Dung Beetles – Critique of the EPA's Assessment of the Benefits and Risks Relating to the Proposed Introduction of Exotic Dung Beetles to New Zealand

Part 1

Theoretical Benefits (Part 1)

Of the theoretical benefits relied on by the Agency, few have been objectively demonstrated at a farm or catchment level in the countries to which exotic dung beetles have been introduced, with the principal exception of the control of several pest fly species that are not present in New Zealand.

The proposed large economic benefits for the introduction of dung beetles into New Zealand (Forgie 2009) are based on prior work in beef range cattle in California (Losey 2006) and rely on a number of dubious assumptions in the original paper itself and in the subsequent extrapolations between dry land beef farming in California (where cow pats persist for 22-28 months) and New Zealand's temperate beef and dairy cattle farming (where cow pats persist for 1-6 months, Weeda 1967).

Even after the successful establishment of dung beetles in Australia, some CSIRO scientists remained sceptical that dung beetles would produce significant impacts on animal production especially in areas of high rainfall (Hughes 1975). They pointed out that estimates of the percentage of pasture covered by faeces (e.g. up to 5% in New Zealand) do *not* equate to estimates of productivity gain following dung beetle introduction (Hughes 1975). Unfortunately, in spite of the dung beetle introductions, anthelmintic use, drench resistance and nitrogenous fertilizer application continue to climb in Australia and water quality continues to deteriorate (Besier 2003, Eckard 2003, Hamer 2004, www.anra.gov.au/topics/water).

In considering the benefits from the introduction of dung beetles ERMA took the approach that given the wide variety of climates, topography, species assemblages and farming practices there could not be an estimate of a gross national benefit. Instead the approach was to accept that there would be significant but localised benefits to those farmers who managed to maximise the benefits from dung beetles. For example farmers could choose to use beetle friendly or unfriendly anthelmintics. Those choosing the former will need to manage more intensively to gain the benefits from dung beetles whereas those choosing the latter will not have dung beetles establishing on their farms. The benefits will accrue to those who wish to manage the dung beetles and for those who do not the status quo will remain.

The small number of field-level experiments (Miranda 2000, Bang 2005, Yamada 2007, Rosenlew 2008) relied on by the applicants to demonstrate pastoral productivity benefits (in response to criticism of their claims by Auckland City Council) were conducted in markedly different environments to New Zealand, demonstrate variable results or are poorly controlled. One field experiment (Brown, 2010) was mistakenly reported by the applicants to show reduced erosion when it actually showed higher soil losses and sediment concentration in the run-off from soil plots subjected to the burrowing activity of dung beetles.

While the above paragraph "*may be right in stating that 'strict' field-level experiments 'directly' related to 'pastoral productivity benefits' may be few, the literature on general benefits of dung beetle on nutrient recycling and dung decomposition etc. is broad and convincing. The real question is then what 'exact' benefits the new introductions are aimed at, and whether there is direct scientific proof such effects in a system like yours [New Zealand]*", (Pers. comm. T. Roslin (of Rosenlew and Roslin 2008)). As noted above the benefits were seen to be localised but significant for those who chose to manage for it.

On the 'mistakenly reported' in Brown et al. (2010) comment:

> "*After dung beetle activity on plots soil losses were higher on plots where dung beetles had been active. This was within a week of their burrowing activity where they bring soil to the surface as they excavate their tunnels. Similar concept to earthworm casts but they are a different consistency.*

Comment [A1]: Part 1of this document focuses on the EPA's analysis of the preliminary concerns of University of Auckland staff regarding the risk-benefits of exotic dung beetles. These concerns were sent to the EPA on 29 June 2011.The EPA has placed excerpts of these concerns in the pale green text boxes. Each text box is followed by the EPA's notes. Part 2of this document contains notes by the EPA on a literature review of dung beetles as vectors and reservoirs. This review was prepared by Prof Guilford in August 2011 to help answer the question posed by the EPA as to whether dung beetles can act as vectors of human or animal diseases. Excerpts from this review are again shown in the green text boxes followed by the EPA's notes. The comments in the margin of this document were made by Prof Guilford in April 2012 after the EPA released their notes in response to an Official Information Act request by Landcare Research Ltd – the Crown Research Institute that prepared the application to introduce the exotic dung beetles. These comments were given to the EPA on 12/4/12. No response was forthcoming. Minor updates to the comments in the margin were made by Prof Guilford on 9/2/13.

Comment [A2]: The EPA notes omit the concluding paragraph of the UoA concerns which reached a similar conclusion to the EPA. Specifically, "While these factors do not eliminate the possibility that some farming regions of NZ and some farming systems may eventually benefit from DBs they serve to show that tangible benefits are far from assured in our complex farming systems and their associated catchments". These conclusions (UoA and EPA) are in stark contrast to those of the applicants and their science provider which make unsubstantiated claims of very large benefits in their application to ERMA and their prior media releases. Many of these unsubstantiated claims are repeated by ERMA in its Evaluation and Review Report and its Decision (dated September 2010).

Comment [A3]: There are many other constraints on the choice of preferred anthelmintic including cost, availability, efficacy and drench resistance. It is likely that this issue alone will significantly reduce the benefits of dung beetles to NZ pastoral farming systems.

Comment [A4]: There are also good quality studies at the field level which question the benefits of dung beetles on herbage growth e.g. Yamada D Grassland Science 53:121-129, 2007.

Comment [A5]: Yes – this is the key question and the answer is that there is no direct scientific proof in NZ farming systems in spite of the opportunity to test for these benefits using the dung beetles established in Northland.

6 months later, the soil losses were lower on the plots where dung beetles had been active (compared to controls) because the increased infiltration rates produced by the dung beetle activity meant a sustained improvement in infiltration rates. Fig 1 d. in the paper shows this very clearly.

It is obvious. If you dig a hole and leave some soil at the surface, it will wash away. But because there is a hole, more water will penetrate the soil resulting in less surface runoff in the long term." (Pers. comm. Brown.)

There are many possible reasons for the discordance between the theoretical promise of dung beetles and the reality at the farm and catchment level. The establishment, maintenance and relative benefits of dung beetles can be affected by a wide array of biological, agricultural, social and economic factors including: anthelmintic and insecticide usage, temperature and rainfall patterns, irrigation, soil types and moisture levels, sanitisation of faecal pats by sunlight, stocking rates, rotational grazing systems, soil compaction by livestock, the watery dung of pasture-fed dairy cattle, beetle pathogens and predators, earthworm abundance, complex interactions with earthworms and other dung dwelling fauna, the relatively greater pollution from urinary versus faecal nitrogen, seasonal periods of pasture surplus, supplementary feeding during seasonal periods of pasture deficit, insufficient predictability of dung beetle benefits to convince farmers to risk changes to management practices, and an uncertain financial model for the introduction and maintenance of the beetle populations (Dymock 1993, Kebreab 2001, King 2007, Nichols 2008, Forgie 2009).

The dung beetle evaluation took this into account but considered that the localised benefits were significant and sufficient for an approval for released to be made.

Comment [A6]: This is misleading; the results of the paper showed a statistically significant $p<0.001$ greater concentration of sediment in runoff from dung beetle treated plots during active burial of dung by the beetles; 6 months after dung beetle activity had stopped there was no statistical difference between the sediment arising from control and dung beetle plots.

Comment [A7]: The Applicants are proposing to introduce dung beetles that will be active all year round, day and night. Thus, there will always be fresh soil to wash away. Brown notes his study does not provide a total comparative soil loss budget over time.

Comment [A8]: It is notable that many of these issues are very difficult for farmers to manage 'to maximise the benefits from dung beetles' as imagined by the ERMA. For instance, the watery dung resulting from ingestion by cattle of lush grass is unworkable by many beetles and is avoided (Forgie 2009).

Comment [A9]: Many of these issues are not taken into account in the Evaluation and Review Report of ERMA nor does the Report discuss whether the *collective* detrimental effect of these issues on the potential benefits of dung beetles was taken into account.

Bovine Tuberculosis (Part 1)

Dung beetles may participate in the ecology of tuberculosis by driving more wildlife-to-livestock contact, thereby enhancing TB transmission. Many mammals eat invertebrates and prefer invertebrates of wide availability and higher nutritive value (Redford, 1984).

Redford and Dorea (1984) studied mammals feeding on ants and termites in Brazil and “concluded that most invertebrate-eating mammals choose prey based on availability and other aspects of prey biology and not on gross nutritional factors”. They also say “Differences in nutritional value may affect a predator’s choice between two specific prey items but availability and abundance of prey probably determine the type of prey taken by most invertebrate-eating mammals”.

The exotic dung beetles are quite large (up to 22 mm) and are likely to be a good source of high quality protein and energy (perhaps up to 2 kilojoules per beetle - Calver 1982).

Although the largest beetle (*Geotrupes spiniger*) to be approved is 22 mm in length the average length of the 11 species (range 9-22 mm) is 13 mm. Using the formula $E_1=0.5L^{2.6}$ where E_1 is available energy in joules (Calver and Wooller, 1982) the average energy value is 0.40Kj with a range of 0.16 - 1.55Kj. Cowan and Moeed (1987) found that that one beetle species, *Stethaspis longicornis,* predominated in the diet of possums, constituting 44.9% of beetles eaten. *Stethaspis longicornis* at 24 mm in length (Parkinson and Horne, 2007) has an E_1 of 2Kj. In this study beetle remains were found in 10% of faecal pellets examined compared to 23.5% for stick insects, 19.6% for cicadas, and 14% wetas. Of the total stomach content invertebrates constitutes less than 2% of the volume. There is no indication that possums actively target specific insect species and Cowan and Moeed (1987) showed marked seasonality in the consumption of specific species, e.g. *Stethaspis longicornis* mostly eaten in summer and early autumn when the adults were in the tree canopy (larvae are subterranean root feeders). They also suggest that these are chance encounters for possums while browsing in the canopy rather than active insectivory.

TB vectors like hedgehogs, pigs, mustelids and possums (as well as rodents and birds) are known to eat beetles (King 1982, Cowan 1987, Thomson 1988, O’Donnell 1995, Smith 1995, Jones 2005). These species frequently visit pastures and are likely to prey on the newly introduced dung beetles on, in or under the faecal pats of farm animals. In one New Zealand study, beetles were the most commonly eaten prey by hedgehogs (Jones 2005).

King and Moody (1982) examined the gut contents of stoats (mustelids) and found carabid beetles (ground beetles) in 3.1% of 1250 stoats. Seventy weasels were also examined and carabid beetles found in 3%. Insects were found in 41% but contributed less than 10% of the biomass consumed. They noted that “*For insects other than wetas the samples are generally too heterogenous or too small to analyse with respect to season or habitat, and some may have been ingested with insectivorous birds or lizards*”. In the analysis of insect content King and Moody (1982) did identify scarab beetles (dung beetles are scarabs). In total 13 beetles were identified: 2 *Odontria* (brown beetles), 2 *Stethaspis* (syn: Chlorochiton, chafer), 4 *Pyronota* (manuka chafer), 3 *Costelytra* (grass grub), and 2 unidentifies melolonthines (chafers). The data does not suggest that mustelids target beetles as preferred food but rather the consumption of beetles is the result of random encounters.

See comments on Cowan and Moeed (1987) above. Specifically Cowan and Moeed (1987) comment “*opportunistic consumption of invertebrates fits well with previous descriptions of the varied food habits of possums*”.

Comment [A10]: It is noteworthy that the following discussion on the impact of the potential risks of dung beetles (DBs) to NZ’s bovine tuberculosis eradication programme (Pages 2-8) was eventually superseded by the Animal Health Board (the government agency that is responsible for eradicating Tb in cattle) which concluded the potential risks of dung beetles contributing to Tb epidemiology were sufficient to require empirical research to clarify the risks. Landcare asked one of their staff with infectious disease experience (Dan Tompkins) to look into the matter. He too concluded that some of the specific concerns regarding tuberculosis risk were justified and did require experimental work to clarify the risk. His study on the bacteriological culture of dung from naturally infected cattle 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 NZ. He went on to warn that 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. Dr Tompkins also reported (Landcare Internal Report March 2012) on a preliminary study to examine whether possums would forage for DBs (thus increasing their bush to pasture movements and hence potentially also increasing rates of Tb transmission) but the experimental design used was adequate only to test the palatability of dung beetles in comparison to the possums’ captive diet of apple and cereal and didn’t allow secure conclusions to be made about foraging behaviour. Nevertheless, this disciplined process of attempting to resolve the areas of uncertainty compromising effective risk assessment is to be applauded. It is exactly the type of scientific rigour that those wishing to see a more precautionary approach to the introduction of exotic dung beetles wish to see adopted by Landcare and the EPA.

Comment [A11]: The relevance of these references was to show that generalist foragers like hedgehogs, pigs, mustelids, possums, rodents etc are known to include invertebrates in their diet when the opportunity presents. The aim of the Applicants is to greatly increase the number of dung beetles in NZ. If they are successful, this will increase their availability to wildlife – potentially encouraging more wildlife-to-domestic animal interaction on farms. In addition, the consumption of dung beetles will increase the potential risk that wildlife will be infected by faecal pathogens from domestic animals (e.g. E. coli, salmonella, campylobacter, MAP etc., and to a lesser extent M. bovis).

Comment [A12]: The rate of which may increase following the large scale introduction of dung beetles.

Comment [A13]: Opportunism that has the potential to increase following the large scale introduction of dung beetles.

Thomson and Challies (1988) found that for pigs "*invertebrate foods were mainly earthworms, with the rest being the larvae, pupae, and adults of insects, and other arthropods. Representatives of 14 insect families were identified in stomach samples, but together they formed only 2.7% of the pigs' diet*". There is no indication that pigs actively seek out beetles as a food source.

Comment [A14]: Pigs are opportunistic and curious omnivores highly motivated to explore their environment by rooting, sniffing and chewing (Studnitz et al Appl Behav Sci 107:183-197, 2007). It is likely they will discover and take advantage of a large scale introduction of dung beetles to NZ just as they have been observed to eat dung beetles in other countries like the USA (see below). The nutritional demands in spring resulting from pregnancy and lactation result in wild pigs in NZ seeking out higher protein diets through predation of soil and litter invertebrates such as earthworms (McIlroy, J Royal Soc NZ, 225-231, 2001) a behaviour which could bring them into contact with dung beetles.

O'Donnell (1995) is a review and only refers to the findings of Cowan and Moeed (1987).

Smith et al. (1995) examined the gut content of ferrets from pastoral habitats and found invertebrates "*in 14.3% of guts but their contribution by weight was minimal (0.1%)*". Also that "*All indications from this and previous studies in New Zealand is that ferret are opportunistic, generalist predators*". There is no indication that ferrets actively seek out beetles as a food source.

Jones et al. (2005) found 81% of hedgehogs' gut content contained the remains of scarab and carabid beetles and "*These were mainly grass grubs* (*Costelytra zealandica*, *C. odontrea*)". Hedgehogs are insectivores and beetles comprise a significant proportion of its diet and they have been shown to focus foraging on a locally abundant food source (Parkes, 1975):

Comment [A15]: Hedgehogs are particularly likely to seek out dung beetles following their large scale introduction.

> "*Concentrations of food become foci of hedgehog activity. During mid March 1970 the effluent from a pigsty spilled over 0.1 ha of pasture in two places … and became infested with maggots of the shed fly, Eristalis tenax. Large numbers of hedgehogs – 21 on 24 March for example – were seen to be eating them. In early May the larvae of the armyworm moth, Pseudaletia separata, were present at an estimated density of 2-3.m^2 in some areas around the central pine plantations, and the hedgehogs gathered to feed. In late November an area which had recently been flooded supported a dense population of slugs of various species, and on 1 December 1970, 9 hedgehogs were observed feeding on these molluscs.*
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> *Conversely, unproductive areas such as the pine plantations were avoided by feeding animals. Within the pasture the hedgehogs avoided long grass, so indirectly the grazing pattern of the herd of cows influenced hedgehog movements.*"

Parkes (1975) indicates that hedgehogs are already feeding in pastures and interact with livestock. The addition of dung beetles is unlikely to increase this interaction as the adult and larvae are subterranean where as maggots, armyworms and slugs are all on the surface.

Comment [A16]: Dung beetles are vulnerable to predation when they emerge from the brood balls and are 'working' faeces on the pasture surface.

These species frequently visit pastures and are likely to prey on the newly introduced dung beetles on, in or under the faecal pats of farm animals. In one New Zealand study, beetles were the most commonly eaten prey by hedgehogs (Jones 2005).

As noted above there is no good evidence, with the exception of hedgehogs (Jones et al., 2005), that pigs, mustelids or possums will deliberately target dung beetles as food source. The evidence suggests that they may eat them if they encounter them but this will be during general foraging rather than targeted foraging.

Comment [A17]: Dung beetles may initially be consumed during opportunist foraging but learned behaviours may eventually supersede the opportunism if the food resource is sufficiently abundant and available.

The invertebrates preferred by possums are sluggish, nocturnal and easily detectable (O'Donnell 1995) suggesting the nocturnal dung beetles proposed for introduction would be vulnerable to possum predation.

This comment is from Cowan and Moeed (1987) which in full says: "*Nevertheless, possums must be considered as one more potential threat to native New Zealand invertebrates. Based on the pattern of invertebrate predation by possums in the Orongorongo Valley, invertebrates most at risk are likely to be small localised populations of large-bodied relatively sluggish nocturnal species with high detectability. Possible examples include mainland populations of giant wetas (Deinacrida), large stag beetles in Coromandel, and large weevils of the subfamily Cylindrorrhinae.*" The comment did not refer to predation of individuals but rather to the effect of predation on a population or species. There is no evidence that dung beetles can be described as sluggish and of the 11 species only two are nocturnal (ERMA200599, Table 1). The only time when these beetles are vulnerable to predation is when they first arrive at fresh dung and have yet to begin tunnelling and when they leave the brood tunnels to gather dung to take back into the tunnel.

Comment [A18]: Sluggish is a relevant term; they will be capable of being caught by most predators when they are working dung on the pasture surface.

Comment [A19]: Only two make nocturnal flights but another 5 species fly (to new dung pats) during dawn and dusk. Thus 7 species are potentially exposed to crepuscular and nocturnal wildlife. Similarly, these 7 species are the species that may be phototactic. There are observations in the literature on phototaxis of nocturnal and crepuscular species.

Wild pigs in New Zealand will root for scarab beetles and these have occasional (sic) been found to make up the bulk of pig stomach contents (Thomson 1988).

The exact quote from Thomson and Challies (1988) says: "Twelve [food] items were found in bulk (i.e., >50% of content) in one or more of the pigs; these included all of their main foods (see Table 2) as well as several otherwise unimportant foods such as fungi, scarab beetles, and introduced thistles (*Cirsium* spp.)." This is the only mention of scarabs in the paper. At best, one pig, out of 104 sampled contained scarabs but this is not clear from the description.

