Informing the infectious disease risks of dung beetle releases into New Zealand

Landcare Research
Manaaki Whenua

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Dan Tompkins, Shaun Forgie, Jackie Aislabie, Graham Nugent, Hugh Gourlay, Alexandra McGill, Malcolm McLeod, Ivor Yockney, Quentin Paynter, Simon Fowler, Lynley Hayes

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Landcare Research, Gerald Street, PO Box 40, Lincoln 7640, New Zealand, Ph +64 3 321 9999, Fax +64 3 321 9998, www.landcareresearch.co.nz

Reviewed by:	*Approved for release by:*
Professor Mike Boots Professor of Disease Biology University of Exeter	Professor Dave Choquenot GM Science and Policy Landcare Research
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Summary

Project and Client

- Eleven exotic dung-burying species of dung beetle (with native distributions in Europe and Africa) have recently been approved for full unconditional release onto New Zealand pastures by New Zealand's Environmental Protection Authority. Here we inform the perceived infectious disease risks of such releases
- This document constitutes a Landcare Research internal report. All work was conducted November 2011 – February 2012, and funded by the Ministry of Science and Innovation.

Objectives

- To categorise the perceived infectious disease risks of releasing dung beetles as either *primary*, *secondary* or *negligible*
- To inform the identified *primary* risks surrounding human and animal exposure to microbial agents linked to dung beetle activities (for which there is insufficient existing evidence on which to base sound judgement) through their roles as:
    - Transport hosts for bovine tuberculosis (TB)
    - Food sources for brushtail possums
    - Ecosystem engineers increasing microbe leaching into freshwater bodies
- To identify those disease risks that should be further informed beyond this study.

Methods

- The perceived infectious disease risks of releasing dung beetles were categorised as either *primary* (high potential risks, being based on likely potential mechanisms, that need to be further informed), *secondary* (low potential risks, being based on unlikely potential mechanisms) or *negligible* (with no biologically reasonable potential mechanisms) in a Background review
- Seven trials were designed to address specific knowledge gaps relating to the identified *primary* risks, utilising the most high risk beetle species (of those already present in the country or to be introduced) for each knowledge gap where relevant:
    - Whether or not dung beetles would become contaminated with TB through utilising the dung of infected cattle on farms in New Zealand
    - Whether or not dung beetles would become contaminated with TB through utilising the faeces of infected possums
    - Whether or not possums would forage for and eat dung beetles
    - Whether or not dung beetle tunnelling activity would increase microbe percolation through soil

- The implications of trial findings for the identified *primary* risks (also taking the Background review into account), and which (if any) disease risks need to be further informed through either post-release monitoring or other approaches, were discussed.

Results

- The Background review identified three *primary* risks needing to be further informed:
  - That contaminated dung beetles may potentially act as TB transport hosts, disseminating *Mycobacterium bovis* (the causative agent of TB) away from either cattle dung or possum faeces
  - That the utilisation of dung beetles on pasture as a food source by possums may increase their bush to pasture movements and, hence, potentially also increase rates of TB transmission between wildlife and cattle
  - That dung beetles may act as ecosystem engineers, with their tunnelling activity potentially increasing freshwater microbiological loading via increased groundwater contamination.
- The Background review also identified three *secondary* risks:
  - That contaminated dung beetles may potentially disseminate *M. bovis* away from the faeces of infected deer or pigs
  - That contaminated dung beetles may potentially disseminate *M. avium* subsp. *pseudotuberculosis* (MAP; the causative agent of Johne's disease) away from infected ungulate faeces
  - That contaminated dung beetles might potentially expose households to enteric pathogens sourced from cattle dung via guttering and water tanks.
- Bacteriological culture of the dung from naturally infected cattle, sampled from the most infected farm in New Zealand, did not detect *M. bovis*, demonstrating that dung beetles are unlikely to become contaminated with TB through utilising the dung of those infected cattle currently on farms in New Zealand
- Dung beetles barely acknowledged the presence of possum faeces (let alone utilised it for brood-balls) in no-choice trials, demonstrating that they are unlikely to become contaminated with TB through utilising the faeces of infected possums
- No dung beetles were consumed by possums in cage trials with a limited diversity of alternative food present, demonstrating that possums are at least not likely to forage for and eat dung beetles
- Dung beetle presence caused no increase in microbial leaching from experimental soil cores with cattle faeces placed on top, demonstrating that their tunnelling activity is unlikely to increase microbe percolation through soil.

Conclusions

- The need for trials to inform three *primary* risks indicates that some of the specific concerns raised regarding the potential infectious disease risks of dung beetle releases onto New Zealand pastures were justified

- For the *primary* risk of dung beetles potentially acting as TB transport hosts (with respect to either cattle dung or possum faeces), our findings that beetles are unlikely to become contaminated through utilising either the dung of those infected cattle currently on farms in New Zealand, or the faeces of infected possums, demonstrate that the actual risk is *negligible* under current circumstances
- However, should TB herd-testing protocols be altered in such a way that allows the disease in cattle on farms to progress to a more advanced stage, at which *M. bovis* can be excreted in dung, this risk should be reconsidered. Short-term cattle destocking to remove dung beetles from areas of TB concern would be a viable management option should such a risk eventuate
- For the *primary* risk of possums potentially utilising dung beetles on pasture as a food source, our finding that possums are at least not likely to forage for and eat dung beetles demonstrates that the actual risk is *low* (i.e. a *secondary* risk). We are unable for now to classify this risk as *negligible*, since there is a small possibility that the relatively simplistic protocols and short duration of our feeding trials were insufficient for possums to learn that the beetles were potential food
- For the *primary* risk of dung beetles potentially increasing freshwater microbiological loading, our finding that their tunnelling activity is unlikely to increase microbe percolation through soil demonstrates that the actual risk is *negligible*
- There is no current need to further inform the *primary* risks identified here through either post-release monitoring or other approaches. However, the potential future risk of dung beetles acting as TB transport hosts, if TB herd-testing is altered in such a way that allows excretion of *M. bovis* in cattle to occur dung, should be further informed.

