer wet season when most of the 800-1000mm of rain falls. For laboratory trials, moisture levels were maintained at levels sufficient to allow migration of 3$^{rd}$ instar larvae (Bergstrom 1983; Bergstrom et al 1976; Coldham 2002; Lucker 1936, 1938). In some studies rainfall was clearly low for substantial periods of the trials (e.g. Bryan 1973, 1976). However, low rainfall for reasonably long periods of time is quite common in many parts of New Zealand, particularly in summer.

To summarise, although there is considerable overlap in temperatures and rainfall in many studies with likely conditions in New Zealand pastures, clearly some overseas trials were carried out in a number of climate types that are not found in New Zealand. Overall, overseas trials that examined the effect of dung beetle activity on nematode larval numbers were conducted in climates that are warmer on average than most areas of New Zealand. Nevertheless, some of the most detailed studies (e.g. Bryan 1976; Coldham 2002; Reinecke 1960) do contain trials that were conducted under environmental conditions that, at least at certain times, would not be unusual in New Zealand.

What effect are some of these differences in weather conditions likely to have on how relevant their results are for New Zealand? Broadly it seems likely that the effect of the breaking up and desiccation of dung by beetles (section 3.1, above) will be highly dependent on weather conditions. As mentioned in section 3.1, cool, moist conditions are likely to reduce the impact of dung break-up on free-living stages of nematodes. However, it also appears that hot, wet conditions could result in such a rapid development to the desiccation resistant 3rd instar larval stage that surface dung break up could also be relatively unimportant. Given that New Zealand does tend to have dry, warm summers (Rattray 2003) it seems that dung break-up and desiccation from beetle activity could at least be important for reductions of nematode numbers in summer (or in dry periods in other seasons). Cool wet winters are likely to lead to prolonged survival of 3rd instar larvae both on the soil surface and in buried dung (Rattray 2003). In an area with cold damp winters (Denmark), one study showed that the overwintering surface dung (where earthworms had been excluded) led to higher numbers of infective larvae on foliage the following spring compared to the pats where dung had been buried (Grønvold 1987). There are no studies to inform whether the same effect would occur with dung buried by beetles before a cool wet winter, but the above discussion (sections 3 and 4) doesn't present any evidence that the results should be that much different between dung buried by earthworms or dung beetles. It is also clear that surface dung can be a key reservoir for the desiccation resistant 3rd instar larvae even after prolonged periods of drought (Bryan & Kerr 1989).

The effect of dung beetle feeding/processing of dung into dung balls on nematode numbers (section 3.2) would not seem to be directly influenced by the weather, but obviously weather influences levels of beetle activity. Overall, the review of overseas studies on the direct effect of dung beetle feeding/processing of dung on nematode numbers would seem to be relevant to New Zealand.

The ability of nematode larvae to migrate from buried dung to the soil surface (sections 4.2 and 4.3, above) will be critically affected by levels of soil moisture and hence rainfall. Soil temperatures *per se* are probably relatively unimportant although nematode larvae will survive for longer periods in cooler temperatures (Rattray 2003). Many studies had adequate moisture for larval migration so the overall result of this section of the review would seem to be able to be extrapolated to New Zealand.

Overall, the effects of climate on the interaction between dung beetles, gastrointestinal nematodes and stock re-infection rates are complex. However, there does not seem to be strong evidence from overseas studies that the typically cool, wet winters and warm, dry summers in New Zealand (Rattray 2003) will unduly alter the overall conclusion that in most weather conditions dung beetle activity will tend to reduce larval nematode numbers available to re-infect stock.

**6. Acknowledgements**

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Note: The report by Skipp et al (2000) was accessed but contains the same information as Waghorn et al (2002) and Popay & Marshall (1996).