Comment [A20]: Thomson and Challies (1988) note that rooting provided approximately 31% of the diet and invertebrates acquired during rooting made up 13% of the diet. The majority of these invertebrates were earthworms. The sentence quoted above appears to mean that scarab beetles were found in one or more pigs to occupy greater than 50% of the stomach contents.

Unlike the risk scenario below, this risk does not require the dung beetles to be infected by M.bovis – just to be sufficiently abundant to attract wildlife into close proximity to livestock more frequently, or for additional seasons, or for longer periods than currently (e.g. see Green 1986 for an estimate of the current frequency of pasture visits by possums).

Green and Coleman's (1986) research showed how much movement by possums there was between forest and pasture and they proposed that "the control of possums in Tb-problem areas will be required over forest at least 1 km in from the forest-pasture margin". This has been incorporated into the Tb control strategy (Green 2004).

The following comment comes from Landcare Research (pers. com David Choquenot): "*At issue is whether the presence of dung beetles would, by changing the foraging behaviour of possums, increase the potential for contact between possums and livestock (noting that the issue of dung beetle consumption of possum faeces was robustly addressed during the ERMA process). Increased contact between possums and livestock is highly unlikely, because in the very areas of concern (i.e. TB-infected farms, where possums are the most likely source of initial infection in the first place), active management of wildlife vector populations (particularly possums) to maintain them at extremely low levels, is undertaken as a primary management priority. It is worth noting that people not directly involved in TB management often do not realise what is meant nowadays by 'low possum populations' – tens of thousands of hectares of farm-forest boundaries with possums at near-zero densities.*"

This scenario is similar to the role proposed for the dung beetle in the badger-bovine TB cycle in the UK (Little 1982, Hancox 1997, Gallagher 2005).

Little (1982) says "*Although the earthworm is the preferred diet of the badger, at times of scarcity badgers will search for other food including dung beetles (Geotrupes sp) in cow pats and during May and August may eat considerable numbers*". Given New Zealand's milder climate it is difficult to see when scarcity of food would be sufficient to drive possums or any other mammals to seek out dung beetles. The exception here is hedgehogs which Parkes (1975). Parkes observed "*It was thought that the study area might be too small to accommodate the hedgehogs' movements, but during the monthly surveys of the surrounding areas only 4 marked animals were discovered more than 500 m from the edge of the study area*". In other words they do not tend to wander far from a home range. He also observed that they tended to congregate around a food source when it was available. If his observations are correct then the animals need to take advantage of localised food availability. This suggests that if dung beetles where available and could be caught by hedgehogs then they would take advantage. However, this also suggests that they will not transport any disease derived from the beetles very far at all.

Hancox (1998) says "*It is perfectly obvious that cattle are infectious at any stage of the disease … but it will be necessary to re-discover that NVL/VL cattle may be infectious and real source of TB to both cattle AND badgers in order to dispel the belief of badger guilt.*" In other words Hancox considers that it is infected cattle that are infecting other cattle and badgers, and that badgers play no role in disease spread. He does not mention dung beetles at all. Hancox (1997) links the infection of badgers from feeding on dung beetles and earthworms in Tb contaminated cow faeces. As earthworms are a preferred food the implication is they transmit Tb to badgers. As New Zealand has earthworms associated with dung a pathway for infecting already exists yet earthworms are not implicated in Tb transmission in New Zealand.

Gallagher (2005) says "*The predominant feeding behaviour of badgers is foraging for earthworms, which are most abundant on pasture. In addition, badgers are particularly fond of beetles, which they forage for under cow-pats*". No particular mention is made of dung beetles or any involvement of dung beetles in attracting badgers into pastures or in 'vectoring' disease.

The counter argument by the applicants that the introduction of the new species of dung beetle will reduce dung quantities on pasture and therefore decrease the overall invertebrate biomass available for predation in faeces is also plausible.

The applicant did not make this claim. The argument put forward by the applicant was that "*Dung burial decreases pest populations such as nematodes, flies, and diseases, thus reducing parasiticide use*" and "*Burial of dung reduces populations of biting flies, improving mental well-being of stock*" while at the same time "*The introduction of dung beetles is expected to enhance soil biodiversity and increase the numbers of other beneficial organisms such as earthworms*".

However, given the efforts by the applicants to ensure they introduce a range of large dung beetles that will be abundant day and night all year round, it seems likely predators will soon associate fresh cattle dung with a consistent, high quality, easily accessible food supply.

Comment [A21]: The use of overseas literature during the ERMA process to verify whether exotic dung beetles will consume the faeces of wild possums consuming NZ flora and fauna could not be described as 'robust'. This requires well designed no-choice or pair-fed feeding trials.

Comment [A22]: Agreed – in areas where possum numbers are successfully kept low by intensive and on-going interventions the likelihood of possums being attracted in high numbers to pasture by dung beetles is a low risk and this scenario would relate primarily to other potential (and less effective) Tb reservoirs.

Comment [A23]: The diet of possums varies significantly with the seasons as preferred foods come and go. During periods of the year when preferred foods are less plentiful and each night at the start of their foraging (before their daily caloric requirements have been met) it is likely possums will take advantage of the dung beetles they encounter.

Comment [A24]: Estimates of hedgehog home ranges vary widely with most falling between 2 ha and 50 ha but a few reaching >100 ha. Hedgehogs can travel long distances with up to 10 km in a 6 month period being recorded in NZ (Moss and Sanders, J Royal Soc NZ 31:31-42, 2001.

Comment [A25]: It is now generally accepted that badgers are the primary reservoir of tuberculosis in Britain (Gallagher, Res Vet Sci 69:203-217, 2000).

Comment [A26]: The implication made by Gallagher and others is that badgers forage for dung beetles - a food they are 'fond of' - under cow-pats. The unanswered question is whether the large scale introduction of dung beetles to NZ will draw more wildlife into contact with domestic animals and their faeces (and faecal pathogens) than currently are drawn by the present dung fauna.

Comment [A27]: The Applicant didn't make this claim in the EPA application but did at a later stage make this claim as a counter to the suggestion that wildlife may be attracted to faeces on farms by DBs.

Comment [A28]: As noted here, the Applicant also made the claim that the introduction of exotic dung beetles will decrease populations of pest flies and biting flies. However, few (if any) of the pest fly species in NZ are obligate dung breeders instead using decaying vegetable matter or carrion This hypothesis is thus untested and considered implausible by the recognised NZ expert in agricultural flies (Dr Allen Heath of AgResearch) who states "There are no fly species in this country of such a pest status that dung beetles need to be introduced and I have found no merit in any of the arguments proposed by the supporters of the idea."

This was not the reason for the large range of species applied for by the applicant. The applicant said "*Each species has been selected for its predicted climatic suitability to specific regions of New Zealand (Edwards, 2010), so that ultimately the majority of pastures used for farming livestock in New Zealand will contain at least one or more species of dung beetle*". The applicant referred to a report on the climatic suitability of dung beetles species for New Zealand (Edwards, 2010) which showed quite clearly the limited distribution that these species would have. This was the basis of the ERMA conclusion that the beetles would only be locally significant. Any increase in food availability to pest mammals would be very localised and ephemeral.

Comment [A29]: Agreed – but nor was this implied by the above sentence.

Comment [A30]: Yes – precisely – what is proposed is a national scale initiative that results in the majority of pastures used for farming containing at least one or more species of dung beetle. In a press release Landcare states "…we expect that in the long term there will be millions [of dung beetles] chewing and burying dung …". It is difficult to see how the EPA can conclude the beetles would be only locally significant.

The Agency states it is unaware of any published evidence that dung beetles carry or vector *Mycobacterium bovis*. However, it is also true that there is no published evidence to *disprove* the carriage of *M. bovis* by dung beetles. The literature on pathogenic and conditionally pathogenic mycobacterial species suggests beetles and other invertebrates can carry the mycobacterial species to which they are exposed (Beerwerth 1979, Fischer 2004, Matlova 1998, Matlova 2003, Kazda 2009).

Comment [A31]: The relevance of these references is to highlight the potential for invertebrates including beetles to carry the mycobacterial species to which they are exposed in faeces.

Beerwerth et al. (1979) found that insects, both adults and larvae, which live in the soil contained mycobacteria, whereas when only the nymph lived in the soil and the adults were winged these species contained far fewer mycobacteria. Interestingly they conclude "*The epidemiological importance of arthropods spreading pathogenic mycobacteria should not be overvalued*" rather than undervalued.

Comment [A32]: See later comment; Beerwerth concluded that arthropods living in substrates containing mycobacteria would be contaminated by them; in the case of dung beetles – both winged adults and larvae dwell in 'substrates containing mycobacteria'.

Fischer et al. (2004) examined 229 adult beetles from 29 species from 14 localities in the Czech and Slovak Republics. They did not find mycobacteria in any of the beetles tested. However they were able to recover mycobacteria in beetles deliberately fed food contaminated with mycobacteria. These same researchers have shown that mycobacteria can be isolated from earthworm and by implication could be involved in the transmission of these organisms.

Comment [A33]: See later comment – the beetles tested were not dung beetles and the prevalence of mycobacteria in the environmental samples was also very low.

Matlova et al. (1998) – this paper is Czech and the abstract provides no detail that we can assess.

Matlova et al. (2003) found that in a sample of 430 invertebrates from farms where mycobacterial infection had occurred that mycobacteria were isolated from 7.9% or 34 individuals. The infected invertebrates were hoverflies, flies, fruitflies, dung flies, biting flies, and earthworms but no beetles. All of the invertebrates that were found to be carrying mycobacteria are already present in New Zealand.

Comment [A34]: It is unclear whether these were pathogenic mycobacteria and how many of the environmental samples were taken on pasture.

Kazda et al. (2009) is a book to which we do not have access.

Comment [A35]: Kazda 2009 suggested dung beetles can act to spread pathogenic mycobacteria including M. bovis in the soil when they bury dung balls with their offspring.

Mycobacteria, because of their cell wall structure, are thought to be resistant to the digestive enzymatic activity of insects and can be excreted in their saliva and faeces (Kazda 2009).

Kazda et al. (2009) is a book to which we do not have access and so have not evaluated.

The literature relating to dung beetles, badgers and TB implies but does not prove carriage of *M. bovis* by dung beetles.

From the review of the information above the implication that TB is carried by dung beetles is dubious.

Comment [A36]: Yes – there is as yet little data to support or disprove that dung beetles can carry M. bovis. It remains, plausible, however, that dung beetles will carry M. bovis if they are exposed to it in the faeces of ruminants or wildlife with advanced tuberculosis.

Dung beetles may increase the prevalence of tuberculosis in wildlife reservoirs within and outside vector-control areas by increasing intra-specific and inter-specific transmission in these reservoirs. In pastures bordering marginal land, beetles may seek out the faeces of wildlife including species that can act as reservoirs or amplifier hosts of TB (such as pigs, deer, possums, goats, lagomorphs, ferrets and hedgehogs, Coleman 2001, Machackova 2003, de Lisle 2008). Beetles consume fluid from the faeces and bury faeces to form brood balls into which they lay their eggs. They produce between 50-200 eggs and may have many generations over their 3 month-3 year life spans (Dymock 1993). If the faeces are from wildlife with advanced respiratory, retropharyngeal or gastrointestinal tuberculosis, the adult beetles may encounter sufficient quantities of *M. bovis* to become infected. Perhaps more importantly, because *M. bovis* can survive for up to 42 weeks buried in soil admixed with faeces (Duffield 1985), the *M. bovis* contaminated brood balls may subsequently infect beetle larvae, potentially creating a much larger new generation of infected beetles. TB-infected dung beetles may then fly on to new faecal pats in neighbouring farms or in other forest clearings frequented by wildlife.

Duffield (1985) notes pot trials conducted by Maddox in 1933 that reported survival up to 42 weeks. Duffield conducted pot trials but was unable to retrieve any *M. bovis* after 8 weeks. One treatment was to expose the pots to sunlight but as the pot temperatures reached 43˚C it is more likely that *M. bovis* was killed by heat and not sunlight. Interestingly *M. bovis* was never retrieved from the treatment containing dung. There are a number of studies that show that pathogenic *Mycobacterium* can survive in soil, e.g. Lavania et al. (2008) showed *M. leprae* was retrieved after 45 days. However these studies do not give any quantitative measure so there is no indication whether there is

Comment [A37]: Menzies and Neil (Vet J, 160:92-106, 2000) reviewed the environmental survival of M.bovis and note the negative effects of sunlight. They conclude M. bovis usually survives in the environment for only a few weeks but cite previous work showing that when buried in shaded soil M. bovis cultures mixed with faeces, blood and urine can survive for 700 days. It would seem plausible, therefore that rapid shallow burial of M. bovis-containing faeces by dung beetles may prolong M. bovis survival sufficiently to expose the next generation of larvae.

sufficient inoculum present in the soil to cause disease. Scantlebury et al. (2004) have examined badger cattle interactions and have suggested that badger latrines could be a source of infection when cattle stocking is high and they are forced to graze these areas. The only mode of infection suggested is by pulling soil up with the grass when grazing. Even if this is a plausible pathway for infection between badgers and cattle there is no evidence that alternate hosts in New Zealand, e.g. possums, have latrines (Paterson, 1993).

Given that very large dispersal distances have been recorded (see later), beetles may fly beyond the vector control areas. On arrival at the new faeces, infected beetles may be predated by wildlife (Hughes 1975) potentially infecting wildlife both inside and outside the vector control area and creating unpredictable intra-specific and inter-specific transmission routes.

Macqueen (1975 [not Hughes]) speculated that a wide range of mammals, birds and cane toads would feed on dung beetles introduced into Australia. In the following 36 years this has never moved beyond anecdotal information and there is no evidence that any new disease dynamics have eventuated.

The Agency takes comfort from the lack of evidence that earthworms are involved in the epidemiology of bovine tuberculosis. This view ignores the reality that we rely on a good understanding of the epidemiology of bovine tuberculosis, movement control and vector control to contain the disease to particular regions of New Zealand (Ryan, 2006). Through their ability to emerge in large numbers (Hughes 1975, Flene [Fiene] 2011), to fly long distances unlike earthworms (Dymock 1993, Appendix 1, ERMA 200599) and to be eaten by a variety of wildlife vectors (see above), dung beetles have the potential to complicate *M.bovis* epidemiology and compromise vector control strategies at the heart of New Zealand's bovine tuberculosis control programme.

There is a lack of scientific evidence to identify a hazard and consequently a lack of evidence to assess risk.

The Agency relies on the suggestion that the risk of bovine tuberculosis will be decreased by the possibility that dung beetles will reduce the amount of *M. bovis* on the soil surface. Unfortunately, it could equally be true that rapid burial of dung will increase the amount of *viable M. bovis* in topsoil (and associated bodies of water) by reducing desiccation, increasing microbial adherence to organic matter and providing beneficial nutrients. As mentioned above, *M. bovis* can survive for up to 42 weeks buried in soil admixed with faeces (Duffield 1985). The impact of soils on the infectivity of bacteria is highly complex (Weinberg 1979 [1987]). More importantly, the Agency is ignoring the strong evidence that the most important bovine tuberculosis transmission pathway in New Zealand is from wildlife reservoirs to stock, not from faecal pats to stock or from soil to stock (Ryan 2006).

As noted above pathogenic *Mycobacterium* can survive for varying periods of time dependent on a number of environmental variables, e.g. temperature, moisture, and exposure to UV. Rapid incorporation into the soil could prolong the longevity of spores in the environment. However, given that the spores are sequestrated in the soil, there is no evidence that arthropods amplify the bacterium, there is no evidence for vectoring, and given the very low occurrence of Tb in the national herd, and the very low numbers of alternative hosts in Tb control areas it is very difficult to construct a plausible source of inoculums or pathway of infection. Ryan et al. (2006) has identified mammal host to mammal host as the most important pathway for disease transmission. In New Zealand the policy has been to eliminate the primary hosts, diseased cattle, possums and mustelids, to control disease. The removal of these animals could result in the disease dying out in the secondary hosts. The same argument has been used in Britain where evidence is that the primary path of Tb infection is from cattle to badgers with a secondary spread back from badgers to cattle. The management of diseases cattle will eventually see the disappearance of the disease in badgers. It is interesting to note that badgers are do not appear to be considered a significant sources of Tb in Europe indicated by the lack of literature on this topic.

The Agency relies on the "highly preferential association" between large herbivore faeces and the dung beetles proposed for introduction. However, 'no choice' tests to prove the introduced dung beetles will not feed on the faeces of New Zealand wildlife vectors have not been undertaken. In fact, there is considerable evidence that, when required, dung beetles will utilize the dung of deer, pigs, carnivores, humans and many other species including the North American opossum (Fincher 1970, Appendix 1 and Response to Submissions ERMA 200599).

Both the applicant's and the ERMA analysis clearly stated that there are, within the large number of dung beetles species, very distinct groups that have evolved for specific habitats and hosts on different continents. The applicant selected species that have coevolved with large ungulates of open savannah/steppe grasslands. In contrast Fincher et al. (1970) were looking at native dung beetles of the open woodlands and forest of south eastern North America with a different suite of mammals and habitats.

Comment [A38]: Agreed – this is an important area of uncertainty that should be resolved.

Comment [A39]: The transmission of Tb from badgers to cattle appears most likely to occur when infected badgers contaminate pasture while searching for invertebrates (Gallagher, 2000).

Comment [A40]: Hughes was the convenor who prepared the cluster of conference papers referred to here for publication. Macqueen's observations included in the compilation were entitled "Dung as an insect food source: dung beetles as competitors of other coprophagous fauna and as a target for predators" . In this paper, he includes direct observations such as dung beetles often being found in the stomachs of native frogs, notes that cane toads 'feed voraciously on beetles at dung pads' and notes that dusk and night-flying beetles appear to be most at risk. He goes on to speculate about the possible predation of DBs by a number of bird species. He also notes DBs periodically become very abundant in Queensland and suspects this will become an annual phenomenon. These specific observations do not appear to have received further attention in the literature but many other authors note predation of dung beetles by birds, rodents and other wildlife.

Comment [A41]: As mentioned above, UoA, AHB and eventually Landcare Research disagreed with this view and empirical work was undertaken to examine the likelihood of Tb-infected cattle in NZ shedding M.bovis in their faeces and to undertake a preliminary investigation of the palatability of dung beetles to possums.

Comment [A42]: Yes – as pointed out above, this is clearly the most important pathway in NZ. Thus, ensuring dung beetles don't complicate Tb epidemiology in the species of wildlife that can act as reservoirs or spill-back hosts of Tb (such as possums, pigs, deer, mustelids, hedgehogs), don't themselves act as an amplifier host, don't influence transmission between reservoirs and domestic species, and don't enhance environmental contamination are all important precautions given the importance of bovine tuberculosis to NZ.