Recommendations

- Our experimental evaluation indicates there is no current need to further inform the *primary* infectious disease risks of releasing dung beetles onto New Zealand pastures. The planned unconditional releases can thus proceed without concern of high potential disease risks
- A captive trial in which dung beetles are reared on *M. bovis* contaminated cattle dung should be conducted as a first step to informing the potential future risk of dung beetles acting as TB transport hosts, should TB herd-testing protocols in New Zealand be altered in such a way that allows the disease in cattle on farms to progress to a more advanced stage
- In light of the above, trials to identify and ground truth efficient protocols for removing dung beetles from farms by short-term cattle destocking should also be considered, so that such a management option would be readily available if needed
- If further assurance regarding dung beetles and infectious disease risks was required, post-release monitoring could be used to inform the *secondary* disease risks identified. For the risk of possums potentially utilising dung beetles on pasture as a food source, such monitoring could take the form of the ‘Assessment of wild possum gut contents’ trial described herein (see 4.6).

1 Introduction

Eleven exotic dung-burying species of dung beetle have recently been approved for full unconditional release onto New Zealand agricultural pastures by New Zealand's Environmental Protection Authority (ERMA 2010). These will join four exotic dung-burying species already present in the country: the Mexican dung beetle *(Copris incertus)* that has persisted in Northland since its intentional introduction in 1956 (Thomas 1960), two Australian species (*Onthophagus granulatus* and *O. posticus*) that were accidently introduced over a century ago and are now patchily distributed over much of the country (Emberson & Matthews 1973), and one South African species (*Epirinus aeneus*) likewise accidently introduced and now established near Christchurch (Dymock 1993). Approval for the new releases was based on there being clear benefits of dung beetle activity on agricultural land (Fincher 1981; Dymock 1993; Nichols et al. 2008; ERMA 2010). These include increased soil health and fertility, reduced nutrient runoff and waterway pollution, reduced greenhouse gas emissions, and reduced parasitism of livestock, all principally due to the rapid mechanical transport of cattle dung underground for the creation of brood balls (Hanski & Cambefort 1991). The aim of releasing a further 11 species was to enable at least one species to persist on each of the different pasture types and climate stocked with cattle in New Zealand (DBRSG 2010; Edwards 2010).

As is required by any risk assessment, the ERMA review process considered potential risks and costs of releasing further dung beetle species onto New Zealand pastures, including greater nutrient leaching leading to increased eutrophication, the displacement of native beetle species, and increases in some parasites (DBRSG 2010). These potential adverse effects were considered either unlikely or negligible based on (1) evidence from literature reviews and modelling, (2) there having been no observed adverse effects of *C. incertus* introduction to New Zealand in over half a century (and, indeed, no observed adverse effects of the three dung-burying species accidently introduced either), and (3) as with *C. incertus* (still restricted to farmland around its introduction sites in Northland), all species chosen for release having both narrow habitat preferences (open grassland) and specific host-preferences (ungulate dung; DBRSG 2010, and references therein). In addition, over 20 exotic dung beetle species have been introduced to both Australia and the United States with no adverse effects to native or beneficial species being reported (DBRSG 2010). As a result, the approval for release was made unconditionally (i.e. with no requirement for controls of any form).

In spite of the formal risk assessment approach taken by ERMA (as standard practice), the decision to grant approval for unconditional release has been protested by several parties (Guildford 2011). Aside from general concern about the quality of the release application and the ERMA approval process, specific concerns include potential risks (both to native biodiversity and regarding infectious diseases) for which it is perceived that insufficient information is available on which to base sound judgement regarding unconditional release.

Initial releases of the dung beetles are planned to occur during 2012. Here we present a set of considerations and trials designed to inform the perceived infectious disease risks of such releases. This document constitutes a Landcare Research internal report. All work was conducted November 2011 – February 2012, and funded by the Ministry of Science and Innovation.

2 Background

Two key categories of infectious-disease-related concern linked to the release of dung beetles onto New Zealand agricultural pastures have been raised:

- Enhanced livestock disease risks due to potential increases in the persistence and dissemination of *Mycobacterium bovis* (the causative agent of bovine tuberculosis, TB) and *M. avium* subsp. *pseudotuberculosis* (MAP, the causative agent of Johne's disease in livestock)
- Potential public health risks primarily due to possible increases in freshwater (including drinking water) microbial contamination.

For the purpose of this Background review, *primary* risks are defined as high potential risks, being based on likely potential mechanisms, that need to be further informed, *secondary* risks are defined as low potential risks, being based on unlikely potential mechanisms, and *negligible* risks are defined as those with no biologically reasonable potential mechanisms.

2.1 Livestock disease risks

The application to release further dung beetle species into New Zealand (DBRSG 2010) and the Environmental Protection Agency's assessment of the application (ERMA 2010), did not consider either TB or Johne's disease related risks specifically. Rather 'infectious disease' risks were considered generically. Given that both TB (in cattle) and Johne's disease (in deer) are major concerns for New Zealand's agricultural industry, with TB management being the focus of an ongoing national strategy managed by the Animal Health Board, this could be viewed as an important ommission. Indeed, three specific areas have been raised following the regulators decision as needing to be considered and assessed (Guildford 2011). These are outlinned below.