**8. Appendix 1: supplementary information**

**Bryan 1973 and Bryan 1976** are important studies, so were reviewed in detail in the main text. The re-plotted data from Bryan 1976 are shown in Figure 1 in section 4.3. Data were also extracted from Bryan (1976) and re-plotted here (Figure 2). Bryan 1976 is the only study found that measured larval nematode numbers in brood balls. Data from the histograms in Bryan (1976) have been extracted and presented in Table 2. Dung burial by beetles does result in nematode larvae in the brood balls, however the numbers are dramatically reduced compared with dung remaining on the surface (Table 2).

| | day 3 | day 8 | day 18 | day 84 |
|---|---|---|---|---|
| pat | 100000 | 24938 | 590 | 750 |
| 2 beetles | * | 900 | 150 | 150 |
| 10 beetles | 300 | 220 | 550 | 300 |
| 30 beetles | 800 | 320 | 180 | 30 |
| Mean | 550 | 480 | 293.3 | 160 |
| % reduction | 99.5% | 98.1% | 50.3% | 78.7% |

Table 2. The numbers of nematode larvae (per 100g dung) in the control pats (no dung beetles) versus brood balls dug out of the ground in the three dung beetle treatments. The mean numbers of larvae in the brood balls on each sampling date were used to calculate the percentage reduction versus numbers in the control pat. The most important comparison is at day 3 because no larvae would have reached the potentially migratory 3rd instar stage. This 99.5% reduction is presumably caused by the dung beetle burying activity which probably aerates and desiccates the dung (which will kill 1st and 2nd instar larvae) but also perhaps due to the processing of the dung by the specialised filtering mouthparts of the adult beetles. *No dung balls by day 3 in the 2-beetle treatments

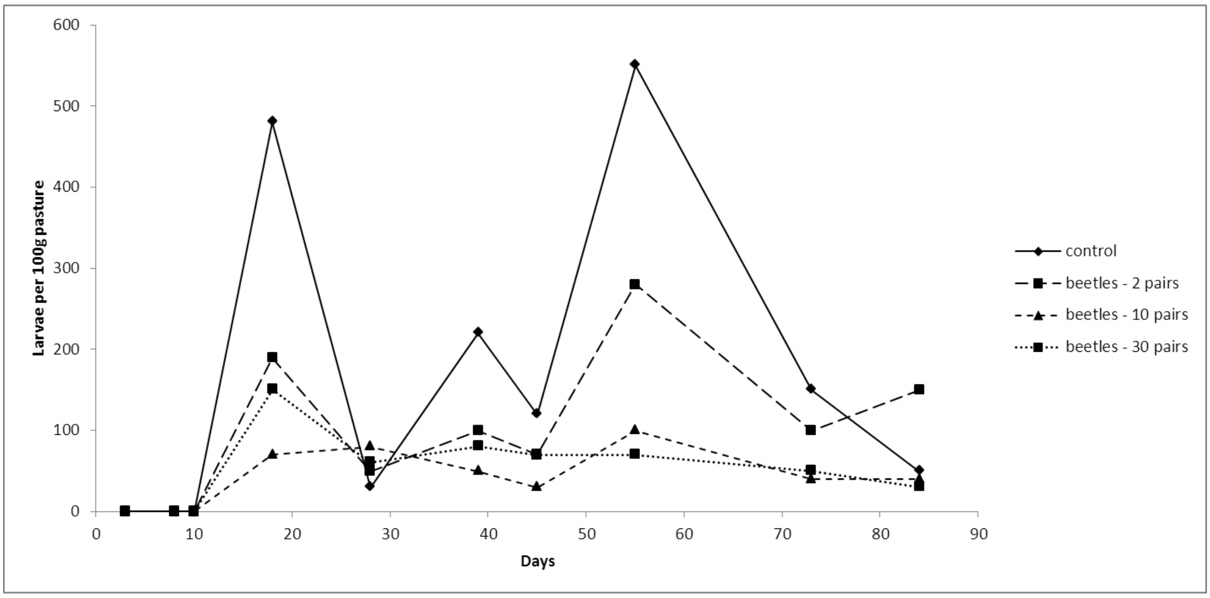

Figure 2. Numbers of 3rd instar larvae recovered from pasture foliage. Data extracted from cumulative graph in Bryan (1976) and re-plotted non-cumulatively. Control pads had no dung beetles. Moderate rainfall events occurred early in the trial and also on days 14 and 53 (just preceding the two main peaks of larval migration). There was only 1 sampling date (day 84) when larval recoveries were substantially higher in one of the dung beetle treatments versus the controls (see main text, section 4.3, for the author's explanation).