Comment [A43]: This has been a major area of criticism of Landcare's work for this application. All other recent biocontrol importations involve a series of careful 'no-choice' feeding trials to prove the imported organism is specific to the target invasive species. These have not been performed for the dung beetles and there is plenty of evidence including in the EPA application (see JP Humare) that they will utlise non-ruminant faeces when they must. For example, O. taurus, the dung beetle planned for release in Northland, has been shown to be able to switch from ruminant to carnivore faeces (Carpaneto et al, Biological Conservation 123:547-556, 2005). The performance of no-choice tests also needs to take into account the diet of the animal whose faeces are being tested as the dietary macronutrients impact on stool quality and may affect preference. Thus, natural diets would be preferred when evaluating the ability of the introduced dung beetles to utilise wildlife faeces.

The Agency relies on the ‘ample quantities’ of large herbivore dung in New Zealand implying that this plentiful resource will make it ‘very unlikely’ the dung beetles will seek out the faeces of wildlife vectors of tuberculosis. This conclusion ignores the fact that most New Zealand pastures are rotationally-grazed. This farming practice, in combination with our plentiful rainfall, will result in many occasions when newly emergent dung beetles will encounter a paucity of faecal material in their immediate environs and will need to rely on their highly developed sense of smell to guide their flight to whatever faeces are available in the region. Accordingly, beetle ‘shuttle flights’ between the faeces of domestic and wild animals in adjoining pastoral and marginal land would seem likely.

These comments make the assumption that when insects do not find their food source they will move on to some alternative foods. This is far from reality and most insect simply do not feed and consequently do not reproduce unless the correct food is available. All the indications are that dung beetles are unlikely to move more than a few kilometres in search of food. Some species will in all likelihood only move a few hundred metres. This lack of movement is seen a limiting factor on their successful establishment. The ERMA review highlighted that dung beetles were likely to be localised and farmers would need to manage their farms to facilitate establishment.

Comment [A44]: Many species of dung beetles have broad tastes in faeces and aren’t focused only on one specific type. It is quite reasonable that if cattle faeces aren’t available they may take advantage of the faeces of other species. See Mathison (1999) for an example of the switch between cattle and deer pasture and Carpaneto (2005) for an example of a switch between ruminant and carnivore faeces.

Comment [A45]: See the later debate on flight distance. Most dung beetles will fly considerable distances upwind using their strong sense of smell to locate the next resource patch.

The Agency relies on the high preference of the introduced dung beetles for an open pasture environment. Although the species of dung beetle proposed for importation are unlikely to penetrate far into dense bush, they can be found in broken scrub, forest fringes, forest clearings bush remnants or regenerating forests with relatively open canopies. (Galante 1995, Appendix 1, ERMA 200599, Jay-Robert 2008) - an environment that is not uncommon in the marginal land around farms in the tuberculosis vector control areas

These comments show limited appreciation of the complexity of the Mediterranean grassland/ shrubland/ woodland habitats (Galante 1995; Jay-Robert 2008). There is a complex of plant species, mammalian species, and dung beetles that is not replicated by the New Zealand environment. At best there will be some open and patchy scrubland in which cattle are grazing where there are possums and possibly pigs. But where Tb is being controlled these animals will be at very low levels. No evidence based hazard could be identified.

Comment [A46]: This type of marginal land is common in NZ and can be frequented by a wide variety of previously domesticated and wildlife species.

The Agency relies on the lack of international evidence to implicate dung beetles in the persistence or transmission of bovine tuberculosis. There is some international evidence of such a link (Little 1982, Hancox 1997, Gallagher 2005). There is also a lack of research disproving the link and no research that examines this question in the light of the specific vector-related issues that complicate the eradication of bovine tuberculosis in New Zealand.

The lack of evidence was one factor, but just as importantly the lack of concern from overseas researchers and jurisdictions that dung beetles exacerbate disease problems. The content of these citations are dealt with above.

Comment [A47]: There is an important maxim in disciplines like veterinary science that are confronted with a vast array of research questions and insufficient resources to do any more than barely scratch the surface – “if you don’t look you will not find”. In other words, it is important not to confuse the lack of *evidence* of risk with the lack of risk.

Johne’s Disease (Part 1)

The Agency concludes that the burial of dung may reduce exposure of stock to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and decrease the risk of Johne’s disease. However, stock avoid grazing near faecal pats and the burial of MAP does not inactivate the bacterium nor necessarily reduce the exposure of stock to MAP. Soil samples are still frequently positive for MAP at up to 20 cm below the soil’s surface (Pribylova, 2011). Rapid burial of dung may increase the amount of viable MAP in topsoil by reducing exposure to light (Whittington 2004). Overtime, this may enhance MAP density in grass, roots and – through runoff, dust and groundwater contamination – in farm ponds and water troughs.

In the presence of animals shedding MAP propagules Pribylova et al. (2011) were able to detect MAP (using PCR) from pasture plant sample and soil sample as deep as 20cm. Note that this test did not distinguish between living and dead propagules. Pribylova et al. (2011) found a correlation between presence of MAP and moisture and clay content of the soil. It is not certain whether or not these conditions enhanced the survival of MAP propagules or the survival of DNA from dead propagules. Pribylova et al. (2011) observes that when mycobacteria were deliberately inoculated onto soil, they were only able to re-extract 3.5%; the remainder stayed irreversibly bound to soil particles. This suggest that MAP propagules where not free to move soil water.

Comment [A48]: MAP is hydrophobic and tends to clump and adhere to particles. This doesn’t prevent MAP being washed into waterways with sediment nor mean the MAP that is clumped or adhered to sediment is necessarily less infective following ingestion in sediment-containing water.

Pribylova et al. (2011) say: “In soil, the population size of bacteria generally declines rapidly over time depending upon biotic and abiotic factors. Predation, competition, and root growth are the most important biotic factors, while the presence of clay minerals, water tension, organic and inorganic nutrients, temperature, pH, and chemicals (toxic waste) represent crucial abiotic factors. The survival of bacteria in soil is mainly enhanced by a slower turnover of organic matter, small pore size, soil type and finer-textured soil—clay.” One of the characteristic dung beetle activity is the increased turnover in organic matter, and an increase in soil porosity which are factor that do not favour the survival of MAP propagules.

Comment [A49]: Yes but microorganisms are also killed on the pasture surface due to exposure to light, drying, temp fluctuations etc. The issue is whether burial significantly slows the decline of the MAP population in comparison to the rate of decline on the soil surface as it does with many other pathogens (see Hutchison et al, Appl Environ Micro 70:5111-5118, 2004).

Comment [A50]: Yes – the balance of these biotic and abiotic factors will be affected by DBs and the results of this change in balance are uncertain and likely to be complex. For this reason, effective risk assessment requires empirical research to determine whether DBs increase or decrease the soil load of bacteria like MAP and to what degree.

Whittington et al. (2004) did not find that exposure to light was a contributing factor in the decline of MAP but rather diurnal temperature flux within dung pats and in the top 1cm of soil. They found MAP survived longer when the contaminated substrate was shaded thus reducing temperature flux. When MAP in faeces becomes mixed with soil, there is a reduction of 90 to 99% in *the apparent* viable count of the organism probably caused by binding of bacteria to soil particles. MAP has an obligate requirement of mycobactin for growth. Mycobactin is an iron-chelating growth factor, thus needs the presence of another organism producing this agent before it can multiply.

MAP can be spread by various contaminated materials, e.g. manure, soil, milk etc., and numerous organism vectors that have been investigated. With all this research on MAP it is interesting that an organism such as the dung beetles, a known obligate feeder of dung has never positively shown to carry MAP from environmental sampling, while earthworms, flies, cockroaches and nematodes have been implicated by association.

The Agency states there is no evidence that dung beetles can amplify MAP within their gastrointestinal system or spread MAP. This view is weakened by the experimental evidence that MAP has been shown to replicate in protozoa (Mura 2006, Gill, 2011) and to remain viable in the gut of beetles (Fischer 2004). MAP clearly has the potential to exploit the intracellular existence in protozoa, nematodes and insects for its survival (Mura 2006) and may even acquire enhanced virulence (Rowe 2006).

Mura et al. (2006) demonstrated under laboratory conditions that MAP is able to live and multiply within an amoeba, a common soil and water single cell organism. A number of pathogenic bacteria, i.e. Legionella, Listeria, Chlamydia and mycobacteria, are also known to survive phagocytosis and remain viable in intracellular protozoan vacuoles. Replication in a single celled protozoan is hardly comparable to replication in a complex multi-cellular organism such as a dung beetle and we consider that this evidence does not weaken our initial view.

Gill et al. (2011) postulated the transmission of MAP from food (meat and milk). The only discussion on protozoa was that they are found at meat processing plants no reference was made to MAP replication in protozoa. The relevance of this paper to the argument is not known. Fischer et al. (2004) failed to isolate MAP from beetles caught in this study from sites where MAP occurred. However, MAP could be recovered from the intestinal tract of beetles that had been fed contaminated food. This studied showed that beetles could, in theory, mechanically carry MAP propagules but did not demonstrate that MAP could replicate within a beetle. Also of note is that newly emerged adult insects, from pupal stage, did not have intestinal mycobacteria.

Rowe and Grant (2006) is a review paper in which they reported a study by Cirillo et al. which found that MAP cells engulfed by amoeba, *Acanthamoeba polyphaga*, had increased virulence but that “increased virulence was not because of selection but induction of a more virulent phenotype”. This induction of virulence has only been observed in protozoa and not in nematodes or insects.

The Agency’s observation that MAP can be spread by multiple pathways does not reduce the significance of environmental sources of infection and wildlife reservoirs in the epidemiology of Johne’s disease. Environmental reservoirs of MAP in soil and water are considered important in the epidemiology of the disease (Whittington 2005, Pavlik 2010, Pradhan 2011). Wildlife reservoirs of infection would be considered to have major implications for the control of MAP in New Zealand (de Lisle, 2003).

The ERMA risk assessment took into account the environmental reservoirs of MAP *already* present in New Zealand. The assessment found the pathway of MAP transmission from dung beetle to cattle to be so limited, i.e. negligible, it would not be additive to all the other known modes of transmission already present in New Zealand.

Interestingly Pradhan et al. (2011) did not invoke an environmental reservoir for the chronic level of MAP in the three dairy herds he studied in the north-eastern US. They considered that the faecal-oral route between animals to be the most important, and the failure to recognise that low shedding animals were infected, and not just passive vectors, and thus a source of infection to the herd.

MAP-infected dung beetles (Fischer 2004) may infect wildlife by seeking out the faeces of wildlife in scrub, forest margins, clearings, etc. Over time, the arrival of numerous dung beetles may build the numbers of viable MAP present in these environments, potentially infecting rabbits, hares or wild ungulates (which in turn may act as reservoirs of infection – Beard 2001, Judge 2005, Judge 2007, Kopecna 2008, Stevenson 2009). In addition, mustelids, rodents, birds, cats, opossums and pigs may ingest MAP-infected dung beetles on farms or in forest clearings and become carriers of MAP. There is evidence these wildlife species can carry MAP (Beard 2001, de Lisle 2003, Corn 2005, Judge 2005, Judge 2007, Kopecna 2008, Stevenson 2009) and that they are likely to predate dung beetles (Hughes 1975, King 1982, Cowan 1987, Thomson 1988, O’Donnell 1995, Smith 1995, Jones 2005).

Comment [A51]: Shading is a measure of ‘exposure to light’ which in turn impacts on temperature flux etc.

Comment [A52]: Aitken Pers. Com . (2011) a medical microbiologist tested Mexican Dung beetles in NZ and found them to be highly likely to have MAP in their intestinal tracts.

Comment [A53]: Perhaps, but if MAP can replicate inside the cells of simple protozoa as well as inside phagocytic cells in the mammalian digestive tract on what basis does the EPA rule out the possibility that MAP can replicate within cells of beetles?

Comment [A54]: Gill (2011) does make reference to MAP replication in protozoa. He states “Map probably cannot grow outside a host except when cultivated in the laboratory, but both members of the M. avium complex and Map can survive ingestion by and may grow within protozoa.” He goes on to say that protozoa can be found in water and soil. He also notes MAP may be able to grow in biofilms and that it can survive for prolonged periods in soil, water and sediment although survival is affected by soil and water composition and is reduced by exposure to sunlight. He also notes that people may be exposed to MAP from natural waters (especially with suspended sediment) or by dust from contaminated soils.

Comment [A55]: Yes but the beetles examined by Fischer weren’t obligate dung eating beetles and the number of positive environmental samples was also very low (4 positive MAP samples out of 75 samples from the floors of Johne’s disease infected cattle). He does conclude that his work on the experimentally infected darkling beetles ‘proved that mycobacteria ingested by beetles remained viable in their intestines.” And goes on to say they are potential mechanical vectors of mycobacterial infections.

Comment [A56]: Agreed.

Comment [A57]: The pupa weren’t tested (i.e. not cultured). However, by Day 4 post-infection no more MAP was cultured suggesting under the experimental conditions (a sterile peat diet) infection was not maintained.

Comment [A58]: Has it been looked for in insects?

Comment [A59]: Yes – it is likely that the direct infection of cattle accidentally ingesting MAP-infected DB’s during grazing will occur relatively infrequently; the greater risk may derive from the impact of DBs on the environmental load of MAP through rapid burial of faeces or via the infection of reservoir species (e.g. hedgehogs, other wildlife spp) that augment the environmental contamination with MAP. This would be particularly significant if the strains spread to the wildlife are novel to a particular farm. It is important to note in this regard that the risk of MAP infection strongly depends on the infectious dose to which young animals are exposed in grass, soil or water.

Comment [A60]: Environmental contamination by faeces is implicit in faecal-oral transfer.

The dung beetle species that have been approved for New Zealand were assessed from their high preference for pasture habitats and the dung of large ruminants. There is no obvious or viable pathway for wildlife to be infected in any significant numbers. It is possible that mustelids, rodents, birds, cats, possums and pigs could occasionally eat a dung beetle but for infection to occur the beetle would have to be contaminated with MAP and MAP would need to be present in sufficient numbers to be an infective dose to the mammal eating the dung beetle. It is far more likely that mustelids, rodents, birds, cats, possums and pigs will become infected by other means, e.g. the greatest risk of transmission comes from the faecal contamination of feedstuffs and drinking water.

The Agency's observation that dung beetles will not be eaten by cattle does not prevent cattle becoming infected by a reservoir species capable of ingesting dung beetles.

The etiology of Johne's disease and MAP is not the same as bovine TB, in which a reservoir is needed. Animals that spread MAP from contaminated dung to food for cattle and sheep are already present in New Zealand in large numbers. Dung beetles will not increase this pathway of infection but is more likely to reduce it by the removal of dung reducing the opportunity of MAP to spread.

Clinically significant MAP infection appears more likely when young stock are exposed to large amounts of the virulent strains of MAP (O'Brien 2006, Norton 2009, Mackintosh 2010, Pradhan 2011). There is evidence that some strains are more pathogenic than others (Motiwala 2005, Marsh 2006, Stevenson 2009, Pradhan 2011). Dung beetles are strong fliers, can emerge in large numbers and may have the potential to transfer virulent strains long distances.

As discussed above dung beetles tend not to travel far and when colonising a new area they tend to move along a front. As also discussed above there is no evidence that dung beetles vector mycobacteria.

Dung beetles may also be able to amplify MAP in the environment by burying MAP-infected faeces into brood balls and infecting a new and expanded generation of dung beetles.

Beerwerth et al. (1979) found that larvae and adults of beetles permanently living in substrates contaminated with mycobacteria were also contaminated with mycobacteria. However flying adults were less contaminated. Fischer et al. (2004) did not find any MAP in any of 229 beetles captured from areas where diseased animals occurred. There is no evidence that beetles either mechanically transfer, are vectors of, or can amplify mycobacteria.

The Agency's observation of the proposed link between nematodes and Johne's transmission is not material; nematodes are not required for the environmental transmission of Johne's disease.

The assessment of nematodes focused on bovine tuberculosis however, nematodes have been found to contain MAP (see discussion above). As nematodes eat amoeba there is a possibility of dung beetles affecting this chain of transmission with their actions, which in itself is very small compared to transmission from the faecal contamination of feedstuffs and drinking water.

Comment [A61]: As previously noted – no-choice tests to establish the faecal specificity were skipped. They should have been performed for each DB species to help evaluate the palatability to dung beetles of the faeces of other species.

Comment [A62]: Yes – the most plausible transmission path to wildlife reservoirs would be the ingestion of MAP-infected beetles by wildlife. Given the proposal to introduce 'millions of dung beetles' to pastures throughout NZ it is difficult to agree with the EPA's assessment that wildlife will only occasionally eat a dung beetle. The current pathways by which wildlife are infected by MAP have not been clearly elucidated in NZ's pastoral environments but if that proves to be directly from soil or water, DBs may again increase this transmission.

Comment [A63]: Yes – a wildlife reservoir is not needed but the potential for wildlife reservoirs to augment or maintain herd infection or to introduce new strains is very much still in consideration (Norton S, PhD thesis, Massey University, 2007).

Comment [A64]: It has not been established that burial of dung reduces the opportunity of MAP to spread. As the Applicants note themselves, grazing animals give a wide berth to pasture contaminated by faeces (the so-called zone of repugnance). Rapid burial of MAP may increase rather than decrease soil (and water) load of MAP. Empirical research is required to resolve this uncertainty.

Comment [A65]: The uncertainty over how far the exotic dung beetles fly is discussed elsewhere. Once again, the fundamental biological behaviour of the exotic dung beetles (e.g. how far they fly) should be better understood by the applicant and EPA before they are introduced.

Comment [A66]: Yes – this is what Beerwerth reported. Winged arthropods were less frequently mycobacteria positive than larvae. Beerwerth did not attempt to quantify the infectivity or examine transmission but cautions against 'over-valuing' the epidemiological significance of arthropods for transmitting the particular mycobacterial biotypes he studied. Notably, Beerwerth's study was not focussed on MAP or dung beetles, however it suggests that both the larvae of dung beetles and the adults could carry MAP because both are directly exposed to 'substrates contaminated with mycobacteria'.

Comment [A67]: See comments above.

Comment [A68]: There is good evidence that beetles will harbour mycobacteria if exposed and may do so at a high prevalence; there are plausible potential routes of transmission to various species; these have yet to be explored to determine if they can produce transmission; the 'lack of evidence' is not therefore compelling .

Quarantine & Disease Transmission (Part 1)

The Agency's conclusion that it is very unlikely dung beetles will 'factor' a disease-causing organism out of a quarantined area relies on the Agency's view that there is no evidence that dung beetles can spread infectious agents. This is a curious conclusion given the ample evidence (in spite of the relative paucity of research investigating their roles as vectors) that dung beetles and carrion beetles carry or spread a variety of pathogens (Stewart 1963, Lonc 1980, Solter 1989, Saitoh 1990, Du Toit 2008, Xu 2003). Similarly, darkling beetles are known to transmit a wide range of viruses, enteropathogenic bacteria and parasites in poultry leading Goodwin (1996) to conclude that "the threat of severe adverse economic impact from beetle-vectored disease should not be overlooked or casually dismissed".