2.1.1 Dung beetles as disseminators of livestock-disease-causing agents

It has been raised that contaminated dung beetles may disseminate *M. bovis* and MAP by their own movements (i.e. as 'transport' hosts). The close association of dung beetles with faecal material, if this was derived from infected animals and contained mycobateria, would indeed create a potential risk of dissemination via internal or external contamination. In the context of the current report, this possibility constitutes a *primary risk* with respect to TB and faeces from infected cattle or brushtail possums (*Trichosurus vulpecula*).

The possum is not an ungulate, and thus its faecal material should not be utilised by the dung beetle species proposed for release. However, the possum is the key reservoir species allowing the persistence of TB in the wild in New Zealand (Nugent 2011), and therefore the potential contamination of beetles through utilisation of its faeces needs to be considered. For TB and faeces from infected deer or pigs, this possibility constitutes a *secondary* risk since, while individuals can be infected with TB, they are not reservoirs of infection (i.e. species in which infection can persist; Nugent 2011). Additionally, with MAP already commonly found in environmental reservoirs (Whittington 2005; Pavlik et al. 2010), this possibility also constitutes a *secondary* risk with respect to this mycobacteria and any infected ungulate host.

It has additionally been raised that dung beetles might amplify MAP in the environment if it can replicate in their guts. However, with mycobacteria being obligate vertebrate pathogens (O'Reilly & Daborn 1995), the risk of such replication is in our view *negligible*.

2.1.2 Dung beetles as food

It has been raised that the utilisation of dung beetles on pasture as a food source by vertebrate wildlife reservoirs of *M. bovis* may increase bush-to-pasture animal movements and, hence, potentially also increase rates of TB transmission between wildlife and cattle. Beetles are utilised globally as a food resource by many vertebrate species, thereby creating a potential risk of increasing wildlife reservoirs of TB. In our view this constitutes a *primary risk*.

It has also been raised that other foragers such as rats, bats and birds may ingest TB-contaminated dung beetles and occasionally transmit the infection throughout the region. However, mycobacteria are efficiently broken down during passage through the vertebrate gastrointestinal tract (Aldwell et al. 2003). Hence, in our view there is *negligible* risk that either *M. bovis* or MAP could be disseminated in such a way.

2.1.3 Dung beetles increasing infective-stage environmental loading

It has been raised that the burial of cattle dung by dung beetles may possibly increase the environmental loading of those pathogenic microbes for which the infective stages remain viable longer when buried than when exposed on the pasture. Potential candidates for such effects include not only *M. bovis* and MAP, but also other agents of concern to both livestock and public health including *Escherichia coli, Cryptosporidium, Salmonella, Campylobacter, Giardia, Listeria* and *Yersinia* (Guildford 2011). Although such a risk was considered in the ERMA application and review process for parasitic helminths (and assessed as *negligible*), it was not explicitly considered for microbes. The survival of *Cryptosporidum* has been shown to be reduced over five-fold when buried by dung beetles (as opposed to remaining on the surface; Mathison & Ditrich 1999; Ryan et al. 2011), and ingestion by certain dung beetles can significantly reduce the abundance of viable *Giardia* cysts (Miller at al. 1961). However, as far as we are aware there is currently no similar data with which to assess the other agents of concern.

In conjunction with the possibility of dung-beetle activity increasing infective-stage longevity, there is also the potential offsetting benefit of infective-stage removal from the pasture surface. This would decrease their availability for both direct infection of subsequent hosts and dissemation by contamination of dung-utilising fly species. Studies showing that dung beetle activity removes up to 90% of helminth infective stages from the pasture surface (Waterhouse 1974; Fincher 1975; Bryan 1976) indicate that the beetles could be simililarly efficacious for the removal of pathogenic microbes.

In our view the evidence supports dung-beetle induced <u>reductions</u> in infective-stage viability for the diseases of concern. Hence, the possibility that burial of dung will increase infective-stage environmental loading is considered *negligible*. Even if infective-stage survival was increased, it would need to be by at least an order of magnitude to have consequences for animal infection or infective-stage dissemination by flies, and then only if all of the buried material was returned to the pasture surface.

2.2 Public health risks

The application to release further dung beetle species into New Zealand (DBRSG 2010) considered the potential benefits to public health of the removal of dung from the ground surface reducing both risks from diseases originating in faeces (such as campylobacteriosis; a benefit classified as *unlikely*) and nuisance fly numbers (being potential disease vectors; a benefit classified as *likely*). However, two main public health concerns have been raised that were not considered (Guildford 2011).

2.2.1 Dung beetles as disseminators of human-disease-causing agents

Similar to the livestock issues discussed in 2.1.2, dung beetles could also potentially act as disseminators of human-disease-causing agents from dung such as *E. coli, Cryptosporidium, Salmonella, Campylobacter, Giardia, Listeria* and *Yersinia* (in addition to *M. bovis* and MAP). If dung from infected animals contained infective stages of disease organisms there is indeed clear potential risk of dissemination via internal or external contamination of the beetles. However, evidence from dung beetle behaviour (i.e. the seeking of ungulate dung for breeding; Hanski & Cambefort 1991), indicates that the risk of increased exposure of humans to infection via this process is far less than the risk of increased exposure of cattle. There are also likely offsetting benefits associated with a resource-limited driven reduction in nuisance fly numbers. In our view, the risk of an overall increase in human infection sourced from dung beetle activity on cattle faeces is thus *negligible*.