Although these studies were located at Glenlogan Field Station in SE Queensland, because they ran from April-June, the mean monthly temperatures and rainfall were a reasonable match for a northern NZ summer (Table 3).

| | January | February | March |
|---|---|---|---|
| Whangarei – temperatures | 24.4/15.4 | 24.2/15.4 | 23.0/15.0 |
| Whangarei - rainfall | 90 | 112 | 142 |
| Napier - temperatures | 24.4/14.6 | 24.1/14.5 | 22.6/12.8 |
| Napier - rainfall | 48 | 62 | 85 |
| | April | May | June |
| Glenlogan temperatures | 26.9/14.2 | 23.8/10.9 | 21.3/6.8 |
| Glenlogan - rainfall | 75.8 | 78.5 | 40.3 |

Table 3. Mean monthly maximum and minimum temperature for the warmest summer months in Whangarei and Napier, compared with the experimental period (April-June) at Glenlogan Field Station, the field site for trials reported in Bryan (1973, 1976).

The Glenlogan Field Station climate data were obtained from:
http://weather.ninemsn.com.au/climate/station.jsp?lt=site&lc=40454

New Zealand climate data were obtained from:
https://en.wikipedia.org/wiki/Whangarei
http://en.wikipedia.org/wiki/Napier,_New_Zealand

**Houston et al 1984b**

The data from this paper were plotted to illustrate the effect of burial depth more clearly (Fig. 3).

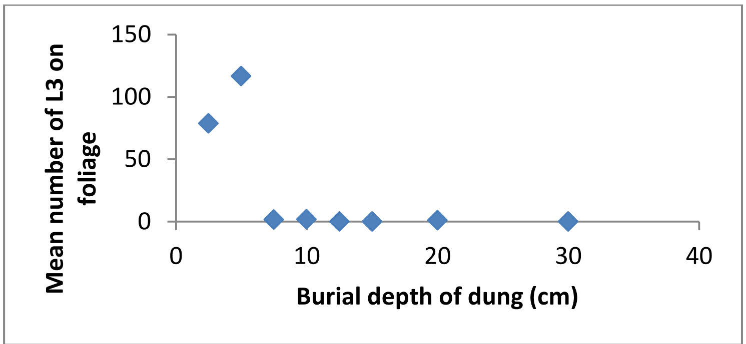


Figure 3. The numbers of 3rd instar larvae migrating onto pasture foliage from dung artificially buried to various depths.

**Hutchinson et al (1989) and Mfitilodze & Hutchinson (1988)** show reductions in 3rd instar larvae of horse strongyloids in dung open to dung beetles compared with dung protected from beetles for 7 days. They claim these are minor, although this might underestimate the differences because numbers were expressed per gram wet weight of dung. Hence they assume that dung beetle activity (which they say shredded dung within 24h) didn't reduce the total weight of the 1 kg artificial dung pat versus dung pats protected from beetles. However, Hutchinson et al (1989) went on to measure larvae on the grass surrounding the pats after 7 days and then at monthly intervals. They found that numbers of infective larvae were slightly higher on average on grass around pats protected from dung beetles, but numbers were quite variable over time, with no clear pattern, suggesting the dung beetle processing horse dung would be epidemiologically insignificant for strongyloids in this system. However dung beetles do not show any indication of making the situation worse. It seems that the eggs develop to the L3 stage so rapidly in the hot, wet summer conditions that the action of beetles dispersing the dung is not effective at killing larvae in the first two instars. At other times of the year, conditions are usually so dry that the larvae cannot migrate from the dung to the grass anyway.