Stewart and Kent (1963) showed that beetles feeding in and around mammalian dung contained mammalian intestinal nematodes. How the beetles came to contain nematodes was not demonstrated, that is did they eat the eggs or the young larval instars? Although claiming that the beetles were intermediate host they did not show that the nematodes could complete their life cycle or that there was any other mechanism for the nematodes to be passed from a beetle to a mammal.

Lonc (1980), Solter et al. (1989), Saitoh and Itagaki (1990), Du Toit, et al. (2008) are as for Stewart and Kent (1963) in that pathogens and parasites can be demonstrated as present but what role if any they play is not shown. We do not have access to Xu (2003).

Goodwin and Waltman (1996) like Stewart and Kent (1963) show that it is possible to isolate vertebrate pathogens from a beetle living in a substrate contaminated with pathogens. However, they fail to show that the beetles can vector the disease.

It is agreed that insect-related biosecurity risk already occurs in New Zealand through flies and the exotic dung beetles that are currently present in New Zealand. The change to this risk following the introduction of 11 new species of dung beetles will depend on such matters as the abundance of the new beetles, the influence of the beetles on the abundance of New Zealand fly species (many of which are not dependent on dung), the comparatively large distances beetles can fly, the potentially greater infective dose carried by larger beetles and the reduced opportunity for pathogens in buried faces to be killed by sunlight, drying and high temperatures (see below).

The typical dispersal distance of the introduced beetles is germane to an understanding of their risks to biosecurity and vector control. Unfortunately, little specific information is available. Reviews (Dymock 1993) and the ERMA 200599 consultation document variably refer to the exotic dung beetles as being 'proficient' or 'strong' fliers capable of dispersing between fifty to 'several hundred' kilometres. In comparison, flights typical for domestic flies are 0.5-2 kilometres (Alam, 2004) and most flights for the small *Aphodius* sp. dung beetles (which are currently present in New Zealand) are less than a kilometre and are relatively infrequent (Roslin, 2000). Roslin (2000) observed marked interspecies differences in dispersal distance in dung beetles and noted that the larger beetle species and those with specialist feeding requirements tended to fly greater distances. The author also observed evidence of active search behaviour and concluded that dispersal distances will ultimately depend on the configuration of their resource patches over the landscape (Roslin, 2000). The dung beetles *E. Intermedius* and *D. Gazellea* that were introduced into the USA soon spread to Mexico (Montes 1998) and those released in Australia to off-shore Islands, highlighting the likelihood the dung beetles proposed for introduction to New Zealand will similarly be capable of widespread dispersal.

The Agency's observation that use of anthelmintics has been demonstrated to be lethal to dung beetles and therefore will affect the magnitude of all effects is somewhat circular and provides a better reason to question the likely benefit of the introduction to New Zealand than to rule out biosecurity risks.

Comment [A69]: The EPA agrees it is not surprising dung beetles will harbour pathogens they are exposed to in faeces; the EPA doesn't dispute that dung beetles can fly significant distances nor that they may be periodically abundant. In addition, the EPA doesn't dispute that DBs have the potential to be exposed to domestic animals, wildlife and people. With that degree of agreement it is difficult to follow how the EPA sees the factoring of disease from a quarantined area as implausible. Other expert bodies such as the OIE (World Organisation for Animal Health) recognise the possibility of arthropod vectoring insisting that countries wishing to compartmentalise their trade in the face of disease outbreaks must prove separation including geographic, movement and arthropod vectors.

Comment [A70]: These observations were extended by a comprehensive study by Fincher et al J Parasitol 55:355-358, 1969 entitled "Beetle intermediate hosts for swine spirurids in Southern Georgia" in which they documented a high incidence of infection of some but not all species of dung beetles with swine nematode larvae and reported their observation that swine are attracted to and eat insects. They also suggest larvae may over-winter in dung beetles.

Comment [A71]: Saitoh and Itagaki showed Toxo oocysts in dung beetle faeces and also adherent to their body surface where they remained infective for up to 25 days. When the infected dung beetles were fed to mice, the mice developed toxoplasma cysts in their brain. When mice infected by dung beetles with toxoplasma were fed to kittens the kittens developed toxoplasma oocysts in their faeces. Thus, this paper provides clear evidence dung beetles can vector toxoplasma.

Comment [A72]: Du Toit describes the life cycle of Spirocerca lupi and makes it clear embryonated eggs are ingested by dung beetles and the infective L3 larvae develop in the beetle as a true intermediate host. He makes further observations on dung beetles and spirocerca in a more recent paper (Du Toit et al, Med Vet Entomol 26:455-457, 2012).

Comment [A73]: They did provide strong evidence beetles can vector viral and protozoal disease in poultry by feeding specific-pathogen-free chicks in sterile incubators beetle homogenates and following the development of disease via serum and faecal diagnostic tests. Mathison (1999) provides an extensive discussion of the numerous other publications that suggest dung beetles can be transport or intermediate hosts for gastrointestinal parasites of man and livestock.

Comment [A74]: AgResearch experts advise that dung beetles are unlikely to reduce 'filth fly' numbers in NZ (and thus to reduce insect-related biosecurity risk). This is not disputed by the EPA.

Comment [A75]: Citing Bornemisszа 1976, Dymock 1993 states "Some species have made recorded flights of up to 50 km."

The distance that dung beetles fly is species dependent and generally the information is generic. We note that the comment above “capable of dispersing between fifty to ‘several hundred’ kilometres” in ERMA 200599 Appendix 1 actually says “known to travel several hundred kilometres in a year”. It does not refer to a single flight but the potential of a moving front of the expanding population to become established in new areas. So the front may move several hundred kilometres not necessarily individual beetles. In contrast Roslin (2000) found that larger beetles species dispersed greater distance than small beetles. However this is qualified as he found that the majority of beetles moved only short distances often only moving between a few dung pats within 1-2 km in their lifetime. This poor dispersal lead him to be concerned that in fragmented pastoral landscapes dung beetles could become locally extinct due to this low dispersal ability.

Euoniticellus intermedius and *Onthophagous gazelle* are described as being “introduced into the USA soon spread to Mexico”. It is difficult to establish how much of this spread is natural, i.e. through beetle flight, rather than human assisted dispersal. Wood and Kaufman (2008) note that *E. intermedius* was released in California in 1978, Texas in 1978 and Georgia 1984 and thus it appears to have crossed the North American continent in six years. How it expanded from these establishment points is unknown but given what occurs in other countries, i.e. Australia, it seems reasonable to assume that much of the spread was human assisted.

The movement of dung beetles in Australia to off-shore islands refers to *Onthophagous gazelle* establishing on Magnetic and Palm Islands, 8 and 30 km off the coast of Queensland (Bornemissza 1976). These islands are immediately adjacent to the primary establishment area of *O. gazelle* in Australia. Palm Island has 4000 residents and an unknown number of livestock, it is serviced by four ferries a week plus a twice weekly barge service bringing food, machinery and fuel to the island. Magnetic Island is a suburb of Townsville with 2100 residents as well as tourist hotels, and has a frequent passenger and vehicle ferry service. As a pathway for establishment on these islands human assistance either deliberate or unintentional, is as likely as the beetles having flown to the islands. Also of note is that these islands are in the cyclone corridor for Queensland and beetles could easily be transported by these events (Flood et al., 2006; Holzapfel and Harrell, 1968).

Comment [A76]: Yes – generic and incomplete. More should be known before introduction. However, in spite of the uncertainty it is clear dung beetles are capable of flying long distances actively searching for new resource and the likelihood of a controlled or limited release is very low.

Comment [A77]: Yes that is why the word ‘dispersing’ was used above. A single uninterrupted flight of several hundred kilometres would be unlikely.

Comment [A78]: The EPA is not disputing here that dung beetles have the potential to spread their population range by several hundred kilometres in 1 year.

Comment [A79]: Roslin noted both short flights between pats and long flights between pastures which led him to the conclusion noted above and supported by others that dung beetles fly from resource patch to resource patch with their flight distance being ultimately determined by the configuration of their resource patches over the landscape.

Comment [A80]: There are many other examples of the rapid spread of dung beetles. For instance, Onthophagus taurus the dung beetle to be released in Northland is suspected to have arrived accidentally in the US and to have spread rapidly in several states. Deliberate introductions were made in California in the late 70’s and the species has since spread into Northern Mexico (Navarrete-Heredia J, Entomological News, 117:211-218, 2006). Four of the six most commonly collected species of dung beetles in Florida are exotics and include O. taurus none of which were intentionally released (Kaufman 2012). Another dung beetle, D. gazella has spread rapidly in North and South America and is now referred to as a highly invasive species’ in Peru where it is thought to have spread from Brazil (Nortega JA Acta Zool Mex, 2010).

Comment [A81]: Human assisted spread has been hypothesised to be both accidental and deliberate. Without doubt, both can contribute to rapid spread of dung beetles alongside vigorous natural expansion.

Public Health (Part 1)

Invertebrates may act as reservoirs and vectors of pathogens capable of horizontal transfer of virulence factors to humans and animals, and potentially also as a pool of future emerging pathogens (Waterfield, 2004).

Waterfield et al. (2004) is a highly speculative article on the role of invertebrates, in general, as an environment in which bacterial strains might develop novel virulence factors that could be spread to existing human commensual or pathogenic bacteria. They say "We believe that invertebrate pathogens act not only as reservoirs and vectors for horizontally transferred virulence factors, but could also provide a potential pool of future emerging pathogens". Their thesis is that all insects that are in some close relationship with humans are a potential source of new diseases. Given the large number of insect species already present in New Zealand it is difficult to see that dung beetles will significantly alter the present risk.

As with the biosecurity risk above, the Agency's view that public health risks are unlikely relies on the Agency's confidence that dung beetles do not spread infectious agents. While dung beetles are unlikely to be direct vectors of disease in people, they can harbour infectious agents that are of risk to people [Lonc 1980, Saitoh 1990, Xu 2003] and there exists the possibility that they might contribute to environmental contamination akin to the role played by carrion beetles (Solter 1989) and cockroaches.

These papers as discussed above simply show that if invertebrate lives in a substrate containing mammalian pathogens and parasites then, not surprisingly, it is possible to isolate those same microorganisms from the insect. They have not demonstrated that these invertebrates vector these pathogens and parasites. Solter et al. (1989) speculated on pathways for pathogenic bacteria picked up by carrion beetles and deposited in the soil could then be transferred from soil to humans. While possible they remain unproven as a source let alone a significant source of such infections. A number of native and introduced species of carrion beetle exist in New Zealand and these have not been linked to any public health risks (Miller, 1971; Kuschel,1990).

In contrast the use of earthworms have been shown, in vermicultural composting, to significantly reduce pathogens in human waste (Eastman et al., 2001). As dung beetles have a similar mode of action and encourage earthworm activity it is like that dung beetles will also reduce mammalian pathogens and parasites found in dung.

Edwards and Subler (Vermiculture http://www.crcnetbase.com/doi/abs/10.1201/b10453-17

The various microbial components of dung tend to survive better when protected from light, dehydration and temperature fluctuations – conditions provided by rapid burial in soil vs surface exposure (Lewis 2011 [Lewis, G. Professor of Microbiology, University of Auckland. Personal Communication. 2011]). Enteric bacteria like *E. coli*, Enterococci and Campylobacter survive longer in damper and cooler sheep and cattle faeces (Sinton 2007, Moriarty 2011). The survival of *E. coli* in cattle faeces is reduced by solar radiation (Meays 2005). Therefore, the likely result of moving dung rapidly into soil is an increase in the soil load of enteric organisms including pathogens such as enteropathogenic *E. Coli*, Campylobacter and Salmonella (Yokoyama 1991, Lewis 2011 [pers. com.]). The soil load of enteropathogens is important because high loads make it more difficult to clear pathogens from herds and make water contamination through near-surface runoff and drains more likely (Lewis 2011). Notably, drains and near-surface seepage are much more prevalent sources of water contamination by microorganisms than surface flow in all but the heaviest of rains (Lewis 2011 [pers. com.]).

Comment [A82]: Yes – this is the hypothesis advanced by the authors. It is the species of insects that associate most closely with human activity (like dung beetles) that are proposed as the more likely source of future pathogens. This is a reasonable hypothesis in light of current knowledge that many recent epizootic human pathogens have emerged from animal species. E.g. AI, SARS etc

Comment [A83]: The large scale of the proposed dung beetle introduction (11 new species across all pastoral farming landscapes in NZ) means the proportional increase in risk may be material.

Comment [A84]: It is clear from this sentence the EPA agrees dung beetles are likely to harbour faecal pathogens of potential significance to people. By definition therefore, they pose a hazard. In turn this means their risk to people should be carefully assessed by empirical studies to quantify the risk.

Comment [A85]: The papers quoted did not set out to undertake transmission experiments (which of course are ethically difficult to undertake in people).

Comment [A86]: NZ has a plentiful supply of earthworms and they play a beneficial role in soil health. They will interact in complex ways with dung beetles. It is difficult, however, to make too many inferences to pastures from the vermicultural study referred to here which calculated the quantity of earthworms at a 1:1.5 wet weight earthworm biomass to biosolids ratio.

Comment [A87]: Lovell and Jarvis Soil. Biol. Biochem 28:291-299, 1996 have shown that the short-term effect on soil microbial biomass of dung pats deposited on the surface of pasture is minor (at least under dry conditions) whereas the effect of burial of dung on microbial biomass is substantial. Hutchison et al, (Appl Environ Micro 70:5111-5118, 2004) have clearly shown that the amount of time livestock faeces remains on the soil surface influences the rate at which enteropathogens decline. Bacterial decline rates of Salmonella spp., E. coli 0157, and Campylobacter were significantly more rapid when faeces were left on the soil surface than when faeces were immediately incorporated into the top 10-15 cm of soil. The D values (number of days required for a 1-log decline in bacterial numbers) for these pathogens in cattle and pig waste were usually 2-3 times lower when faeces was left on the surface. The authors note the need to take into account exposure risk but concluded their results indicate that *not* immediately incorporating contaminated livestock wastes into soil may help to limit the spread of zoonotic agents further up the food chain.

While this section appears reasonable it fails to take into account the ecology of soil and its use in bioremediation of waste water. In simple pasture systems with many animals carrying disease it is reasonable to assume that pathogen loads in the environment will increase as propagules of pathogens and parasites are shed. However as the system becomes more complex, e.g. as for instance with the introduction if dung beetles, the interactions between species becomes more frequent and maintains ecological balance. There is also an assumption that there are many diseased animals shedding propagules which will cause the increase in pathogen load but this is not the case as the occurrence of Tb and Johnes disease is low as is the number of propagules produced. The description above implies that every cattle beast and dairy cow is shedding propagules and dung beetles are frequent enough to be burying every pat. In reality currently there are approximately 80 herds identified with Tb, of which the vast majority contain a single-reactor animals rather than whole infected herds and active management keeps the number low (pers com Choquenot).

Comment [A88]: In a review of enteric pathogens and soil, Santamaria and Toranzos (Intl. Microbiol 6:5-9, 2003) note that the role of soil as a reservoir of certain bacterial pathogens is not in question and the link between enteric diseases of humans and soil has been under-studied and possibly underestimated. They point out there is concern for public health from the application of sewage to soil because the fate of enteric pathogens in soil is not well understood, the infective dose of some pathogens like Crypto is low, and there is the possibility of regrowth of pathogenic bacteria. They note work demonstrating that viruses are inactivated by sunlight on the soil surface but not in the deeper layers. They report several outbreaks of water-borne enteric disease outbreaks associated with contamination from grazing land, on-site disposal systems and wildlife.

Comment [A89]: A poetic story but not reflective of the empirical evidence. For example, see Guan and Holley J Environ Qual 32:383-392, 2003 to understand the growing problems of water contamination from livestock faeces and the serious zoonotic diseases resulting. They note that in general zoonotic pathogens survive longer in water, followed by soil followed by manure and in each of these environments they survive better at lower rather than at higher temperatures. For example E. coli 0157:H7 can survive in soil for up to 99 days, salmonella for several months, crypto for 8 weeks etc. See also Hutchison et al (Appl Environ Micro 70:5111-5118, 2004) as above who clearly show the protective effect of soil vs surface on faecal pathogens.

Comment [A90]: This is incorrect. While the prevalence of faecal M.bovis is low the prevalence of other faecal pathogens including E.coli, salmonella, campylobacter, Yersinia, MAP, rotavirus, cryptosporidia, giardia etc is very high.

Comment [A91]: Tb is not the focus of this section on public health; enteropathogens and MAP are more significant in modern day NZ than M. bovis.

The water run-off scenario is also simplistic in that it assumes only one change and that is the an increase in pathogen load. It does not factor in the increased porosity of the soil holding the water rather than allowing it to run-off quickly, increased root development due to deeper richer soils retaining water longer, and the interaction with an increased soil fauna microbiota interacting with pathogen propagules. This is the basis of bioremediation of waste water (Eastman et al., 2001; Kadam et al., 2008).

Not only are dung beetles that bury faeces likely to increase the soil load of bacteria (Yokoyama 91, Lewis 2011 [pers. com.]) there is also evidence that the burrowing activity of dung beetles can produce periods of increased soil loss following rainfall (Brown 2010) – increasing the risk waterways could be contaminated by enteropathogens from soil.

> “After dung beetle activity on plots soil losses were higher on plots where dung beetles had been active. This was within a week of their burrowing activity where they bring soil to the surface as they excavate their tunnels. Similar concept to earthworm casts but they are a different consistency.
>
> 6 months later, the soil losses were lower on the plots where dung beetles had been active (compared to controls) because the increased infiltration rates produced by the dung beetle activity meant a sustained improvement in infiltration rates. Fig 1 d. in the paper shows this very clearly.
>
> It is obvious. If you dig a hole and leave some soil at the surface, it will wash away. But because there is a hole, more water will penetrate the soil resulting in less surface runoff in the long term.” (Pers. comm. Brown.)

As noted above, the burial of dung by beetles may increase the MAP ‘load’ in soil. The greater exposure of humans to environmental sources of MAP through contaminated dust or water may predispose to Crohn’s disease (Pickup 2005, Gill 2011).

This is dealt with above. There is no evidence to show that there would be an increase in MAP ‘load’ in the soil as a result of dung beetle activity.

Accordingly, the counter-view expressed in the ERMA application that the burial of faeces by dung beetles may reduce public health risk via reduced fly and pathogenic protozoal populations, reduced run-off and less contamination of waterways by microorganism seems unlikely and would certainly require empirical confirmation at a catchment-level scale.