There is one specific human disease risk pathway, however, that deserves further consideration. As Guildford (2011) notes, 'nocturnal and crepuscular dung beetles…have been reported to be attracted to the lights of homesteads and barns. These dung beetles have the potential to collect in guttering and water tanks…this may create…higher exposure of households to enteric pathogens.' With 10% of New Zealanders deriving their water from roof collection (Guildford 2011), this represents a disease-risk pathway that cannot be classified as *negligible*. Rather, accounting for the offsetting benefit of reduced nuisance fly numbers,we suggest it constitutes a *secondary* risk.

2.2.2 Dung beetles increasing freshwater microbiological loading

If dung burial *were* to increase subsoil loading of microbial-disease-causing agents (see 2.1.3 above), one potential risk pathway for increased human exposure to infection sourced from dung would be via increased microbial percolation through soil into groundwater, and eventually into freshwater running through or adajcent to pasture (Aislabie et al. 2001). Tunnelling activity by dung beetles may provide biopores that enhance microbial-bypass-flow processes in soil through acting as conduits for water (McLeod et al. 2001). Microbial percolation and the enhancement of such activities by biopores are both proven mechanisms, and clear effects of agricultural contamination of freshwater by such routes are already evident in New Zealand (MfE 2007). Therefore, in our view increased microbial loading of freshwater due to dung beetle activity constitutes a *primary* risk. Although the likely benefit of such contamination being reduced by decreased runoff from the soil surface (due to dung burial) was considered by both the application to release further dung beetle species into New Zealand (DBRSG 2010) and it's assessment (ERMA 2010), the potential risk of increased contamination via groundwater was not.

3 Objectives

- To categorise the perceived infectious disease risks of releasing dung beetles as either *primary*, *secondary* or *negligible*
- To inform the identified *primary* risks surrounding human and animal exposure to microbial agents linked to dung beetle activities (for which there is insufficient existing evidence on which to base sound judgement) through their roles as:
  - Transport hosts for bovine tuberculosis (TB)
  - Food sources for brushtail possums
  - Ecosystem engineers increasing microbe leaching into freshwater bodies
- To identify those disease risks that should be further informed beyond this study.

4 Methods

- The perceived infectious disease risks of releasing dung beetles were categorised as either *primary* (high potential risks, being based on likely potential mechanisms, that need to be further informed), *secondary* (low potential risks, being based on unlikely potential mechanisms) or *negligible* (with no biologically reasonable potential mechanisms) in the Background review (see 2 above)
- Seven trials were designed to address specific knowledge gaps relating to the identified *primary* risks, utilising the most high risk beetle species (of those already present in the country or to be introduced) for each knowledge gap where relevant:
  - Whether or not dung beetles would become contaminated with TB from utilising the dung of infected cattle on farms in New Zealand (see 4.1 below)
  - Whether or not dung beetles would become contaminated with TB from utilising the faeces of infected possums (4.2 – 4.4)
  - Whether or not possums would forage for and eat dung beetles (4.5 – 4.6)
  - Whether or not dung beetle tunnelling activity would increase microbe percolation through soil (4.7)
- The implications of trial findings for the identified *primary* risks (also taking the Background review into account), and which (if any) disease risks need to be further informed through either post-release monitoring or other approaches, were discussed.
- Assessment of TB presence in the dung of infected cattle

Working with the cattle herd in New Zealand that has by far the highest number of TB-infected individuals (AHB 2009a), for those animals identified as infected at slaughter between November 2011 and February 2012, faeces were removed and assayed for the presence of *M. bovis* using the ‘gold standard’ culture methodology (Denis et al. 2005; Wedlock et al. 2005). Detection of *M. bovis* in the faeces of these individuals would demonstrate that there is a potential risk of increased TB dissemination from infected cattle on farms in New Zealand due to dung beetles becoming contaminated through utilising their faeces; no such detection would demonstrate that there is little such risk.

4.1 Assessment of the use of possum faeces by dung beetles

To investigate the ability of introduced dung-beetles to utilise possum faeces, no-choice host range tests were performed. *Onthophagus taurus* was selected for testing; being the smallest of the species earmarked for introduction (body length c. 9 mm; Tyndale-Biscoe 1990), it was considered the species most likely to be capable of nest building using small-volume possum faeces (the larger a dung beetle is, the more material it generally requires for brood-ball construction; Hanski & Cambefort 1991). The possum faeces used for the trial comprised large scats (with a mean wet weight of 10 g) obtained from wild-caught possums held in the Landcare Research captive facility at Lincoln, Canterbury, and smaller pellets (with a mean wet weight of 3 g) obtained by dissecting the bowel contents of freshly killed wild possums from Northland. The larger scats of the captive animals is due to the unnatural diet of cereal and apple on which they are maintained.

Ten-litre ventilated plastic containers were two-thirds filled with moistened compact sandy loam soil, into each of which two pairs (one male and one female per pair) of *O. taurus* adults were added along with either possum faeces (treatment) or cow dung (control). Four beetles per container is sufficiently few, with respect to the volume of dung resource provided, to minimise competitive interactions (Hanski & Cambefort 1991). Eight experimental replicates containing possum faeces (four with large scats and four with smaller pellets), and eight control replicates containing cow dung, were run between 24 November and 14 December 2011. One scat or four pellets were used per possum faeces replicate (simulating a typical amount produced in a defaecation event; G. Nugent, unpubl. data), dropped from 20 cm above the container. Control dung was obtained from organically reared Angus beef cattle; 250 g (wet weight) of dung was used for each control replicate, also dropped from 20 cm into the containers.