**Krecek & Murrell (1988)** used buried barriers in the form of 30 cm diameter steel tubes to show that large numbers of nematode larvae could migrate from surface dung to at least 15 cm deep and then return to the surface outside the barrier. This result seems unusual in two respects. Firstly the numbers of nematodes migrating around the 15 cm deep barrier in particular is large compared with other studies above. Secondly most studies do not report nematodes penetrating soil very deeply without dung burial (e.g. Popay and Marshall 1996). It seems possible that barriers might encourage nematode movement in a similar way as Fincher & Stewart (1979) suggest for their glass tube experiment, and Lucker 1938 suggests for experiments with wooden barriers i.e. the damp barrier provides an easy route for larval travel. In Krecek and Murrell (1988) larval movement may also have been exacerbated by the heavy rain they report in the latter part of their trial: it may be that nematode larvae were virtually 'washed' down the inside of the steel cylinders. Bryan (1973) also makes the comment that heavy rain can wash nematode larvae deep into soil, although presents no data on this.

**Lucker (1936, 1938)** showed few nematode larvae reached the soil surface from burial depths of >10 cm (Figure 4).

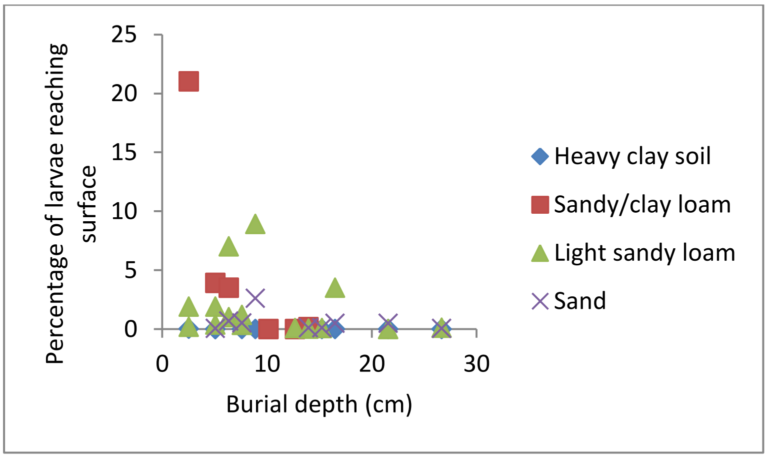

Figure 4. The percentage of 3rd instar larvae migrating to the soil surface from either direct burial of 3rd instar larvae (Lucker 1936) or burial of dung infested with known quantities of nematode eggs or larvae (Lucker 1938).

**Reinecke (1960)** is a huge study and presents a large amount of data, but as published is difficult to follow. The data were re-evaluated here.

Of the 58 trials - only some had dung beetle activity. Trials were excluded if Reinecke's (1960) comments made it clear that atmospheric conditions were unsuitable for nematode larval development, or the trial ran for an unusually long time, or there were other unusual circumstance (e.g. a dripping trough in one trial; no grass near pad in another). Dung beetle activity was scored as 0 (no mention), 1 (dung beetle activity mentioned) and 2 (major impact mentioned). Firstly, the number of nematode larvae in the dung pats was examined in relation to dung beetle activity across both dry and wet trials (as dung beetles were active in both). Dung beetle activity clearly reduced the number of nematode larvae in dung pats (Fig 5). The mean number of larvae collected from unattacked pads (back-transformed mean) was 1308.8 and from pads affected by dung beetles (activity levels 1+2 grouped) was 12.9, a reduction of 99.0%.