More empirical evidence would give finer resolution to the risk assessment but is not necessary for a risk assessment to be conducted.

Comment [A92]: Many public health problems from waterway contamination occur following heavy rain events. There is little likelihood that the modest increases in soil porosity shown in experimental dung beetle plots (often without earthworms) will have a practical impact in sodden pastures subjected to heavy rain.

Comment [A93]: Jamieson et al Can Biosystems Engineering 44:1.1-1.9, 2002 provides a thorough analysis of the complexities surrounding waterway contamination from agricultural manures. Amongst many other observations the authors note that subsurface injection of liquid manure has been recommended to reduce losses of bacteria in surface runoff but that this may increase survival of pathogens and their transport to subsurface drainage systems.

Comment [A94]: Semenov et al Appl Env Micro 75:3206-3215, 2009 also observed that surface application of manure decreases the risk of groundwater contamination with pathogens compared to injection of slurry.

Comment [A95]: Zaleski et al J Residuals Sci & Tech 2:49-63, 2005 provide an in depth analysis of the fate in soil of enteric pathogens from sewage with a particular focus on the conditions that promote survival and regrowth of pathogens. Moisture content of soil promotes regrowth; the critical mass of the pathogen at initial application or augmented via re-inoculation is also important with larger numbers favouring survival and growth. Large populations of indigenous non-pathogenic microbes decrease pathogen survival. Empirical research on the impact of dung beetles on soil pathogen numbers is required to determine which of these effects will predominate.

Comment [A96]: As discussed above, this comment is misleading. The issue of increased sediment contamination of water resulting from dung beetle burrowing is addressed above.

Comment [A97]: Addressed above. There is good evidence MAP survival is enhanced in shaded faeces, soil and sediment and that contaminated water may be a significant reservoir of MAP (e.g. Whittington et al Appl Env Microbiol 71:5304-5308, 2005.

Comment [A98]: There are clearly far too many uncertainties re the impact of rapid, shallow burial of dung on faecal pathogen numbers in soil and water for the EPA to have conducted an effective risk assessment.

Ten percent of New Zealanders derive their water from roof collection – especially in rural areas. Nocturnal and crepuscular dung beetles in Australia and Northland have been reported to be attracted to the lights of homesteads (see ERMA consultation document). These dung beetles have the potential to contaminate roofs and collect in guttering and water tanks. Given the propensity of dung beetles to undergo so-called 'mass occurrences' when very large numbers take to the wing in mid-summer (Hughes 1975, Flene, 2011) this may create short time points of higher exposure of households to enteric pathogens.

> “The biological quality of roof water in New Zealand is usually poor (Ministry of Health 2001). Potential microbiological contaminants in roof collected water include E coli O157, Cryptosporidium, Campylobacter, Giardia and Salmonella. Salmonella and campylobacter bacteria are increasingly
>
> - detected in roof water supplies in the Auckland region. Likely sources of microbiological hazards in roof collected water include:
> - soil and leaf litter accumulated in gutters particularly if kept damp for long periods of time due to poor drainage and/or maintenance.
> - faecal material from animals including cats, birds and rats.
> - dead animals and insects in the guttering or the tank itself” (Owen and Nickolic, 2002)

Household rainwater tanks have been identified for many years as a significant hazard for householders. Abbott et al. (2006) found that:

> “At least 50% of the roof-collected rainwater samples [560 samples] from private dwellings in New Zealand exceed the minimal acceptable standards for contamination and 30% of the samples showed evidence of heavy faecal contamination. The likely sources of the faecal contamination were faecal material deposited by birds, frogs, rodents and possums, and dead animals and insects, either on the roofs or in the gutters, or in the water tank itself.”

Comment [A99]: A relatively straightforward step in the approach to this risk assessment would be to evaluate the phototaxis of the 7/11 nocturnal or crepuscular beetles proposed for introduction. How strong is the draw to lights? Note up to 50 Mexican dung beetles can be caught per trap per night in Northland and up to 3000 per trap in Australia.

Comment [A100]: It is noteworthy that Landcare (Tompkins Internal Report, March 2012) eventually also agreed that the impact of DBs on roof water collection was a non-negligible risk but discounted it in the belief that this risk would be off-set by reduced nuisance fly numbers – something which is unlikely to happen in NZ.

Comment [A101]: The risk posed by dung beetles is based on the likelihood they will be additional to these other contaminants, may occur periodically in large numbers, are quite large, and are highly likely to be contaminated by the faecal pathogens of domestic animals. Also, Saitoh and Itagaki (1990) make the observation that dung beetles usually defaecate when immersed in water.

Comment [A102]: Given more than 10% of the NZ population relies on roof-collected rainwater (Abbot et al 2007), this figure of 50% (and 56% in Auckland region – Simmons 2001) suggests that upwards of 200,000 people are already drinking water that exceeds minimal acceptable microbial standards. Abbot suggests the gastrointestinal consequences of this exposure is likely to be sporadic and under-reported. Of interest in this regard, is an epidemiological study of campylobacter in NZ (Eberhart-Phillips et J Epidemiol Comm Hlth 51:686-691, 1997) that noted a campylobacter infection odds ratios of 2.2 for people with rainwater sources for home water supply and 3-5.5 for people who have handled bovine faeces in the last ten days. Similarly, a study of a recent epidemic of Salmonella typhimurium in people in NZ found an association between contact with dead wild birds and found S. typhimurium in the roof-collected rainwater drunk by 5 patients (Thornley Emerg Infect Dis 9:493-495, 2003). It is notable that many bird species (e.g. starlings) are likely to predate dung beetles which (as with darkling beetles and poultry - see below) could be a plausible source of infection to the birds.

A number of agencies provide information on the best way to mitigate the risk from collected rainwater (e.g.: Gaw, 2004 [Auckland Regional Public Health Service] Ministry of Health 2011; Abbot, 2007 [Massey University]). Unprotected drinking water is already at risk from contamination by a wide range of insects and other organisms and the addition of dung beetles does not make the likelihood any greater. The measures suggested to mitigate the current risks would be the same for dung beetles, that is, as outlined by Owen and Nickolic (2002) above.

Comment [A103]: Why not? The dung beetle effects would be added to the current poor quality baseline water. Their periodic abundance may create points of higher exposure of households to enteric pathogens beyond minimum infective doses. Recent UoA research shows dung beetles carry greater than 100,000 E.coli per beetle. Given NZ water standards set a minimum acceptable value for E. coli of 1 per 100 ml, even a small number of dung beetles gaining access to a tank may make a significant contribution to the microbial burden. The indirect impacts of dung beetles on faecal pathogen content in soils may also be relevant as dust is an important source of contamination.

Comment [A104]: Agreed but these measures do need to be adopted to be effective. Abbot et al 2007 make the points that information on safe water collection systems seems not to be reaching many users and over 50% of users did not have even simple measures in place to safeguard water. They also note that changing the behaviour of consumers of roof-collected rainwater is not always easy.

Biodiversity (Part 1)

The Agency’s view that the biodiversity impacts of the introduction of the beetles has been adequately examined is difficult to sustain when permission has been granted to introduce species of dung beetle reputably capable of utilising the faeces of large herbivores in New Zealand sub-alpine native grassland and scrubland ecosystems. While it is recognised that exotic herbivores will be doing damage on their own accord in these ecosystems, this does not justify introducing exotic dung beetles with the potential to further destabilise the native flora and fauna in these ecosystems by gradually and cumulatively altering nutrient cycles in the areas frequented by wildlife.

If sufficient large ungulates are present in these habitats to support high numbers of beetles then the habitat is already severely compromised by browsing and by nutrient runoff from dung. The addition of dung beetles is likely to at least reduce nutrient runoff in these compromised habitats. However, the best option would be the removal of the ungulates, as ungulates numbers are reduced, the quantity of available dung will also be reduced, and the beetles will consequently reduce in numbers.

As noted above, the introduced dung beetles may become an excellent quality, consistently available, food source for generalist predators like hedgehogs, rodents, mustelids, opossums, pigs and birds such as magpies, plovers, starlings and crows. If this nutritional boost is sufficient to result in an expansion in the numbers of these predators, there is a significant risk of attendant repercussions on native wildlife in the natural ecosystems bordering pastoral land.

There is no evidence to indicate that the introduction of dung beetles in any jurisdiction has resulted in an increase in pest vertebrates.

Comment [A105]: However, it doesn’t appear that this has been investigated. Unfortunately, no countries who have introduced dung beetles have collected baseline data to allow such before and after comparisons of risk and benefit. Given the proposed scale of this introduction, the nutritional value of the beetles (see previous discussion) and observations that species such as starlings, hedgehogs and rodents will actively forage for beetles, it is implausible that no predator species will benefit. There is also evidence of potential harm to wildlife via aberrant parasitism following consumption of infected dung beetles (see Auk 47:380-384, 1930). Aberrant parasitism from consumption of dung beetles is also suspected to occur in owls, hawks and cranes. The widespread introduction to New Zealand of such a large group of proficient invertebrate intermediate hosts creates the risk that indigenous wildlife species that consume beetles (e.g. bats, moreporks etc) may be exposed to similar risks of aberrant parasitism. Dung beetles also carry many microbial pathogens derived from the livestock faeces they eat. The risk to native species from increased exposure to these pathogens is unknown. It is noteworthy, however, that salmonella species (not necessarily livestock-derived) have been associated with high mortalities in sparrows and other birds. The consumption of dung beetles by wildlife species provides another route through which wildlife can be exposed to the chemical residues excreted in animal faeces. This potential link has been explored in one study of burrowing owls which concluded the risk to these particular birds was low – principally because they had a low degree of dietary reliance on dung beetles.

PART 2

Introduction (Part 2)

Vector and reservoir competence - Invertebrates may act as both reservoirs and vectors of pathogens capable of transmission to humans and animals, and potentially also as a pool of future emerging pathogens (Waterfield, 2004). Most vector-borne pathogens are transmitted among several host (reservoir) species, but different species vary considerably in their importance to pathogen transmission. Overall disease incidence – and the risk of infection to humans in the case of zoonotic diseases – is a function of the reservoir host community's composition (Brunner 2008). Reservoir competence is the product of 1) the probability the individual reservoir host is infected i.e. *prevalence*, and 2) the probability that if the reservoir host is infected, it will transmit the infection i.e. *infectivity* (Brunner 2008). Similarly, vector competence refers to the ability of arthropods to acquire, maintain and transmit microbial agents.

Waterfield et al. (2004) is a highly speculative article on the role of invertebrates, in general, as an environment in which bacterial strains might develop novel virulence factors that could be spread to existing human commensual or pathogenic bacteria. They say "We believe that invertebrate pathogens act not only as reservoirs and vectors for horizontally transferred virulence factors, but could also provide a potential pool of future emerging pathogens". Their thesis is that all insects that are in some close relationship with humans are a potential source of new diseases. Given the large number of insect species already present in New Zealand it is difficult to see that dung beetles will significantly alter the present risk.

Comment [A106]: See comments above. The issue is that the large scale introduction of dung beetles into close proximity to humans may have significant additive effects.

Brunner et al. (2008) examine the 'realised reservoir competence' of ticks, blood feeding parasites, feeding on ten species of vertebrates and their ability to transmit *Borrelia burgdorferi* (Lyme disease agent), between these vertebrate species. While not disputing Brunner et al.'s findings the direct relevance to dung feeding beetles that have not been implicated in the transmission of disease, other than under unusual circumstances, is difficult to accept. Ticks move directly between host mammals, intimately feeding on their blood where as dung beetles move between dung pats and may occasionally be eaten by non-insectivorous mammals.

Comment [A107]: The relevance of the Brunner article was to provide a useful framework to examine the reservoir/vector competence of dung beetles (i.e. to consider matters like prevalence, infectivity etc. However, what is missing from this paragraph is another important consideration 'exposure'. When the issue of prior exposure to faecal pathogens is considered, it implies the added risks to NZ communities from direct contact with dung beetles fall mainly to groups like children and peri-rural urban communities into which dung beetles may fly.

The *prevalence* of gastrointestinal pathogens in dung beetles is likely to be very high all year and in all regions because of their feeding habits. The *infectivity* of dung beetles is likely to be higher to species that deliberately ingest the beetles (poultry, pigs, human infants, some species of wildlife) than to species that accidentally ingest the beetles or parts thereof (pastoral livestock, human adults). The probability of transmission may also be enhanced by the relatively large size of the beetles (increasing the number of organisms or 'dose' carried per beetle), their periodic abundance in farming regions, their attractiveness to curious children and to predators, and the strong flight, attraction to light, high vagility and wide distribution of some species.

While not disputing that dung beetle can become contaminated with gastrointestinal pathogens from feeding in dung there is little or no evidence to show that dung beetles are a source of infectivity to 'poultry, pigs, human infants, some species of wildlife' anymore than invertebrates already present in New Zealand that feed in or around dung. The claimed enhanced probability of transmission is dubious. The dung beetle species in question are not particularly large in comparison with the existing insect fauna, they are not likely to be spectacularly abundant at any given time although there may be some localised peaks in numbers, their attractiveness to children and predators unsupported, their strong flight, attraction to light is unsupported by the evidence and is discussed elsewhere in this review, and evidence for high vagility and wide distribution is again not supported by the evidence.

Vector and reservoir competence has been more carefully studied in darkling beetles than dung beetles. Darkling beetles inhabit the faecal-contaminated litter of poultry sheds and, as described below, are known to be effective vectors and reservoirs for many infectious agents of poultry. They have the ability to internalize enteropathogenic bacteria within their haemolymph and they can be highly infective with the ingestion of one beetle being sufficient to infect a bird. It would seem unwise to assume that dung beetles have any less vector and reservoir competence than darkling beetles. For instance, one recent study of dung beetles showed the beetles to be capable of carrying well in excess of the minimum infective dose of *Cryptosporidia* for people (Conn 2008).

Comment [A108]: The following paragraph appears to be a statement of a prior position or mind set rather than a considered analysis of the potential risks.

Comment [A109]: Many of these invertebrates (e.g. earthworms) don't fly and most aren't likely to come into contact with communities naïve to domestic animal faecal pathogens. Dung beetles are likely to add to this current baseline risk from other invertebrates assuming the Applicant's hypothesis that they will reduce 'filth fly' numbers proves to be incorrect.

Comment [A110]: There is no empirical evidence because these transmission studies have not yet been undertaken. However, there is good quality empirical evidence at each of the steps in the above vector/reservoir competence framework that suggests transmission is plausible – and is a not insignificant risk. There is also evidence from other beetle species (e.g. darkling beetles) that beetle-mediated transmission of disease occurs.

Comment [A111]: They are very large compared to the flighted arthropods that currently visit dung in NZ.

Comment [A112]: There are many references in the literature to the periodic abundance of dung beetles. For example Tyndale-Biscoe et al, Bull ent Res 71:137-152 , 1981 reporting on exotic dung beetles in Australia state "In January and February, a mass emergence of beetles occurred and the beetle numbers increased dramatically so that on 9 February over 3000 came to each trap".

Comment [A113]: There are several reports in the literature of children ingesting other types of beetle that have more immediate (and therefore more easily diagnosable) adverse effects than dung beetles. There is also an enormous volume of literature on the curiosity of children and their innate hand to mouth behaviour. It is implausible that NZ children will not come across dung beetles given the intent of the applicants to ensure that the majority of pastures used for farming will contain at least one or more species of dung beetle and that "in the long term there will be millions chewing and burying dung."

Comment [A114]: There are numerous reports of NZ predators eating beetles.

Comment [A115]: Their strong flight, attraction to light, high vagility and wide distribution are strongly supported in the literature as discussed above.

Comment [A116]: This empirical work in darkling beetles shows it is entirely plausible other beetle species like dung beetles could also be biological as well as transport vectors of high infectivity.

Darkling beetles role in disease is discussed below. Conn et al. (2008, see Xiao 2009) did show that dung beetles in their study sites carries a mean of 93 oocysts per beetle. Lowery et al. (2000) note that in feeding trials with a calf the minimum infective dose was ≤30 and a median dose of 132 oocysts. They also note that in gnotobiotic animals the minimum infective dose could be as low as one oocyst. Given such low infective doses any invertebrate feeding in material containing oocysts could be capable of carrying an infective dose. Conn et al. did not claim that dung beetles were a source of transmission but simply a means of mechanical dissemination.

Comment [A117]: It appears from this paragraph that the EPA does not dispute that dung beetles could transport sufficient cryptosporidia to infect people. Instead, the EPA seems to be implying this is not an issue because crypto can be picked up from other invertebrates. This of course ignores the matter of exposure. The key issue is that urban (and other) people with no exposure to crypto may come in contact with phototaxic dung beetles that fly into their houses, sports-grounds etc. These random aerial transfers of crypto and other faecal pathogens into urban communities is not currently a typical exposure pathway in NZ and the significance of this risk should be clarified.

Public Health (Part 2)

Humans - transmission of infectious agents from beetles to humans is most often thought to occur via accidental ingestion of beetles (or parts thereof) or their excreta in food or water (Jordan 1974, Wilson 2001, Sterling 2006). Transmission via water may pose a greater risk in New Zealand than other developed countries because of the comparatively high reliance of New Zealanders on water from roof collection – especially in rural areas. Nocturnal and crepuscular dung beetles in Australia and Northland have been reported to be attracted to the lights of homesteads (see ERMA consultation document). These dung beetles have the potential to contaminate roofs and collect in guttering and water tanks during the night. Given the propensity of dung beetles to undergo so-called 'mass occurrences' when very large numbers take to the wing in mid-summer (Hughes 1975, Flene, 2011) this may create short time points of higher exposure of households to enteric pathogens through tank water.

There are 36,000 species of beetle (USGS, 2011) of which Jordan (1974) records 10 genera carrying cestodes and nematodes “which may be transmitted by some intermediate invertebrate host that accidentally falls or crawls into food”. The only beetle discussed is a dung beetle feeding on dog dung then transmitting *Spirocerca lupi* to humans. No approval has been given for dung beetles that utilise dog dung which is very different from herbivore dung.

Comment [A118]: As stated above, the point of these references was to show that the transmission of infectious agents from beetles to humans is most often thought to occur via accidental ingestion of beetles (or parts thereof) or their excreta in food or water. The last sentence is incorrect. Some of the beetles approved for importation can utilise (and maintain populations on) dog faeces.

Wilson et al. (2001) reports on a *Gongylonema* (nematode) infection in humans in noting that there have been about 40-50 cases reported worldwide including Europe, North Africa, China, New Zealand, Sri Lanka, and the US. The source of infections is considered to be insect contaminated food. Eleven cases have been reported for the US in a population of 312 million people and an extensive beetle, including dung beetle, fauna. It would seem unlikely the introduction of dung beetles to New Zealand is going to have any effect on such contamination of food stuffs.