Each replicate was left undisturbed for seven days, after which the surface dung was photographed in situ and overturned to reveal any feeding damage and tunnelling evidence in the dung and soil directly beneath the dung. The following variables were recorded: total number of tunnels; number of tunnels associated with dung; number of tunnels not associated with dung. The soil beneath the dung was excavated and any brood balls (i.e. dung balls containing beetle egg chambers) were counted. Evidence of dung beetles utilising possum faeces, particularly for brood-ball construction from which offspring would subsequently emerge, would demonstrate that there is a potential risk of TB dissemination from infected brushtail possums due to dung beetles becoming contaminated from utilising their faeces; no such evidence would demonstrate that there is little such risk.

4.2 Assessment of beetle contamination through the use of possum faeces

Contingent upon the demonstration of a high potential risk of increased TB dissemination from infected brushtail possums through dung beetle activity in 4.2 above, an assessment was planned of whether beetles become contaminated with *M. bovis* through the use of possum faeces containing *M. bovis* bacilli. Beetles were to be provided with possum faeces artificially contaminated with a 100-fold range of *M. bovis* bacilli. Upon brood-ball completion (or 1week post-ingestion, if investigating contamination through feeding only), emerging adults were to be assayed (again using the 'gold standard' culture methodology; Denis et al. 2005; Wedlock et al. 2005) for the presence of *M. bovis* both on the carapace and within the gut.

Evidence of dung beetles becoming contaminated with *M. bovis* from using infected possum faeces would demonstrate that the potential risk of increased TB dissemination from infected possums through dung beetle activity, as identified in 4.2, may actually be realised in the field; no such evidence would demonstrate that there is little such risk. Assessment of TB in/on adult beetles constructing brood balls with contaminated faecal material would also provide a conservative assessment of the contamination of later emerging offspring.

4.3 Assessment of TB presence in the faeces of terminally infected possums

Contingent upon the demonstration of high potential risk of increased TB dissemination from infected possums through dung beetle activity in both 4.2 and 4.3 above, an assessment was planned of the level at which *M. bovis* is actually present in the faeces of terminally infected possums. For this, it was planned to obtain faecal pellets from the bowel contents of freshly killed wild possums in the terminal stages of natural infection with TB, from the Orongorongo Valley, Rimutaka Forest Park, New Zealand (Tompkins et al. 2009). As for the assessment of cattle faeces in 4.1, pellets were to be assayed for the presence of *M. bovis*, again using the 'gold standard' culture methodology (Denis et al. 2005; Wedlock et al. 2005).

Evidence of *M. bovis* presence in the faeces of infected possums, at levels demonstrated in 4.3 to result in beetle contamination with *M. bovis* from using such faeces, would demonstrate that the potential risk of increased TB dissemination from infected possums through dung beetle activity (as identified in 4.2 and 4.3) is likely to be realised in the field; no such evidence would demonstrate that there is little such risk.

4.4 Assessment of whether possums will eat dung beetles

To investigate whether possums will forage for and eat dung beetles, feeding trials were conducted whereby wild-caught possums held in the Landcare Research captive facility at Lincoln, Canterbury, were provided with live adult dung beetles in addition to their normal captive diet of apple and cereal.The larger Mexican dung beetle (body length c. 15mm) was used for this assessment, based on the assumption that larger beetles would be a more attractive food source than smaller beetles to omnivorous possums, and that this beetle is nocturnally active (as are possums).

Beetles were exposed to a total of nine adult possums in December 2012. For each replicate, a patch of soil and grass (30 × 20 × 10cm) was dug up and placed into a plastic container. The grass surface was covered in cow dung onto which four adult beetles were placed, and then exposed to a single possum in a small cage for 2 days. The number of beetles remaining was counted after each exposure, by gently pulling apart the turf patch, and any evidence of possum foraging in the patch noted.

Utilisation of beetles as food, or evidence that possums actively forage for them, would demonstrate that the presence of dung beetles would potentially increase possum movements onto farmland, and hence potentially increase TB transmission rates from this wildlife reservoir to cattle; no such evidence would demonstrate that there is little such risk.

4.5 Assessment of wild possum gut contents

Contingent upon the demonstration of a high potential risk of new dung beetle species presence increasing the movement of possums onto farmland, an assessment of the gut contents of wild possums was planned. Possums were to be trapped in bush patches adjacent to farms where high abundances of the Mexican dung beetle already occur in New Zealand, and their gut contents analysed. The presence of dung beetle mouthparts and exoskeletons would demonstrate that the potential risk of new dung species' presence increasing the movement of brushtail possums onto farmland, as identified in 4.5, may actually be realised in the field; no such evidence would demonstrate that there is little such risk.

4.6 Assessment of dung beetle effects on microbial percolation through soil

To investigate whether the tunnels (i.e. biopores) that dung beetles build can enhance microbial-bypass-flow processes in soil (through acting as conduits for water), we conducted leaching experiments using experimental soil cores (undisturbed barrel lysimeters; McLeod et al. 2001). Specifically, we used this previously validated approach to test whether the concentration of *E. coli* and MAP in soil leachate (with the microbes sourced from naturally infected cattle dung placed on the tops of cores) was altered by dung beetle activity. The larger Mexican dung beetle (body length c. 15 mm) was used for this assessment because the larger tunnels constructed by this species would be more likely to influence soil drainage properties than those of smaller species.