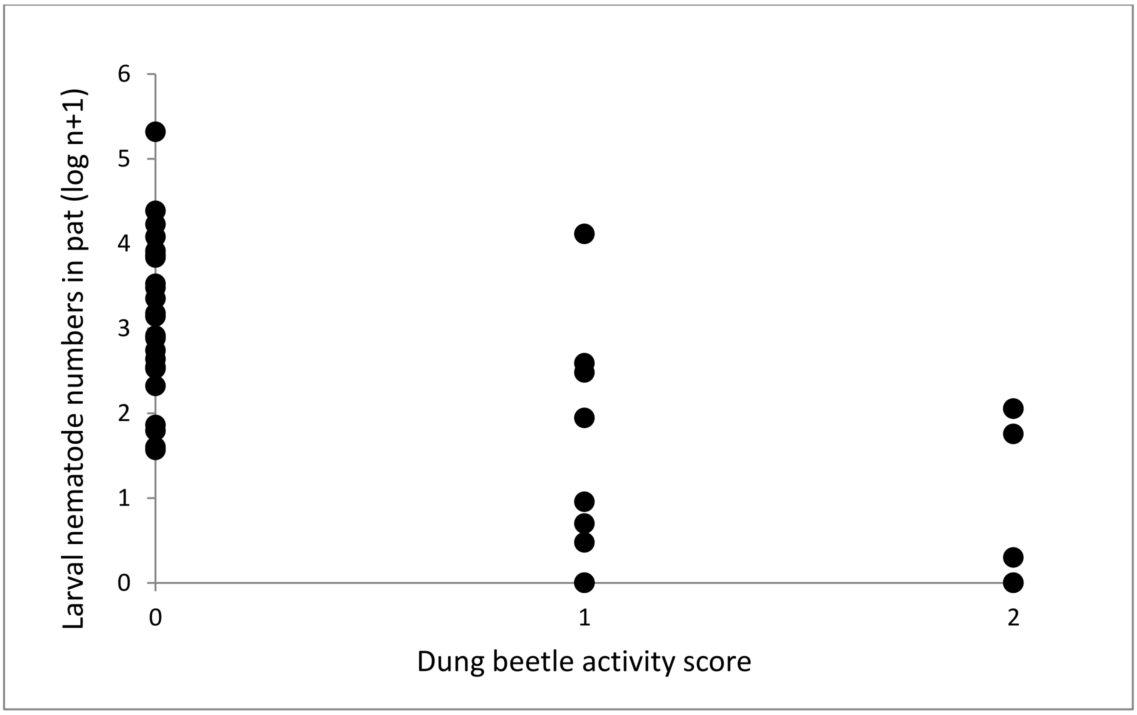


Figure 5. The total number of nematode larvae in dung pats with levels of dung beetle activity scored as 0, 1 or 2 (see text for details). Y = -1.23X + 3.02, $F_{1,38} = 28.76$, $P<0.00001$, $r^2 = 0.42$.

Soil under intact pats with no beetle activity also had slightly more nematode larvae than soil under pats where beetles had been active, but the effect was not statistically significant.

Trials were then divided into those where Reinecke (1960) considered that insufficient rain had fallen to enable nematode larvae to migrate from dung pats, and those where rainfall was sufficient. In trials where sufficient rain fell to allow migration of 3rd instar larvae out of the dung pads, there were more larvae recovered from adjacent pasture foliage around pads with no beetles than pads with beetle activity levels of 1 or 2 (grouped here because only 1 trial with sufficient rain had a beetle activity score of 2). Back-transformed means (180.8 and 22.8 for beetle activity levels of 0 and 1+2) reveal that this is a percentage reduction of 87.4% (Fig 6).