Comment [A119]: It would be worthwhile considering whether dung beetles could contaminate agricultural food stuffs during harvesting. For example, will they be attracted to the lights of horticulture and cereal crop harvesters that commonly work throughout the night.

Sterling (2006) reviewed food-borne nematode infection in humans and the only one involving insects is *Gongylonema* infection. He notes that there have been 50+ infections reported in the world literature up until 1999. It is very unlikely the introduction of dung beetles into New Zealand will change the rate of infection by this parasite.

Comment [A120]: Yes this is currently a relatively uncommon parasite. The number of actual cases will exceed the number of reported cases and given the apparent ability of dung beetles to act as an intermediate host (and to be exposed to people through direct or indirect means) it seems likely that more New Zealanders (and livestock) will be diagnosed with this unpleasant parasitic infection over time. The scale of this adverse impact is unlikely to be large but for the affected individuals it will be very unwelcome.

Dung beetles contaminating drinking water has been addressed above.

The deliberate ingestion of beetles by inquisitive children is also reported reasonably regularly. Not surprisingly, most reports of this nature arise when the ingestion of a beetle causes the child to rapidly develop symptoms which in turn increases the likelihood of a temporal association being recognized between the clinical signs and the ingestion of the beetle (e.g. acute vomiting due to cantharidin poisoning from the ingestion of blister beetles) (Wertelecki, 1967, Mallari 1996, Tagwireyi 2000, Al-Binali 2010). Because beetles can carry infectious agents on their exterior surfaces as well as in their intestinal tracts (Mathison 1999, Graczyk 2005), handling of beetles is another likely source of infection. This may pose a public health risk to children who collect dung beetles as has been previously reported in New Zealand with children collecting skinks (de Hamel 1971). It is reasonable to assume that the large size of dung beetles will result in a comparatively large dose of infectious agents should the beetles be ingested or handled.

New Zealand is reported to have 41 species of cantharid beetles (Klimaszewski and Watt 1997). I have not been able to find any records of poisoning or other adverse effects to children through the eating of insects in New Zealand.

Comment [A121]: This comment misses the point of the above references to blister beetles. These papers were quoted to provide evidence that curious infants do from time to time ingest beetles. It is implausible to argue that NZ children will not encounter dung beetles given the massive scale of the proposed introduction.

De Hamel and McInnes (1971) found that native skinks and geckos had *Salmonella Saintpaul* infections in their intestinal tract and that skinks from Otago had higher infection rates than from other areas. This correlated with the higher infection rate in humans from this same area. and McInnes said “[T]his paper does not pretend to show conclusive evidence that the lizards themselves are always responsible for all human cases of *S. saintpaul* infections. The evidence, however, is strong that human infection rates are highest where skink carrier rates are highest”. They also say “[U]ndoubtedly the actual handling of lizards is not necessary for infection to be obtained. It is apparently sufficient for close contact with earth or rocks in an area abounding with lizards to cause infection. Since it has been established that lizard excreta may contain very large numbers of the organisms it is possible that *S. saintpaul* might be picked up on hands or clothing and thereby cause infection by the oral route”. This suggest that it is the presence of the infected animal in the local environment and the failure of people to wash their hands before putting them in or near their mouths that is the major source of infection. The logical conclusion should be to discourage people sitting on grass where herbivores have been or even on lawns where dogs and cats have been because of the possibility of picking up an infection.

Comment [A122]: Again, this misses the relevance of the above reference which was to demonstrate that public health problems from enteric pathogens have been previously recorded as a result of children collecting wildlife as pets. This particular issue with skinks required public authorities at the time to undertake a campaign to warn children and parents of the hazard of collecting skinks. A similar hazard may exist if children collect dung beetles.

Comment [A123]: Goodness me. It might be slightly more practical to discourage people from keeping dung beetles as pets.

Dung beetles may also contribute to a higher risk of enteropathogenic infections in humans by increasing the prevalence of infection in livestock and wildlife reservoirs (see the 'One Health' concept below) and by increasing soil bacterial load (Yokohama 1991). The various microbial components of dung tend to survive better when protected from light, dehydration and temperature fluctuations (Meays 2005, Stinton 2007, Moriarty 2011). Therefore, the likely result of moving dung rapidly into soil is an increase in the soil load of enteric organisms including pathogens such as enteropathogenic *E. Coli*, *Campylobacter* and *Salmonella* (Yokoyama 1991, Lewis 2011). The soil load of enteropathogens is important because high loads make it more difficult to clear pathogens from herds and make water contamination through near-surface runoff and drains more likely (Lewis 2011).

These matters are dealt with above. However, to reiterate the studies cited do not take into account of dung beetle processing of the dung pats, the interaction of dung beetles and other soil fauna with the processed dung, nor do they provide a mechanism for re-infection of livestock from the soil 'reservoir'. This scenario also overlooks the studies of the effectiveness of soil filters to clean up pathogens in waste water (see above).

Livestock and companion animals – transmission of infectious agents from beetles to these species is thought to occur principally by deliberate (e.g. swine, poultry, dog, cat) or accidental ingestion of beetles during grazing (e.g. ruminants). Ingestion of pasture and water contaminated by decomposing beetles or beetle excreta may also play a role. As discussed above, the increase in soil load of bacteria by rapid burial of dung may also enhance risk to livestock (see above).

This has been dealt with above.

Cryptosporidia spp. are zoonotic protozoa that are frequently shed in the faeces of livestock and wildlife in New Zealand and elsewhere. When dung beetles ingest cryptosporidia in faeces the chewing action of their mouthparts destroys a significant proportion of the oocysts but beetles still carry large numbers of oocysts on their external surfaces and in their intestinal tract and faeces (Mathison 1999, Fayera 2000, Conn 2008). All of the beetles examined in one study (Conn 2008) carried potential oocysts of *C. parvum* (mean 93.3 oocysts per beetle) in numbers well in excess of the minimum infective dose for people (10-30 oocysts). The epidemiological importance of dung beetles may be of some significance because of the high vagility and large distribution of some species (Mathison 1999). Many beetles readily search for new sources of dung and migration between deer preserves and cattle pastures has been recorded (Mathison 1999). Mathison (1999) concludes that dung beetles may both aid in the control of pathogens like *Cryptosporidia* by destroying them during feeding or burying them with faeces, or assist in their dissemination by carrying them both externally and internally and should be considered an important aspect of the ecology of gastrointestinal diseases, including cryptosporidiosis.

Mathison and Ditrich (1999) were able to recover oocysts from dung beetles fed contaminated dung under experimental conditions. They concluded:

> *Overall, evidence shows that coprophagous insects may both aid in the control of pathogens, by destroying them during feeding or burying them with feces, or assist in the dissemination of pathogens by carrying them both externally and internally and should be considered an important aspect of the ecology of gastrointestinal diseases, including C. parvum.*

Comment [A124]: The importance of the 'One Health' concept should not be overlooked. It is worth noting that dung beetles have the potential to markedly complicate this epidemiology by creating or exacerbating links between the enteropathogens of domestic animals, wildlife and humans, and soil and water reservoirs, in complex and unpredictable ways.

Comment [A125]: The studies referred to above do take into account the processing of dung by the beetles where the impact of that processing is known (e.g. crypto). However, there does not appear to be any significant studies on the impact of dung beetle 'processing' on the numbers of bacterial enteropathogens. Given that most evidence to date suggests the impact of processing is primarily from physical damage to parasites by beetle mouthparts, it would be unwise to assume that processing by dung beetles also reduces the numbers of enteropathogens.

Comment [A126]: The interaction of other soil fauna with the processed dung is discussed above.

Comment [A127]: The mechanisms by which soil can infect livestock and people is not under-question. See Santamaria and Toranzos Enteric pathogens and soil:a short review. Intl Microbiol 6:5-9, 2003 for a brief general review. In livestock the usual routes are considered to be uptake of bacteria by roots into plants, soil adherent to pasture, pica, and sediment in standing water.

Comment [A128]: There is a large amount of literature on the effectiveness (or lack thereof) of soil in cleaning up faecal pathogens. The over-riding conclusion is its complexity with factors as varied as soil type, soil moisture and water flow, soil pH, the surface properties of microbes, root growth, temperature, micro- and meso-fauna, drainage, and the method of application all having significant effects on vertical and horizontal movement of pathogens through soil (see Mawdsley et al Appl Soil Ecology 2: 1-15, 1995 for a comprehensive review plus the comments above). It is naïve to assume (and misleading to claim) that burial of dung by beetles has a predictably positive effect on this complex picture.

Comment [A129]: This quote from Mathison clearly articulates an important question – what will have the greater impact on public health – the possibility that DB's will reduce crypto in soil (and potentially waterways which public health authorities already have systems in place to 'sterilize') or the possibility of random aerial incursions of crypto laden DB's into crypto naïve peri-rural urban communities?

Although they showed the possibility of dung beetles being mechanical transportes of oocysts they did not suggest any disease pathways. They also comment on the introduction of exotic dung beetles and do not comment on any change in disease frequency or intensity as a result of these introductions. Instead they note the studies on the reduction of pest organisms associated with dung.

Fayera et al. (2000) is a review that simply notes Mathison and Ditrich (1999).

Conn et al. (2008) (note that is a very short conference abstract of 84 words) homogenised 16 beetles collected from facilities and pastures housing livestock. The homogenates were tested using fluorescence in situ hybridization (FISH) 145 and immunofluorescent antibody (IFA) techniques. This is a detection method for oocysts and does not provide any pathways for disease transmission.

Mathison and Ditrich (1999) do not contribute to our understanding of vagility of dung beetles but refer to Hanski and Camberfort (1991, p. 294) who said:

> *The maximum rates of dispersal can be examined in species that have been introduced into areas where they did not occur before. Table 16.2 gives estimates for two medium-sized tunnelers,* Digitonthophagus gazelle *and* Onthophagus taurus*. The observed dispersal rates are surprisingly similar and very high, from 50 to 130 km per year. These values may not be representative for most dung beetles as Onthophagii seem to be exceptionally good dispersers (Section 16.3). It is also possible that the estimates in Table 16.2 are inflated by human transport of beetles on cattle trucks, for instance.*

The figure of 129 km per year for the spread of *Onthophagus taurus* in the southern US (Fincher et al. 1983) is derived from an attempted to survey the spread of this beetle from its deliberate release in Texas and the accidental establishment of it in Florida. Given that the Florida population was an accidental establishment it is likely that more accidental and deliberate establishments also took place over the area that was being surveyed and this would have confounded the results. This is why Hanski and Camberfort (1991) noted that the estimates were probably inflated by human interference.

Further in regard to vagility the statement is made that “Many beetles readily search for new sources of dung and migration between deer preserves and cattle pastures has been recorded (Mathison 1999)”. Mathison and Ditrich (1999) say:

Many beetles readily search for new sources of dung, and 1 of the species studied here, A. stercorosus*, has been observed (data not shown) migrating between deer preserves and cattle pastures.*

Mathison and Ditrich (1999) give no indication of distance as to whether it is metres or kilometres. Very little can be concluded from this anecdotal statement.

Hookworm infection of humans results from both canine and human hookworm species both of which have been recorded in New Zealand. The frequency of hookworm infection in people in poor rural communities overseas has been suggested to depend on the presence of faeces-burying dung beetles (Beaver 1975, Hominick 1987). Hookworms have thin-shelled eggs and free-living larvae that are very sensitive to desiccation; direct sunlight is ovicidal and larvicidal. Dung beetles may play an important role in hookworm survival by protecting eggs and larvae in faecal matter from lethal temperatures on the soil surface (Hominick 1987). Moreover, burial of a faecal mass by dung beetles may provide not only protection for the developing larvae, but also a favoured pathway back to the soil surface (Hominick, 1987).

Beaver (1975) said:

> *The high frequency of heavy hookworm infection in southeastern United States and probably elsewhere may depend largely on the presence of feces-burying dung beetles. Human infection with soil-transmitted helminths of dogs and cats has become a serious public health problem attributable to the persistence of rural mores in the urban setting.*

Unfortunately only the abstract was available for study. However it includes the statement that hookworm has “become a serious public health problem attributable to the persistence of rural mores” indicates that it is the interaction of contaminated cats and dogs with their owners that was the major source of infection. Reading around the topic the Centers for Disease Control and Prevention (no date) says:

> *The growing popularity of dogs and cats in the United States, together with high rates of ascarid and hookworm infections, has resulted in widespread contamination of the soil with infective eggs and larvae. Epidemiologic studies have implicated the presence of dogs, particularly puppies, in a household, and pica (dirt eating) as the*

Comment [A130]: Good to see the potential of DB’s being a mechanical vector of crypto to people is not disputed. On what basis then does EPA consider this mechanical transport a not insignificant risk? Urban people are not very often exposed to crypto from domestic animal species and DB’s offer a plausible method to transmit crypto to them.

Comment [A131]: No but they did note that the epidemiological importance of the beetles may be of some significance considering their high vagility and large distribution and concluded they may assist in the dissemination of pathogens.

Comment [A132]: This wasn’t the subject of their study and could not be commented upon without good quality baseline data to allow comparisons of disease incidence before and after dung beetles. This baseline data is not available. That said, many countries with introduced dung beetles report very high levels of enteropathogenic infections. Australia alone has approximately 17.2 million cases of gastroenteritis each year with 42,000 of these having serious chronic sequelae. The source of these infections is most often not identified. The role dung beetles play in increasing or decreasing this incidence has not been examined.

Comment [A133]: Fayera et al (2000) is an authoritative review providing information on minimum infective dose, and transmission routes of Crypto. He includes the Mathison work on crypto and dung beetles under a section on ‘transport hosts’.

Comment [A134]: The point of this reference was that Conn (2008) demonstrated the number of oocysts on dung beetles can be sufficient to exceed the minimum infective dose.

Comment [A135]: The high vagility of dung beetles has been discussed in depth above. The rapid widespread dispersal of many exotic species of dung beetle is simply not in doubt.

Comment [A136]: The point of including this statement was simply to provide evidence – as clearly stated - that beetles will readily search for new sources of dung and that they will migrate between deer preserves and cattle pastures. It is an observation not an anecdote.

Comment [A137]: The phrase ‘rural mores’ here could mean many things but the most likely is the failure of owners to pick up canine and feline faeces on soil – allowing environmental hookworm infections to build to high levels.

> *principal risk factors for human disease. Children's play habits and their attraction to pets put them at higher risk for infection than adults.*

It is not so much the burying of dung as the close living together of people and pets and the poor hygiene practices of the owners creating a situation of high propagule numbers for repeated infection to occur. Centers for Disease Control and Prevention recommend:

Comment [A138]: Dung beetles are not necessary for hookworm and ascarid infections to develop. However, the suggestion of Beaver (1975) and Hominick (1987) is that the rapid burial of dung exacerbates hookworm contamination.

> *Most cases of human ascarid and hookworm infections can be prevented by practicing good personal hygiene, eliminating intestinal parasites from pets through regular deworming, and making potentially contaminated environments, such as unprotected sand boxes, off limits to children. It is also important to clean up pet feces on a regular basis to remove potentially infective eggs before they become disseminated in the environment via rain, insects, or the active migration of the larvae.*

Also there is only a small number of dung beetles that utilise carnivore dung. In a study in Spain Martín-Piera and Lobo (1996) included three carnivore species' dung in an experiment to see which beetle species used which mammals dung. The dung of two carnivore, lynx and fox, attracted only one beetle species, *Typhaeus momus*, while third species, badger, did not attracted any beetles. In comparison horse dung attracted 21 species of dung beetle. As the case was made in the dung beetle application the dung beetle species selected for New Zealand were those that use herbivore dung and not carnivore dung. It is highly unlikely that these beetles will bury dog and cat dung and as a result not create the public health problem indicated by Beaver.

Comment [A139]: As mentioned previously, the case that the exotic dung beetles will use only herbivore dung was made poorly because the applicant's did not undertake the 'no-choice' feeding tests usually considered a minimum for new organism biocontrol introductions to prove host specificity. There are many species of dung beetle that will consume dog or human dung. In the EPA application,, the overseas dung beetle expert JP Lumaret lists carnivore or human dung as being potentially utilised by G. spiniger, O. taurus, O. vacca, C. hispanus, and B. bison. Publications show the ability of some dung beetles including one proposed for introduction to NZ (O. taurus) to switch from ruminant to dog dung when the former is not available (Carpaneto et al, Biological Conservation 123:547-556,2005). The likelihood of dung beetles utilising dog dung is markedly affected by the nature of a dog's diet. These observations do not support the hypothesis that the dung beetles proposed for introduction to NZ will use only herbivore faeces. This oft-repeated claim should have been tested in containment and is a good example of the substandard biosecurity practices associated with this application.

In a completely different scenario Hominick (1987) considered hookworm infection in West Bengal were there was a lack of latrines and the most people defecated directly on the ground. This human waste was used by dung beetles and perpetuated a disease cycle. Such a situation does not exist in New Zealand and the introduction of dung beetles will not create it.

Comment [A140]: The point of referencing this article was to underscore that hookworm survival (whether human or canine) may be enhanced by the rapid burial of dung. This may have veterinary and public health significance in NZ if any of the exotic dung beetles proposed for introduction prove willing to shift to canine (or human) faeces when they emerge in paddocks devoid of ruminant dung. It is notable that the same concern has been raised by leading NZ parasitologists with regard the potential for rapid burial of dung by beetles to exacerbate nematode parasitism of livestock. The effect of DBs on parasite larvae is likely to be both dung beetle and parasite specific and is yet another area of major uncertainty that should be examined empirically before shallow burying species like O. taurus are released.

***Escherichia coli* 0157:H7** and other pathogenic *E. coli* are food- and water-borne zoonotic pathogens derived from animal reservoirs (Garcia 2010). They cause diarrhoea, haemorrhagic colitis and kidney damage in humans. Fatalities have been recorded and outbreaks of *E. coli* 0157:H7 pose major risks to trade. Like other zoonotic infections, pathogenic *E. coli* (EHEC) are illustrative of the 'One Health' concept as they embody the complex ecology of agricultural animals, wildlife, and the environment in zoonotic transmission of EHEC (Garcia 2010). There is an incomplete understanding of the ecology of EHEC infection in animals and the persistence of EHEC bacteria in the environment (Garcia 2010). Significant aspects of the microbiology, epidemiology, and host-pathogen interactions of EHEC in animals remain undefined. The complexity of these epidemiological interactions is partially captured in the figure below (from Garcia 2010). Dung beetles have the potential to further complicate the epidemiological web shown below by acting as an arthropod reservoir, transmitting infection between livestock and wildlife species, increasing soil load of EHEC (through protection of EHEC from sunlight - Meays 2005), and transmitting EHEC to humans through direct contact and via contaminated food and water. Notably, a Chinese study isolated *E. coli* 157:H7 from the intestine of a small number of dung beetles and found these to be an identical strain to those in ten strains isolated from humans with diarrhoea in the same geographic region (Xu 2003).