Six soil lysimeters (each measuring approximately 500 mm in diameter by 600 mm high) were hand-carved in situ. The soil was a shallow fine sandy loam with 300 mm of fines over gravels, representing a common stony soil type in the Mackenzie Basin (a potential area for conversion to dairy farming), previously ranked as low to possibly medium for microbial bypass flow (McLeod et al. 2012). A 10 mm internal annulus within each core was filled with petroleum jelly to prevent water preferentially flowing at the soil–casing interface, and a sampling port installed in the centre of the base of each core to allow collection of leachate. The lysimeters were transported from the field to Hamilton where they were irrigated with tap water to field saturation then allowed to drain before application of cowpats and dung beetles. Background levels of *E. coli* in the leachate were determined from samples taken at the end of the wetting-up period. Full details on the collection of undisturbed barrel lysimeter cores and leaching experiments are given in McLeod et al. (2001). Fresh dung was collected from a dairy herd known to be infected with MAP, and 2.5 kg (reflecting an average excretion volume) placed onto the surface of each core. Twenty-two adult dung beetles were introduced onto each of three of the cores with dung (cores covered with shade cloth and secured with bungy cords to retain beetles), with three left as similarly covered no-beetle controls. The cores were left outside and irrigated with tap water intermittently to ensure they did not dry out. After 11 days (giving the beetles sufficient time to construct burrows and brood chambers) they were transferred into the laboratory and irrigated continuously with tap water at a rate of 5 mm per hour (a realistic standard for trials of this type; McLeod et al. 2012) using a drip-type rainfall simulator to about one pore volume (PV) or about 250 mm depth of irrigation. One PV is the amount of space in the soil core occupied by soil pores or cracks; this is typically about 45–55% of the total soil volume and for these soils was about 44 litres. Background levels (no dung application) of *E. coli* in the leachate to one PV had previously been established (McLeod et al. 2012).

One-litre leachate samples were collected approximately hourly from each replicate into sterile Schott bottles for a total of 44 samples. Subsamples for *E.coli* assay were stored at 4°C, while subsamples for MAP assay were frozen at −80°C. At the end of the trial a sample was collected aseptically from the centre of the residual cowpat for *E. coli* enumeration and dry weight determination (weight following drying at 105°C for 24–30 hours). Numbers of *E. coli* in soil, soil leachates and cowpats were determined using the Colisure-quanti-tray technique (IDEXX Laboratories, Inc.), with samples diluted in sterile water if required. Evidence that dung beetle activity influences *E. coli* leaching from soil would demonstrate that there is a potential risk of dung beetles increasing microbe percolation through soil; no such evidence (at this small scale, in a soil-type sensitive to such effects, and with a highly labile microbe; Aislabie et al. 2001) would demonstrate that there is little such risk. Frozen samples were stored for follow-up assay for MAP, contingent on evidence of *E. coli* percolation rates being affected by dung beetle activity in the trials.

5 Results

As detailed in the Background review (see 2 above), three *primary* infectious disease risks of releasing dung beetles needing to be further informed were identified:

- That contaminated dung beetles may potentially act as TB transport hosts, disseminating *M. bovis* away from either cattle dung or possum faeces
- That the utilisation of dung beetles on pasture as a food source by possums may increase their bush to pasture movements and, hence, potentially also increase rates of TB transmission between wildlife and cattle
- That dung beetles may act as ecosystem engineers, with their tunnelling activity potentially increasing freshwater microbiological loading via increased groundwater contamination.

The Background review also identified three *secondary* risks:

- That contaminated dung beetles may potentially disseminate *M. bovis* away from the faeces of infected deer or pigs
- That contaminated dung beetles may potentially disseminate *M. avium* subsp. *pseudotuberculosis* (MAP; the causative agent of Johne’s disease) away from infected ungulate faeces
- That contaminated dung beetles might potentially expose households to enteric pathogens sourced from cattle dung via guttering and water tanks.

5.1 Assessment of TB presence in the dung of infected cattle

Faecal samples collected from twelve tuberculous cattle, at least three of which had sufficiently generalised TB for the carcass to be condemned, have failed to yield any positive cultures to date. Although cattle in advanced stages of TB can excrete *M. bovis* in dung (Menzies & Neill 2000), our study demonstrates that under current herd testing protocols in New Zealand any infected cattle are removed well before they develop such symptoms.

Hence, dung beetles are unlikely to become contaminated with TB through utilising the dung of infected cattle on farms in New Zealand.

5.2 Assessment of the use of possum faeces by dung beetles

The beetles in the cow dung replicates dug significantly more tunnels than those in the possum faeces replicates (Fig. 1), both in total (mean of 8.1 versus 3.3 tunnels respectively; Kruskal–Wallis H = 11.58, df = 1, $P < 0.001$) and in the faecal material alone (mean of 7.6 versus 0.3 tunnels per replicate respectively; H = 11.58, df = 1, $P < 0.001$). In addition, an average of one brood ball was constructed per cow dung replicate (range 0–2 brood balls), in contrast to none in the possum faeces replicates (H = 4.885, df = 1, $P < 0.05$). The number of tunnels not associated with dung was significantly lower in the cow dung than in the possum faeces replicates (mean of 0.63 versus 2.8 respectively; H = 7.741, df = 1, $P < 0.01$).

These results demonstrate that dung beetles are unlikely to become contaminated with TB through utilising the faeces of infected possums. The 'Assessment of beetle contamination through the use of possum faeces' and 'Assessment of TB presence in terminally infected possum faeces' trials were thus not required.