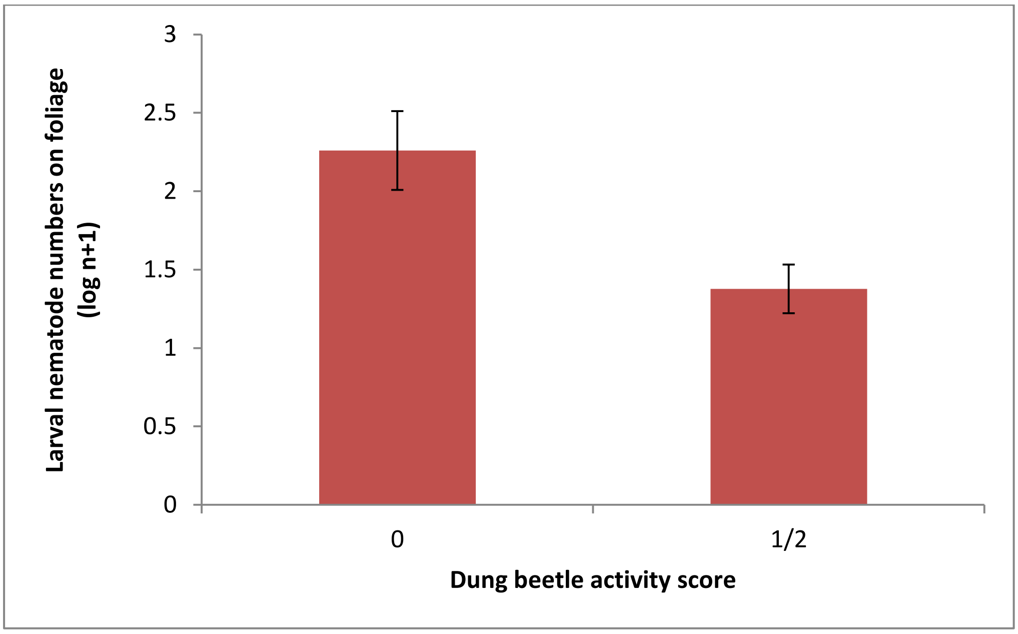


Figure 6. The total number of 3rd instar larvae recovered from pasture foliage adjacent to dung pats where dung beetles had not been active (0) or had been moderately or highly active (1/2), in trials where sufficient rain had fallen to allow migration of larvae away from the dung pat. The difference is statistically significant ($t_{13}$ = 2.99, $P<0.05$). Error bars SEM.

Reinecke (1960) reported that in trials where insufficient rain fell to enable larval migration away from the dung pat itself, then smaller amounts of rain could allow migration from small pieces of dung left behind after dung beetle activity. The re-analysed data do show some evidence that this is happening because in dry conditions there were more nematode larvae recovered on adjacent foliage in the trials that had moderate dung beetle activity (Fig 7), but the effect was not statistically significant. Note that the mean numbers of larvae recovered from these dry trials are much lower than from the moist trials (means of 1.5, 5.1 and 0.9 for dung beetle activity scores of 0, 1 and 2 versus means in moist conditions of 180.8 and 22.8 for beetle activity levels of 0 and 1+2).