According to Garcia et al (2010) nearly all vertebrates are capable of harbouring and shedding EHEC in there faeces. There are effectively two points of infection: one, directly from animal or human faeces and two, from contaminated raw products going into human food. In both cases these can be remedied with normal hygiene practices.

Comment [A141]: The original source of E.coli 0157and other enteropathogenic E. coli infections is ruminant faeces. The sources by which humans are infected include food, water, domestic animals, humans and environmental sources like wildlife, soil, and insects. The source of well over 25% of infections is not identified (Pennington, The Lancet 376:1428-1435, 2010). Dung beetles have the potential to complicate several of these transmission pathways by sharing faecal pathogens between farms and species, altering the soil and water load of O157:H7 and through direct contact with people. Manure-amended soil is capable of harbouring O157:H7 for prolonged periods (Jiang et al, Appl Env Microbiol 68:2605-2609, 2002). Spatial clustering of dairy farms in NZ positive for 0157 has been reported at a distance of around 3-4 km ((Irshad et al, NZVJ 60:21-26, 2012). The linkage of 0157 positive regions with 0157 negative regions via dung beetles is probably undesirable.

Xu et al. (2003) isolated strains of EHEC from the gut of 4 of 113 dung beetles sampled. It is not surprising to find EHEC in this environment given the beetles are feeding in contaminated dung. It is also unlikely that dung beetles will exacerbate the current situation where an already high level of poor hygiene results in most infections. Currently there is no evidence to suggest that dung beetles are a reservoir for EHEC or that there is a viable pathway to cause any increase in infections in humans or any other vertebrates.

Comment [A142]: The Xu study confirms that dung beetles are likely to harbour the faecal pathogens they ingest including E. coli O157:H7. Only very small numbers of bacteria are needed for infection with O157 with one study predicting a probability of infection per bacterium of 0.93% (Pennington, 2010).Given that direct contact between people and dung beetles is likely to occur in NZ following the large scale introduction of dung beetles throughout the country, it seems plausible that immunologically naïve people could be exposed to O157:H7 from contact with beetles.

***Gongylonema pulchrum** (‘the gullet worm’)* is a parasite that infects the mouths of humans (Gutierrez, 1999, Wilson 2001, Mowlavi 2009). It has been reported in New Zealand (Andrews 1976). According to Sterling (2006), this parasite normally occurs in ruminants and swine, with man being an accidental host. Adult worms live in the oesophageal epithelium of their normal hosts. Eggs passed in the faeces are consumed by cockroaches and dung beetles in which larvae mature to the infective third stage. *Copris lunaris* (one of the beetles proposed for introduction to New Zealand) has been shown to carry Gongylonema spp. (Mowlavi 2009). Infection of the definitive host occurs after ingestion of the insect host. Most human infections follow accidental ingestion of cockroaches or dung beetles in food (Wilson 2001, Mowlavi 2009) and some may follow the drinking of water in which larvae had been released from disintegrating insect intermediate hosts (Sterling 2006). In most cases, patients do not recall knowingly ingesting insects (Wilson 2001).

This has been dealt with above.

Moniliformis moniliformis is an intestinal parasite found in most parts of the world including Australia, Polynesia and South East Asia. Common definitive hosts include rats, mice, hamsters, dogs, and cats. The definitive host is infected by eating a beetle or cockroach (Prokopic 1981, Ikeh 1992). These intermediate hosts in turn are infected by eating parasite eggs shed in the faeces of the definitive host. Humans can be incidental hosts with the worm living in the small intestine and producing symptoms such as abdominal pain, vomiting and fatigue (Ikeh 1992, Berenji 2007). Human infections with this parasite have been reported in Australia, Asia, Europe, America, Africa, and the Middle East (Ikeh 1992, Berenji 2007). Toddlers are at risk of infection with this parasite because of their propensity to ingest insects (Bettiol 2000, Berenji 2007, Messina 2011).

Prokopic (1981) was not available for study. Ikeh et al. (1992) reported a of *Moniliformis moniliformis* in a man in Nigeria. They concluded that he had become infected from eating foodstuffs infested with beetles or cockroaches or from eating rats. Berenji et al. (2007) describes the infection of a two-year-old girl with a history of eating dirt and cockroaches. As children with a propensity to eat dirt and cockroaches in New Zealand can already do so it seems unlikely that dung beetles are likely to cause an increase in infection. Similarly, an infection in a 14 month-old child in Australia was traced back to a house the family had been living in being infested with rats (Bettiol and Goldsmid 2000). Messina et al. (2011) reports similar infections from the US were in small children who were exposed to a number of vertebrate species as well as frequently putting objects and insects in their mouths. Interestingly all these cases are reported from regions that have native or introduced dung beetles and these were not invoked as the source of infection.

Comment [A143]: Yes, the key question is whether the large scale introduction of dung beetles will add to the relatively minor risk of M. moniliformis already present in NZ from these other transmission pathways. The EPA presents no plausible arguments as to why an increased incidence will not occur.

Macracanthorhynchus hirudinaceus, the giant thorny-headed worm, is a parasite of pigs and canids that can infect people. The definitive hosts are thought to be infected by the ingestion of dung beetles (Prokopic 1981, Wang 1987, Solaymani-Mohammadi 2003). Human infections have been reported in Iran, Papua New Guinea and Asia (Mowlavi 2006). The parasite is pathogenic to pigs and human infection is thought to occur through contact with the faeces of infected pigs.

Comment [A144]: A human infection has also been reported in Australia in an infant noted by her parents to have occasionally eaten beetles (Prociv et al, Med J Aust 152:215-216, 1990).

Prokopic (1981) was not available for study. Wang et al (1987) was only available as an English abstract of a Chinese paper and the abstract does not provide any information as to whether or not the beetles examined were proven to be vectors of parasites. Both Solaymani-Mohammadi et al. (2003) and Mowlavi et al. (2006) do not provides any new information on beetles as vectors and simply suggests it as a possibility.

Comment [A145]: M. hirudinaceous eggs are infective to a number of beetle species when shed in the host faeces. When ingested by the beetles, they penetrate the intestine and develop in the body cavity into the infective stage over a 65-90 day period. Swine eating the beetles acquire the parasites. (Olsen OW, Animal Parasites: their lifecycles and ecology. 1986). Copris lunaris one of the beetles proposed for introduction has been reported to be an intermediate host of M. hirudinaceous (Sadaterashvili, Trudy Vsesoyuznogo Inst Gel’mintolgii, 16:201-208, 1970).

Hymenolepis diminuta is a common tapeworm of mice and rats (including Kiore) with a widespread distribution including New Zealand (Roberts 1991, Tattersall 1994) that can infect humans. Eggs are passed in rodent faeces and ingested by coprophagus beetles. Ingestion of beetles by rats completes the life cycle. Infection of humans usually occurs in children. It is often asymptomatic but abdominal pain, diarrhoea, irritability and pruritus have been reported (Tena 1998, Easterbrook 2007).

Both Roberts (1991) and Tattersall (1994) report the presence of *Hymenolepis diminuta* in rodents in New Zealand. Tena et al. (1998) note from another publication that “Coprophilic arthropods act as obligatory intermediate hosts”. They do not specifically mention dung beetles and New Zealand already has many species of coprohilic arthropods without having a significant problem with *Hymenolepis diminuta*. Easterbrook et al. (2007) say “Humans and other animals become infected when they eat material contaminated by infected insects or faeces” again without any specific mention of dung beetles.

Comment [A146]: Yes – there are many other intermediate hosts including other beetles, cockroaches etc. The key question is whether the large scale introduction of dung beetles will add to the relatively minor risk of H. diminuta already present in NZ from these other transmission pathways. Once again the EPA presents no plausible reason why an increased incidence will not occur.

Spirocerca lupi occasionally infects humans but is not known to occur in New Zealand. The postulated transmission pathway is either by humans eating infected coprophagus beetles in food or by humans eating infected poultry viscera from birds that have ingested infected beetles (Jordan, 1974).

Jordan (1974) postulated that dung feeding beetles as a pathway of infection but did not provide any evidence that it did happen.

Comment [A147]: There is no doubt Spirocerca lupi uses coprophagus beetles as intermediate hosts and from there may infect a wide variety of paratenic hosts including poultry and rabbits. See Van der Merwe et al, Vet J, 176:294-304, 2008 and Bailey et al, J Parasitology 49:485-488, 1963) for further information. Evidence has recently come to light that S. lupi has been recorded in NZ but has not yet become established .

Canthariasis/Scarabiasis is a condition of humans in which beetle larvae or adults temporarily infest the digestive tract and the beetles are identified in the "fly away" from the anus at the time of defecation (Theodorides 1950, Palmer 1970, Rajapaske 1981, Karthikeyan 2008). This condition is rare and reported most often in children living in tropical or subtropical countries (Karthikeyan 2008). Infection is thought to be from ingestion of dung beetles (or litter beetles) or, more likely, from dung beetles gaining access to the anus of infants playing or sleeping in areas near land contaminated by the faeces of livestock or humans (Rajapaske 1981, Karthikeyan 2008). Rarely the nose and eyes can be infested by the beetle larva causing severe irritation (Karthikeyan 2008).

Theodorides (1950) and Palmer (1970) were not available for review, and Rajapaske (1981) is cited in Karthikeyan et al. (2008). Karthikeyan et al. (2008) report the case of a child with canthariasis in India. The following description is given:

> “The family lived in a small house with cemented flooring and the child slept on bed and at times on the floor. She was an active child and often played without her underclothes in the portico of her house which was facing the road. Occasionally during the daytime she slept on the elevated cement slab in the portico. In the neighborhood, cows and cow dung was a common sight as the neighbors residing opposite her house raised cattle for domestic purposes.”

The conditions described are not common in New Zealand. A study by Majumder and Datta (2010) described similar living conditions of 18 children with canthariasis in India:

> “All children belonged to middle socio-economic class families and were between 2 to 5 years age. Nine (50%) children lived in Kuccha houses and ten (55.56%) children slept on floor in night hours. Most (88.89%) of the children were without their underclothes during playing and daytime. Fourteen (77.78%) children lived nearby cows and cow dung in their neighbourhood.”

The conditions described cannot be considered common in New Zealand.

Comment [A148]: Yes – the condition is rare in tropical countries and is likely to be rare in New Zealand. However, a number of the dung beetle species proposed for introduction to NZ will utilise human faeces.

Miscellaneous other pathogens - coprophagus beetles may serve as hosts for a variety of pathogens that can infect people including *Salmonella* and *Campylobacter* (see section on poultry below) and parasites like *Taenia, Ascaris, Fasciola, Necator, Eimeria, Entamoeba* and *Toxoplasma* (Lonc 1980, Saitoh 1990, Mathison 1999).

Lonc (1980), Saitoh and Itagaki (1990) and Mathison and Ditrich (1999) show that pathogens can be retrieved from beetles fed on contaminated dung, however they do not show that definitively that they are capable of vectoring the pathogens. In all these papers this is speculated but not proven.

Comment [A149]: See previous comments above.

Streptophargus spp. are parasites of primates. They have an indirect life cycle in which dung beetles act as the intermediate host (Munene 1998). Other parasite genera, namely, *Physaloptera, Abbreviata and Protospiruva* which have been found to infect dung beetles experimentally, have also been documented to infect non-human primates. Dung beetles should not be allowed access to primate colonies (Munene 1998).

This is a misrepresentation of the author’s words. Munene (1998) said, with reference to the absence of Streptopharagus infection in a research colony: “Dung beetles should not be available to primates in well-kept colonies. This might explain its absence in CB primates at IPR.”

Comment [A150]: It is unclear whether the authors meant to convey that well-kept colonies are unlikely to have dung beetles (by virtue of the colony being well-kept) or whether they meant to convey those managing well-kept colonies should actively work to exclude dung beetles. This is a matter of interpretation not misrepresentation.

Animal Health (Part 2)

Bovine tuberculosis – as discussed in detail in previous communications with the EPA, dung beetles may pose a risk to tuberculosis eradication by increasing intra-specific and inter-specific transmission of *Mycobacterium bovis* in wildlife reservoirs and by driving more wildlife-to-livestock contact. A recent analysis by the Animal Health Board (AHB) has identified a number of critical components in a putative dung beetle epidemiological pathway that need to be evaluated prior to their introduction to New Zealand including the probability of cattle or deer faeces being contaminated with *M. bovis* and the probability of possums becoming infected from eating infected dung beetles. In addition to these questions identified by the AHB, other issues of interest include: the probability of dung beetles being exposed to Tb-infected faeces of possums, pigs, ferrets etc. shed on pasture and marginal land; the length of time *M. bovis* will remain viable in a brood ball; the risk of Tb 'spill back' hosts like hedgehogs and pigs becoming infected through consuming infected dung beetles; and the possibility of dung beetles becoming vectors for pig-to-pig transmission of *M. bovis* (a transmission pathway which currently, without the new species of dung beetles, is fortunately rare).

This matter has been dealt with in part 1.

Comment [A151]: Yes – see comments in Part1. The empirical work by Dan Tompkins to clarify Tb risks identified by UoA and AHB (and eventually agreed by Landcare) has demonstrated that dung beetles are unlikely to become contaminated with Tb through utilising the dung of those infected cattle currently on farms in NZ. He went on to warn that 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. The likelihood of possums foraging for dung beetles and the probability of dung beetles being exposed to Tb-infected faeces from other species have yet to be clarified but the latter is considered a lesser risk by the AHB.

Johne's disease – *Mycobacterium avium subsp. paratuberculosis* (MAP) has been shown to remain viable in the gut of beetles (Fischer 2004), has the potential to exploit the intracellular existence in insects for its survival (Mura 2006) and may even acquire enhanced virulence (Rowe 2006). As previously raised with the EPA, dung beetles may enhance the risk of Johne's disease by enhancing MAP density in soil, pasture, runoff and groundwater, or by transmitting MAP amongst livestock and wildlife reservoirs. Environmental reservoirs of MAP are considered important in the epidemiology of the disease (Whittington 2005, Pavlik 2010, Pradhan 2011). Wildlife reservoirs of infection would be considered to have major implications for the control of MAP in New Zealand (de Lisle, 2003).

This matter has been dealt with in part 1.

Comment [A152]: Yes – see comments in Part 1. The most important uncertainties compromising the risk assessment of dung beetles and Johne's disease prevalence include the impact of rapid shallow burial of faeces on the soil/water load of pathogenic bacteria like MAP and the impact of DB's on the prevalence of pathogenic MAP strains in wildlife reservoirs (and the degree of these impacts).

Cryptosporidia spp. are a common cause of diarrhoea in calves in New Zealand. As discussed above, dung beetles are likely to assist in the dissemination of *Cryptosporidia* within a region.

Comment [A153]: Cryptosporidia are a common cause of diarrhoea in calves and also affect many other species including humans, wildlife and companion animals. DBs are unlikely to have a significant effect on the already very high rate of calf-to-calf transfer on the same farm. As strong fliers, however, they may play a role as mechanical vectors transferring pathogenic cryptosporidia oocysts from an infected farm to other farms or to other species including humans, wildlife and companion animals (such as cats which are likely to prey on dung beetles and in turn could potentially infect households).

E.coli – as discussed above *E. coli* are food- and waterborne zoonotic pathogens. They may produce little or no discernible disease in their animal reservoirs but can also produce serious enteritis.

This matter has been dealt with above.

Comment [A154]: Yes – see comments in Part1. The most important uncertainties compromising the risk assessment of dung beetles and the prevalence of colibacilosis and pathogenic coliforns include the impact of rapid shallow burial of faeces on the soil/water load of enteropathogenic E.coli, the likelihood of DB's transferring pathogenic strains from one farm to the ruminants, pigs and free-range poultry on another farm, and the impact of DB's on the prevalence of enteropathogenic strains in wildlife reservoirs and companion animals. As discussed above, all of these pathways have potential (indirect) public health consequences. As also mentioned previously, the linkage of 0157 positive farming regions with 0157 negative regions in NZ via dung beetles is probably undesirable. These potential new transmission pathways via dung beetles are additive to the insect-related biosecurity risk already present in the country (see below).

Gongylonema pulchrum as mentioned above this parasite has been reported in New Zealand (Andrews 1976). It is a zoonotic parasite that lives in the oesophageal epithelium of ruminants. Dung beetles are considered to play an important role in its life cycle (Mowlavi 2009).

Mowlavi et al. (2009) found that of 15 species of dung beetle only *Copris lunaris* was infected with a *Gongolenema* sp. Of 231 beetles collected five (2.2%) and the infected beetles were only in one locality. The authors only considered "*C. lunaris* as a potential biological vector for transmission of *Gongylonema* sp. to vertebrates in the surveyed region"

Comment [A155]: Yes and C. lunaris is one of the dung beetles approved for introduction into NZ.

***Rhabditis* sp**. - dung beetles are considered to be important in the life cycle of Rhabditis sp. helminths in cattle pastures in Iran (Mowlavi 2009). This parasite was found in 9 species of beetles. Free-living parasites are also found in soil. The parasite has been implicated in dermatitis and otitis externa.

Mowlavi et al. (2009) has been misrepresented. These authors' said

> "The other kind of nematode detected in this study was *Rhabditis* sp. This nematode exists abundantly in different kinds of soil worldwide. Despite several reports of human infections with free-living nematodes, they are not well considered as the real threatening agents of human and animal health. In the present study, 41 out of 231 (17.7%) collected dung beetles were found carrying these free-living nematodes internally, as well as on external body surfaces."

It is uncertain where the link between the *Rhabditis* sp. in this study and the diseases, dermatitis and otitis externa, came from.

Miscellaneous other pathogens – ruminants are affected by many other pathogens which undergo faecal-oral transmission including *Salmonella spp*., rotavirus, bovine virus diarrhoea, and *Eimeria spp*. The impact of dung beetles on the transmission of these diseases in ruminants is unknown.

As noted in part 1 there are many dung inhabiting insects already in New Zealand all of which could effectively carry these organism and the addition of dung beetles is unlikely to have any impact.

Spiroceca lupi is a parasite of canids that produces lesions in the aorta, oesophagus (sarcomas), spine (spondylosis), stomach and miscellaneous other tissues. Dung beetles are considered the intermediate host (Miller 1961, Brodey 1977, Gottlieb 2011). It is found mostly but not exclusively in tropical or subtropical parts of the world. A decrease in frequency of spirocercosis in dogs from Alabama has been suggested to be due to a decrease in number of dung beetles (Pence 1978).