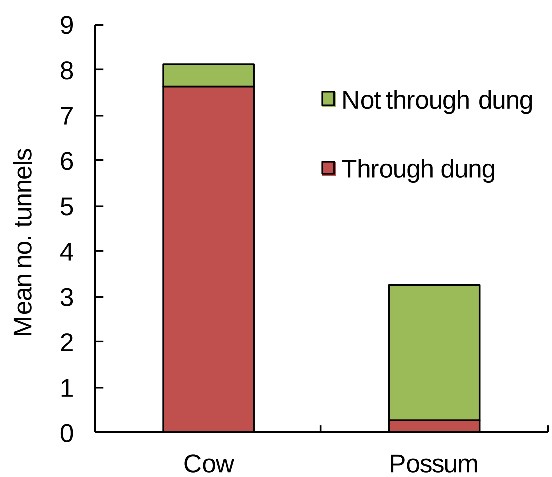

Figure 1 Mean number of tunnels through the dung and not associated with dung for both cow dung and possum faeces treatments.

5.3 Assessment of whether possums will eat dung beetles

All dung beetles were accounted for at the end of all nine possum feeding trial replicates. In addition, there was no evidence of any possum foraging for the beetles. Although there is a small possibility that the relatively simplistic protocols and short duration of these trials (two days only, for animal welfare with respect to cage fouling reasons) were insufficient for possums to learn that the beetles were potential food, this demonstrates that possums are at least not likely to forage for and eat dung beetles. The 'Assessment of wild possum gut contents' trial was thus not required for this report.

5.4 Assessment of dung beetle effects on microbial percolation through soil

Background *E. coli* was only detectable at a low level (<20 per 100 ml) in one of the six lysimeters (dung-only Replicate 1; Table 1). This is unsurprising since the soil cores were collected from alongside an airfield where, to the best of our knowledge, the soil had not been grazed by animals for a number of years. *E. coli* concentrations in the dung ranged from 1.4 to 8.0 ($\times 10^6$) colony forming units (cfu) per gram dry weight at the end of the trial (Table 1).

Ponding of irrigation water was observed on the top of one lysimeter (dung plus beetles Replicate 3), indicating that water movement was restricted in this soil core compared with the other replicates. In the 44 leachate samples collected from each of the remaining five experimental lysimeters during the rainfall simulation trial, *E.coli* was only detectable at any time point for two of the dung-only soil cores (dung-only Replicates 1 and 3) and one of the dung-plus-beetles soil cores (dung-plus-beetles Replicate 2). Of note, the *E. coli* leachate flow from dung-plus-beetle Replicate 2 was of a lower concentration and shorter duration (detected in only one subsample) than that detected from dung-only Replicates 1 and 3 (detected in subsamples 6 and 12 respectively), and additionally was of a lower concentration than the background level detected from cow-dung-only Replicate 1 before the trial (Table 1).

Table 1 Detection and concentration (mean plus range) of *E. coli* colony forming units (cfu) in 100 ml subsamples from 44 one-litre leachate samples collected from stony soil cores treated with either cow dung only or cow dung plus dung beetles, during a rainfall simulation trial. An *E. coli* concentration of less than one cfu per 100 ml indicates no detection on the assay used. One control soil core contained neither dung nor beetles. The *E. coli* concentration of the dung remaining in each lysimeter at the end of the trial was also enumerated (expressed as 10^6 cfu per gram dry weight).

Treatment	Replicate	*E. coli* concentration before trial	Samples *E. coli* detected during trial	Sample with peak *E. coli* concentration	Peak *E. coli* concentration	*E. coli* in dung at trial end
Control	na	< 1	na	na	na	na
Cow dung only	1	11.7 (4.1 – 17.5)	39–44	41	13.5 (7.8 – 23.4)	2.6 (1.6 – 3.8)
Cow dung only	2	< 1	na	na	na	5.8 (4.1 – 7.9)
Cow dung only	3	< 1	33–44	40	50.4 (33.9 – 70.9)	4.1 (2.8 – 5.7)
Dung plus beetles	1	< 1	na	na	na	2.3 (1.4 – 3.6)
Dung plus beetles	2	< 1	43	43	5.2 (2.4 – 12.1)	3.0 (1.9 – 4.4)
Dung plus beetles	3	< 1	na*	na*	na*	9.1 (6.6 – 12.3)

* Dung plus beetles Replicate 3 was non-comparable with other replicates due to ponding of irrigation water

All leachate *E. coli* concentrations detected in this trial were well below the Ministry for the Environment guideline level of 550 cfu *E. coli* per 100 ml (indicating a level of freshwater microbiological contamination that poses an unacceptable public health risk; MfE & MOH 2003). Moreover, *E. coli* concentrations in leachate from well-drained undisturbed soil cores with dairy shed efluent placed on top have been demonstrated to reach as high as 1.1×10^7 cfu per 100 ml (Aislabie et al. 2001). The results from this current trial (at this small scale, in a soil type sensitive to such effects, and with a highly labile microbe; Aislabie et al. 2001) demonstrate that dung beetle tunnelling activity is unlikely to increase microbe percolation through soil. Given this, no follow-up assays for MAP were required.

6 Conclusions

The need for trials to inform three *primary* risks indicates that some of the specific concerns raised regarding the potential infectious disease risks of dung beetle releases onto New Zealand pastures were justified.

6.1 Dung beetles as TB transport hosts

That dung beetles may act as TB transport hosts, potentially resulting in both an increased frequency and severity of herd outbreaks, is one of the key perceived disease risks associated with the release of further dung beetle species onto farms in New Zealand (Guildford 2011). The main mechanism postulated to create this risk is that dung beetles and their progeny will disseminate *M. bovis* away from infected cattle dung or possum faeces via contamination of either the carapace or the gut.

Here we informed this perceived risk by investigating (1) whether there is a source of TB-contaminated cow dung on farms in New Zealand for dung beetles to disseminate in this way, and (2) whether dung beetles will either feed on possum faeces or utilise them in brood-ball construction, providing the mechanism for TB to be potentially disseminated from contaminated possum faeces.