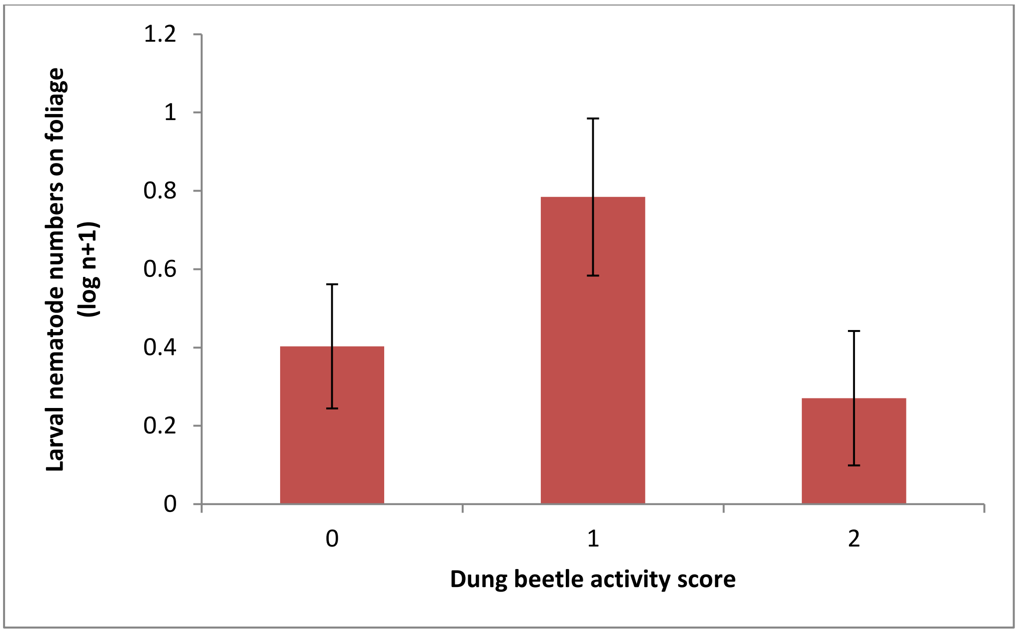


Figure 7. The total number of 3rd instar larvae recovered from pasture foliage adjacent to dung pats where dung beetles had not been active (0) or had been moderately (1) or highly active (2), in trials where insufficient rain had fallen to allow migration of larvae away from the dung pat. The difference between activity levels 0 and 1 is not statistically significant ($t_{11}$ = 1.49, P>0.1). Error bars SEM.

Similar analyses of the number of nematodes recovered from soil under the pat, adjacent soil or from plant roots, revealed no significant differences regardless of whether dry and moist trials were analysed together or separately.

Climate data for Reinecke (1960) used in Table 1 were obtained from the nearby city of Vryburg: http://www.saexplorer.co.za/south-africa/climate/vryburg_climate.asp

**Persson 1974** presents data on several artificial burial trials. This has been summarised here in a simple table (Table 4).

| | | | | total recoveries | | | |
|---|---|---|---|---|---|---|---|
| Date | Depth | No eggs | No L3 | moss soil | clay soil | sandy soil | |
| 15/10/1971 | 10 | 180000 | | 10 | 13 | 31 | 54 |
| 15/10/1971 | 20 | 180000 | | 16 | 18 | 2 | 36 |
| 15/10/1971 | 10 | | 20000 | 308 | 135 | 345 | 788 |
| 15/10/1971 | 20 | | 20000 | 291 | 665 | 278 | 1234 |
| 9/05/1972 | 10 | 180000 | | 539 | 679 | 114 | 1332 |
| 9/05/1972 | 20 | 180000 | | 97 | 307 | 33 | 437 |
| 9/05/1972 | 10 | | 15000 | 722 | 1010 | 52 | 1784 |
| 9/05/1972 | 20 | | 15000 | 663 | 247 | 12 | 922 |

Table 4. Data tabulated from Persson (1974).

There was no obvious pattern of decreased emergence of larvae with increased burial depth. This is unusual compared to other burial studies. The design is similar to the Krecek and Murrell (1988) experiment with aluminium cylinders. Thus the same concern exists, that 3$^{rd}$ instar larval nematodes could be travelling up the inside of the metal cylinders as in other experiments using barriers.

**Popay & Marshall 2002**

This New Zealand study shows that shallow (less than 5 cm) burial of dung by the small Australian beetle *Onthophagous granulatus* results in higher nematode larval numbers in the soil, but there was no significant increase in the numbers of larvae on foliage. The study has not been published in a peer reviewed journal, and does contain several unusual techniques/results e.g. covering containers with cling-film in one trial that resulted in high levels of fungal infection on the dung. The trials also used two marsupial-adapted, adventive dung beetles that do normally process much stock dung in New Zealand. In their report they mention a further set of trials but no details are given.

**Waghorn et al 2002**

This New Zealand experiment used shallow, artificial burial of dung to 5 cm to simulate dung beetle burial activities. Notably it is one of the only experiments to ever show that dung burial can lead to a significant increase in the numbers of 3$^{rd}$ instar larvae on foliage when compared to the control treatment with dung exposed on the surface.