Comment [A156]: There isn't any 'misrepresentation' of Mowlavi in the above sentences. The paper noted Rhabditis spp. were present in 8 (9 in the abstract) species of beetles in 3 regions of Iran constituting 17.7% infection of all collected dung beetles. It also recorded that 7 species of dung beetle did not show any infection with Rhabditis spp. The authors considered this finding to be of 'great importance from the perspective of ecobiology' and note reports of human infections are not 'well considered'. Further literature research on these comments revealed other authors most commonly implicate Rhabditis spp infection in humans as a cause of dermatitis (Pasyk K, BJDerm. 98:107-112, 1978). There are also many reports of Rhabditis spp in association with dermatitis and otitis externa in cattle and dogs. References noting the association between dung beetles and Rhabditis spp were found dating back as far as 1927 (Triffit, J Helminthology, 33-46, 1927). Poinar (Ann Rev Entomology 17:103-122, 1972) aptly describes the relationship between various beetle species and free living nematodes like Rhabditis as 'facultative parasitism'.

Comment [A157]: As with E. coli, the most important uncertainties compromising the risk assessment of dung beetles and the prevalence of salmonellosis include the impact of rapid shallow burial of faeces on the soil/water load of these very serious enteropathogens, the likelihood of DB's transferring pathogenic strains from one farm to the ruminants, pigs and free-range poultry on another farm, and the impact of DB's on the prevalence of pathogenic Salmonella strains in wildlife reservoirs and companion animals. All of these pathways have potential (indirect) public health consequences. As above, these potential new transmission pathways via dung beetles are *additive* to the insect-related biosecurity risk already present in the country. Salmonellosis occurs regularly in NZ livestock and wildlife. Salmonella survives well in faeces especially when admixed with soil (Nicholson et al, Bioresource Technology 96:135-143, 2005); You et al Appl Env Micro 72:5777-5783, 2006) and even appears to be able to replicate in soils (Winfield and Groisman, Appl Env Micro 69:3687-3694, 2003). As mentioned above, Semenov et al (2009) observed that surface application of manure decreases the risk of groundwater contamination with salmonella compared to injection of slurry into the soil. A significant increase in salmonellosis has been observed in people drawing well water from irrigated dairy land in NZ and flows of bacteria (e.g. campylobacter and E.coli) increase following heavy rain or irrigation events which the authors attribute to bacteria being released into the soil from cowpats (Close et al J Water Health, 06.1, 83-98, 2008). As noted above, many bird species (e.g. starlings) are likely to predate dung beetles which (as with darkling beetles and poultry - see below) could be a plausible source of Salmonella infection to the birds.

The papers quoted here do show that some species of dung beetles can be intermediate hosts for *Spiroceca lupi.* If we assume as a basic model a disease triangle there are at least three, if not four, diseases in these publications. Brodey et al. (1977) studied disease in uncontrolled urban dogs in Kenya noting the incidence of diease. They noted that “In areas of high *S. lupi* prevalence, cattle, chickens, dung beetles and dogs were in close association” but did not attribute the disease incidence to dung beetles. Gottlieb et al. (2011) looked at the increase in disease incident in urban dogs in Israel and found that dogs became infected with *S. lupi* when walked in shady irrigated parks where nematodes were able to survive the normally dry conditions. It would appear that there is no dog faeces management or hygiene in these parks and dogs are able to freely interact with accumulated dog faeces. Where pet dogs are routinely ‘wormed’ there are no health problems. Pence and Stone (1978) looked at disease in wild canids (coyotes) in the United States and in particular Alabama. They concluded that “one species of these wild carnivores, the coyote, seems to be the principal host and disseminator of *S. lupi* from this area. Considering the common and widespread occurrence of *S. lupi* in wild carnivores, especially the coyote, in Texas, it seems unusual that the infection is so infrequently observed in dogs from the state.” If dung beetles are important in the vectoring of the disease it is not apparent from this study.

Comment [A158]: As mentioned above, there is no doubt Spirocerca lupi uses coprophagus beetles as intermediate hosts and from there may infect a wide variety of paratenic hosts including poultry and rabbits. See Van der Merwe et al, Vet J, 176:294-304, 2008 and Bailey et al, J Parasitology 49:485-488, 1963) for further information. Also, as mentioned above, information has been recently uncovered that suggests S. lupi has been recorded here but has not yet established.

Moniliformis moniliformis – as mentioned above, this intestinal parasite can infect dogs and cats when they eat an infected beetle or cockroach.

***Macracanthorhynchus hirudinaceus* –** as discussed above this parasite can infect dogs after the ingestion of infected dung beetles.

These matter has been dealt with above.

Hookworms are an important cause of morbidity in dogs housed on soil in New Zealand. As noted above, dung beetles may play an important role in hookworm survival by protecting eggs and larvae in faecal matter from lethal temperatures on the soil surface.

No supporting evidence for this pathway was provided.

Comment [A159]: Evidence for the potential impact of dung beetles on human and canine hookworm infection was given above along with evidence several of the exotic dung beetles proposed for introduction will utilise human and/or canine dung.

Toxoplasmosis and Isospora - dung beetles feeding on cat feces infected with *Toxoplasma gondii* were found to carry infective oocysts both in their faeces and on their bodies (Saitoh 1990). Mice that then consumed these beetles were capable of infecting kittens (Saitoh 1990). *Isopora felis* and *Isopora rivolta* have been found on dung beetles collected from urban dog faeces. These dung beetles were also able to transmit these coccidia to three of four kittens via dung beetle-mouse consumption, suggesting a potential incidental or intermediate host role for some beetle species in feline coccidian infections (Saitoh 1990).

Saitoh and Itagaki (1990) was reviewed in part 1.

Comment [A160]: As discussed previously, Saitoh and Itagaki investigated the role of dung beetles as transport hosts of Toxoplasma gondii. They showed clear evidence dung beetles can vector toxoplasma.

Miscellaneous other pathogens – dogs and cats are affected by many other pathogens which undergo faecal-oral transmission including *Salmonella spp*., *E. coli*, and *giardia*. The impact of dung beetles on the transmission of these diseases in companion animals is unknown.

It can only be reiterated here that humans, cats and dogs occur all over the world in association with dung beetles and there is no publications which indicate that there is a problem.

Comment [A161]: Unfortunately there do not appear to be any studies that have looked at transmission of these pathogens from dung beetles to cats and dogs. Again, this may be a case of “If you do not look you will not find.” It is clear that cats in NZ will eat beetles (Fitzgerald and Karl NZ J Zool 6:107-126, 1979. Thus, there is a plausible direct exposure pathway for cats to the enteropathogens harboured by dung beetles as well as an indirect exposure pathway via rodents which also eat dung beetles (Saitoh and Itagaki (1990). Similarly, it is likely that dogs will eat dung beetles if they come across them.

Swine nematodes - dung beetles are intermediate hosts for several widely distributed swine nematodes (Fincher 1969, Roepstorff 1994). Dung beetles are commonly infected with the third-stage larvae of *Physocephalus sexalatus*, a spirurid stomach worm of swine (Fincher 1969). Other swine parasitic nematodes recovered from dung beetles include *Ascarops strongylina*, *Gongylonema spp*. and *Physaloptera spp*. These parasites are economically important to swine producers because of the necessity of repeated anthelmintic treatments, the lack of efficient gains, and increased mortality.

***Macracanthorhynchus hirudinaceus* –** as discussed above this parasite can infect the small intestine of pigs after the ingestion of infected dung beetles (Roepstorff 1994).

As has been discussed previously with other publications Fincher et al. (1969) show that beetles feeding in contaminated dung will harbour pest nematodes however these publications do not provide any pathway for the disease cycle and the authors make statements such as:

> "It is doubtful that *Dichotomius carolinus* beetles are effective intermediate hosts for nematode parasites of swine although 23.7% of the beetles were infected with *P. sexalatus*."

Roepstorff and Nansen (1994) note that *Ascarops strongylina* and *Physocephalus sexalatus* and *Macracanthorhynchus hirudinaceus* are uncommon disease, in northern Europe, despite the presence of dung beetles and are only mentioned for completeness in this publication.

Miscellaneous other pathogens – pigs are affected by many other pathogens which undergo faecal-oral transmission including *Salmonella spp*., *Treponema hyodysenteriae*, rotavirus, transmissible gastroenteritis virus, and *Eimeria spp*. The impact of dung beetles on the transmission of these diseases in pigs is unknown.

It can only be reiterated here that pigs exist all over the world in association with dung beetles and there is no publications which indicate that there is a problem.

Some dung beetles (such as several *Onthophagus* spp.*)* will consume poultry faeces (Kabir 1990) and they have been implicated in the transmission of parasites in free range birds in third world countries. However, as mentioned above, most research on the reservoir competence of beetles in poultry has been performed on darkling beetles (Coleoptera: Tenebrionidae) which, unlike dung beetles, can live inside poultry houses. This research illustrates how important beetles can be in the transmission of disease in poultry and raises questions about the potential impact of dung beetles on free-range poultry operations.

Comment [A162]: The paper by Fincher et al is entitled "Beetle intermediate hosts for swine spirurids in Southern Georgia". They documented a high incidence of infection of some but not all species of dung beetles with swine nematode larvae and reported their observation that swine are attracted to and eat insects. They also suggest larvae may over-winter in dung beetles. The reason they concluded Dichotomius carolinus, unlike other beetles in their study, was unlikely to be an effective intermediate host was that this beetle species flies at night. They go on to say that in contrast "Phanaeus vindex adults make good intermediate hosts for stomach worms of swine because they are numerous around swine habitats during the day and are available for consumption except during winter." In a later publication (Fincher et al J Entomol Soc Am 64:855-860, 1971) Fincher investigates the flight activity of coprophagous beetles on swine pasture and concludes: "Flight activity, coupled with other biological and ecological factors, indicated that P. vindex was the most important vector of swine spiruids in southern Georgia." In earlier work, Cram (Auk 47:380-384, 1930) showed by feeding experiments that P. carnifex and Canthon laevis were capable of infecting pigs with large numbers of Physocephalus sexalatus. She also demonstrated that this swine parasite could be found encysted in the intestinal tract of birds including owls, hawks and Shrikes which she attributed to the consumption of infected dung beetles. When infected Shrike intestine was fed to pigs, a heavy P. sexalatus infection developed. The author was unable to determine whether the aberrant parasitism adversely affected the health of the birds because she was unable to locate uninfected birds to enable a comparison. These observations of aberrant parasitism associated with dung beetles may have relevance to NZ birds should the exotic dung beetles be introduced.

Comment [A163]: Again, this may simply be a case of if you do not look you will not find. It is important not to confuse the lack of *evidence* of risk with the lack of risk nor to ignore NZ-specific factors. NZ has a relatively high proportion of its pig industry based on free range piggeries. The pork industry recommends strict biosecurity standards to maintain the health of pig farms that includes routine barrier protocols between farms. The isolation of farms from one another is assisted by the geographic separation of farms. At least two of the eleven dung beetles will utlise pig faeces and, as strong fliers, may provide a regular corridor between pig farms in a region transfering pathogens such as these from farm to farm.

It has never been disputed that some dung beetle species have evolved to use poultry dung as Kabir et al.(1990) study from Bangladesh shows. There role if any in poultry disease is not obvious.

Comment [A164]: It is plausible that dung beetles will be an effective vector of disease between free-range poultry farms. As with pigs, there is little doubt that poultry will eat dung beetles and that they can carry parasites and pathogens of significance.

Campylobacter spp. – Darkling beetles can carry *Campylobacter spp*. in poultry houses (Strother 2005). *C. jejuni* was detected on the exterior of larval beetles for 12 hours from exposure, from the interior of larvae for 72 hours, and from the faeces of larvae for 12 hours after exposure (Strother 2005). Ninety percent of the birds that consumed a single adult or larval beetle became *Campylobacter*-positive (Strother 2005). It is recommended that beetles are eliminated to help maintain *Campylobacter*-free poultry facilities (Strother 2005). In a recent study in New Zealand, a set of genetically distinct Campylobacter isolates was found to be common to broiler flocks and to darkling beetles. This research suggests that the beetle may serve as a source of Campylobacter contamination of poultry (Bates 2004). Darkling beetles can transmit *Campylobacter* to flocks in successive rearing cycles (Hazeleger 2008).

While these studies are interesting darkling beetles are not dung beetles and the case cited is specific to high density, indoor production facilities. It can only be reiterated here that chickens are kept all over the world in association with dung beetles and there is no publications which indicate that there is a problem. A specific search for dung beetles in chicken deep litter systems did not find any information.

Comment [A165]: As mentioned above, the importance of darkling beetles in the epidemiology of disease in indoor poultry production facilities demonstrates the existence of plausible biological mechanisms for beetle-mediated poultry diseases.

Salmonella spp.- Darkling beetles infesting broiler chicken rearing facilities can act as potential reservoirs for Salmonella spp. between consecutive broiler flocks (Skov 2004). The beetles are capable of internal carriage of Salmonella. They rapidly acquire the bacteria from external sources and harbor the bacteria within their alimentary canal and haemolymph (Crippen 2009). Ingestion by chicks of as few as 4 or 5 beetles or larvae infected with *Salmonella typhimurium* or *Salmonella enteritidis* is sufficient to produce *Salmonella* positive birds (Roche 2009, Leffer 2010). The beetles can transmit *Salmonella* to flocks in successive rearing cycles (Hazeleger 2008).

The above discussion for *Campylobacter* is relevant here.

Comment [A166]: It is plausible that, like darkling beetles, dung beetles could also acquire Salmonella from faeces and harbour the bacteria in their intestinal tract and haemolymph infecting birds on the same or neighbouring farms following ingestion.

Spirocerca lupi - chickens are transport hosts for *Spirocerca lupi*. Infective third stage larvae encyst in the crop after ingestion of infected dung beetles (Brodey 1977). Dogs and people can then be infected by ingesting the chicken viscera or by direct ingestion of dung beetles.

This matter is discussed above. Given the nature of New Zealand society it is highly unlikely that people will eat dung beetles or raw chicken viscera. New Zealand has the highest rate of food poisoning from *Camylobacter* in the world resulting from the poor handling of chicken in both commercial and domestic kitchens. There are many New Zealand publications and websites that explain this and profide information as to how to avoid food poisoning. The introduction of dung beetles is unlikely to have any effect on the levels of food poisoning in New Zealand. (See Paterson 2012).

Comment [A167]: Yes – as with campylobacter, handling of uncooked chicken or ingestion of poorly cooked chicken would be the most likely transmission pathway.

Comment [A168]: As discussed above, the potential influence of dung beetles on food poisoning in NZ could come through many routes ranging from direct contamination of food, to poor hygiene following the handling of beetles, to greater environmental contamination of soil and water resulting from the rapid shallow burial of dung.

Miscellaneous – Dung beetles have been reported to be intermediate hosts of avian tapeworms (Miller 1961). Darkling beetles have been associated with the transmission of a wide variety of poultry pathogens in addition to *Campylobacter* and *Salmonella* including Escherichia spp, viruses such as the agents of fowl pox, infectious bursal disease, Marek's disease and Newcastle disease, fungi of the genera *Aspergillus*, *Penicillium* and *Candida*, and protozoans such as *Eimeria* (Goodwin 1996, Bates 2004, Retamales 2011). Unsurprisingly, Goodwin (1996) concludes that "the threat of severe adverse economic impact from beetle-vectored disease should not be overlooked or casually dismissed".

These matters are addressed above. Goodwin and Waltman's (1996) comment was specifically about darkling beetles and not about dung beetles or beetles in general.

Comment [A169]: Yes, but as mentioned above, it would seem cavalier to assume the biological mechanisms for beetle-mediated poultry diseases that have clearly been demonstrated in darkling beetles do not occur in dung beetles.

Insect-related disease risk already occurs in New Zealand in particular through a number of fly species of public health significance (so-called 'filth flies'). The change to this risk following the introduction of 11 new species of dung beetles will depend on such matters as the epidemiological effectiveness of beetles as vectors, hosts and reservoirs (see above), the abundance of the new beetles, and the influence of the beetles on the survival of pathogens, the abundance of faeces and the number of 'filth' flies.

As discussed by the Applicants (ERMA 200599), dung beetles may aid in the control of some pathogens by destroying a proportion of fragile parasites and labile infectious agents during feeding or by burial in soil. Whether or not this reduction in some types of pathogens will make a material difference to disease prevalence has not yet been established and will depend on many factors including the exposure thresholds of the various pathogens.

Given the rapid disappearance of dung in New Zealand pastures, it is as yet unclear how effective dung beetles will be in reducing the amount of dung available to coprophagus flies in New Zealand. It is also unclear what impact the introduced dung beetles will have on the number of syanthropic ‘filth flies’ in New Zealand given that most common fly pests in New Zealand (e.g. the house fly and blowflies) are not dependent on faeces. However, at least one fly species *Muscina stabulans* (the false stable fly) is found in dung in New Zealand and is included on lists of ‘filth flies’ of public health importance (Dymock 1993, Graczyk 2005).

Comment [A170]: As noted above, the goal of the applicants is to introduce beetles ‘in their millions’ so they are active across all regions of the country, in all seasons both day and night. The likelihood of this proposed surge in pathogen-carrying insects is highly unlikely to be off-set by reductions in fly numbers because few (if any) of the pest fly species in NZ are obligate dung breeders instead using decaying vegetable matter or carrion. Even the stable fly mentioned above does not require dung to breed (Allen Heath, Pers. Com.).

These matters were full discussed in the E&R and in part 1 of this document. No new information has been presented here.

Summary

In summary, the infectious agents carried by coprophagus beetles appear to pose significant risks to public health (especially children), pastoral livestock, free-range pigs and poultry, and companion animals. Dung beetles have the potential to play an important role in the ecology and epidemiology of human and animal gastrointestinal diseases and to produce adverse economic impacts for some livestock industries.

The review of the publications present here does not support the above summary.

Comment [A171]: In summary, it appears that the EPA’s decision to approve the importation without control of 11 species of dung beetle was based on an overly positive assessment of the possible benefits of the dung beetle introduction coupled with an incomplete consideration of the risks in the NZ context. Contrary to the assertions of the Applicants, few farm-level benefits have been objectively demonstrated in the countries to which exotic dung beetles have been introduced. The establishment, maintenance and theoretical benefits of dung beetles can be compromised by a wide array of biological, agricultural, social and economic factors which conspire to mean that tangible benefits are far from assured in our complex farming systems and their associated catchments. The application-to-import dung beetles prepared by Landcare Research, the EPA’s written record of its decision and the EPA’s notes above suggest that the Applicants and Agency were not fully acquainted with the relevant literature, or of equal importance, fully cognisant of the lack of relevant research to adequately quantify the risks of exotic dung beetles to biosecurity, biodiversity and public health. Given the uncertainties outlined above, the paucity of proven benefits at a farm system level and the gravity of the consequences should any of the potential risks materialise, it would seem prudent for the Applicants to undertake empirical New Zealand-based research to improve the reliability of the risk assessment.

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Comment [A172]: This reference list blends the list attached in the original review of dung beetles as vectors and reservoirs with references added by the EPA. It does not include the references referred to in the comments in the margin – the essential details of which are shown in parenthesis alongside the relevant comment.

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