In our assessment of TB presence in the dung of infected cattle, we conducted 'gold-standard' screening for *M. bovis* of faeces removed from TB-infected cattle at slaughter. These individuals represented the most advanced stage of TB disease progression in New Zealand cattle that current herd testing protocols allow (AHB 2009b), and hence represented the greatest current risk for dung beetle exposure to TB-contaminated cattle dung in the country. We recorded no *M. bovis* in the samples screened, and therefore conclude that there is minimal risk of dung beetles disseminating *M. bovis* away from contaminated cattle dung under current farming conditions.

In our assessment of possum faeces use by dung beetles, adult pairs were exposed to realistic quantities of possum faeces in no-choice trials. With only a single tunnel dug through the possum faeces by a total of 32 beetles (and no brood-ball construction occurring) our trial demonstrates that possum faecal material is rarely explored, let alone utilised, by dung beetles. Furthermore, the increased tunnelling activity of the beetles through the soil in the possum faeces replicates, and the extensive tunnelling though and use of cow dung for brood-ball construction in the control replicates, indicate that even such minor exploration of possum faeces would likely not occur in the field. Hence, since the smallest beetle species proposed for release was used in our trials (considered to be the species most likely to be capable of developing using possum faeces), we can conclude that there is minimal risk of dung beetles disseminating *M. bovis* away from contaminated possum faeces.

These results demonstrate that there is a *negligible* current risk of dung beetles acting as TB transport hosts in New Zealand with respect to both cattle dung and possum faeces. However, should TB herd-testing protocols be altered in such a way that allows the disease in cattle on the ground to progress to a more advanced stage, at which *M. bovis* can be excreted in dung, this risk should be reconsidered. Short-term cattle destocking to remove dung beetles from areas of TB concern would be a viable management option should such a risk eventuate.

6.2 Dung beetles as brushtail possum food sources

That the presence of dung beetles on farms may increase possum movements onto pasture, with such movement potentially influencing the rate of TB transmission from this wildlife reservoir to stock, is a second key perceived disease risk associated with the release of further dung beetle species onto farms in New Zealand (Guildford 2011). The main mechanism postulted to create this risk is that of possums utilising dung beetles as a food resource.

Here we informed this perceived risk by investigating whether wild-caught possums held in captivity would eat a large species of dung beetle presented to them, along with their normal captive diet, in the context that would be encountered naturally (placed, with cow dung, on turf). While one may argue that 'no-choice' trials would be required to obtain a definitive answer, not only would such trials with possums raise ethical issues, they would also not be valid to possums in the wild. Brushtail possums are generalist omnivores able to browse on a wide range of vegetation (Montague 2000), and hence such a 'no-choice' situation would be unrealistic.

In the captive trials conducted here, with the possums being maintained on a relatively protein depauparate diet (apple and cereal), one can argue strongly that if possums could utilise dung beetles as a food source they would at the very least have foraged for them. However, there is still the small possibility that the length of these trials was insufficient for possums to learn that the beetles were potential food, or that some other discriminating behaviour was involved. Our results can thus only be equated to the actual risk of the proposed release of dung beetles onto farms in New Zealand causing increased movement of possums onto farmland as being currently *low* (i.e. a *secondary* risk) rather than *negligible*.

6.3 Dung beetles increasing microbiological contamination of freshwater bodies

That the presence of dung beetles on farms may increase the microbiological contamination of freshwater bodies is a third key perceived disease risk associated with the release of further dung beetle species onto farms in New Zealand (Guildford 2011). The main mechanism postulated to create this risk is that of tunnelling activity creating biopores that will enhance microbial-bypass-flow processes in soil through acting as conduits for water, thus potentially increasing the rate and amount of microbial transport into groundwater from cattle dung and effluent deposited on the soil surface. Here we informed this perceived risk by using leaching experiments to investigate whether dung beetle activity in undisturbed soil cores altered the concentration of *E. coli* in soil leachate (with the microbes sourced from naturally infected cattle dung placed on the tops of soil cores).

We found no evidence of such ‘ecosystem engineering’ effects occurring (1) at this small scale, (2) with a larger dung beetle species (based on the reasoning that the larger tunnels constructed by this species would be most likely to influence soil drainage properties), and (3) with the movement of a highly labile microbe through a soil type that would be sensitive to the enhancement of bypass flow properties being monitored. Our finding that their activity is unlikely to increase microbe percolation through soil thus demonstrates that the actual risk of dung beetles increasing freshwater microbiological loading is *negligible*.

7 Recommendations

- Our experimental evaluation indicates there is no current need to further inform the *primary* infectious disease risks of releasing dung beetles onto New Zealand pastures. The planned unconditional releases can thus proceed without concern of high potential disease risks
- A captive trial in which dung beetles are reared on *M. bovis* contaminated cattle dung should be conducted as a first step to informing the potential future risk of dung beetles acting as TB transport hosts, should TB herd-testing protocols in New Zealand be altered in such a way that allows the disease in cattle on the ground to progress to a more advanced stage
- In light of the above, trials to identify and ground truth efficient protocols for removing dung beetles from farms by short-term cattle destocking should also be considered, so that such a management option would be readily available if needed
- If further assurance regarding dung beetles and infectious disease risks was required, post-release monitoring could be used to inform the *secondary* disease risks identified. For the risk of possums potentially utilising dung beetles on pasture as a food source, such monitoring could take the form of the ‘Assessment of wild possum gut contents’ trial described herein (see 4.6).

8 Acknowledgements

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