Lech, Katharine. A. and Archer, Simon C. and Breen, James E. and Green, Martin J. and Ohnstad, Ian C. and Tuer, Sally and Bradley, Andrew J. (2015) Recycling manure as cow bedding: potential benefits and risks for UK dairy farms. The Veterinary Journal, 206 (2). pp. 123-130. ISSN 1532-2971

Elsevier Editorial System(tm) for The Veterinary Journal
Manuscript Draft

Manuscript Number: YTVJL-D-14-01009R4

Title: Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms

Article Type: Review Article

Keywords: Dairy Cattle; Recycled Manure; Bedding; Udder Health; Risk Management

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Abstract: Material obtained from physical separation of slurry (recycled manure solids; RMS) has been used as bedding for dairy cows in dry climates in the US since the 1970s. Relatively recently, the technical ability to produce drier material has led to adoption of the practice in Europe under different climatic conditions. This review collates the evidence available on benefits and risks of using RMS bedding on dairy farms, with a European context in mind. There was less evidence than expected for anecdotal claims of improved cow comfort. Among animal health risks, only udder health has received appreciable attention. There are some circumstantial reports of difficulties of maintaining udder health on RMS, but no large scale or long term studies of effects on clinical and subclinical mastitis have been published. Existing reports do not give consistent evidence of inevitable problems, nor is there any information on clinical implications for other diseases. The scientific basis for guidelines on management of RMS bedding is limited. Decisions on optimum treatment and management may present conflicts between control of different groups of organisms. There is no information on the influence that such 'recycling' of manure may have on pathogen virulence. The possibility of influence on genetic material conveying antimicrobial resistance is a concern, but little understood. Should UK or other non-US farmers adopt RMS, they are advised to do so with caution, apply the required strategies for risk mitigation, maintain strict hygiene of bed management and milking practices and closely monitor the effects on herd health.

Review

Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms

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Abstract

Material obtained from physical separation of slurry (recycled manure solids; RMS) has been used as bedding for dairy cows in dry climates in the US since the 1970s. Relatively recently, the technical ability to produce drier material has led to adoption of the practice in Europe under different climatic conditions. This review collates the evidence available on benefits and risks of using RMS bedding on dairy farms, with a European context in mind. There was less evidence than expected for anecdotal claims of improved cow comfort. Among animal health risks, only udder health has received appreciable attention. There are some circumstantial reports of difficulties of maintaining udder health on RMS, but no large scale or long term studies of effects on clinical and subclinical mastitis have been published. Existing reports do not give consistent evidence of inevitable problems, nor is there any information on clinical implications for other diseases. The scientific basis for guidelines on management of RMS bedding is limited. Decisions on optimum treatment and management may present conflicts between control of different groups of organisms. There is no information on the influence that such 'recycling' of manure may have on pathogen virulence. The possibility of influence on genetic material conveying antimicrobial resistance is a concern, but little understood. Should UK or other non-US farmers adopt RMS, they are advised to do so with caution, apply the required strategies for risk mitigation, maintain strict hygiene of bed management and milking practices and closely monitor the effects on herd health.

Keywords: Dairy Cattle; Recycled Manure; Bedding; Udder Health; Risk Management

Introduction

The concept of using material described as 'dairy waste solids', 'separated manure solids' or 'recycled manure solids' (RMS) as bedding for cattle (recently termed 'green bedding' in the UK), was established in the US in the 1970s (Keys et al., 1976; Timms, 2008a). Rising numbers of expanding housed US dairy herds increased the amounts of manure produced, but the ability to separate solid and liquid fractions using a screw or roller press facilitated handling the material.

The solid fraction of manure consists mainly of undigested fibres (Menear and Smith, 1976) and the potential of using this fraction as bedding material was explored initially in hot dry areas in the Western United States, in 'dry lot' dairies, where maintaining 'a high dry matter content' (Timms, 2008a) was easy. Due to concerns about high bacterial load, further processing steps were incorporated, initially composting, which aimed to reduce bacterial numbers by raising the temperature (Carroll and Jasper, 1978). Later, it became popular to use as bedding solid material extracted from the products of the anaerobic digestion of manure as a way of offsetting the cost of digesters (Timms, 2008b). Many combinations of separation, digestion and composting are now practised in the USA, allowing successful use of RMS bedding in cooler, wetter regions of the US (Timms, 2008a, b, c).

Increased marketing of high performance slurry separation machinery, that can produce separated manure solids with over 30% dry matter (DM), has generated interest in this practice in Europe, where there are very different climatic conditions (Zähner et al., 2009; Feiken and van Laarhoven, 2012; Marcher Holm and Petersen, 2015). Livestock manures are Category 2 Animal By-products, as defined by EC

Regulation 1069/2009. As such, their use as a 'technical product' (e.g. animal bedding) is only permitted if strict conditions apply which minimise the health risks involved. 'Safe end use' of a product derived from animal by-products is defined as use '*under conditions which pose no unacceptable risks to public and animal health*' (EC Regulation 1069/2009). Member State jurisdictions are approaching this requirement in different ways. In the UK, the Department for Environment, Food and Rural Affairs (Defra) and the Scottish Office have allowed the use of this bedding under controlled conditions, while research is carried out, whilst in Wales and Northern Ireland the practice is currently (May 2015) prohibited.

This review article considers in a UK context the scientific basis for the opportunities and challenges presented by RMS bedding. In view of the limited peer reviewed literature on the subject, we also draw on conference proceedings and unpublished research reports.

Potential benefits

Farmers' interest in RMS is based largely on economics, availability and cow comfort and this is true in UK as elsewhere (Leach et al., 2014). Economic calculations must be made at individual farm level, considering the capital cost of equipment, management time and running costs, set against the purchase and management costs of current bedding materials. Availability is more under the farmer's control than when depending on an external bedding supplier. UK farmers, for example, perceive 'more comfortable cows', longer lying times and fewer hock lesions than on previous bedding materials including paper, sawdust, or even sand (Leach et al., 2014).

Physical attributes of RMS suggest potential advantages for cow comfort. It is soft, non-abrasive, and readily available. DM content appears to influence cow preferences; cows chose to lie less on stalls with ‘dewatered manure solids’ (29% DM), compared with ‘dehydrated manure solids’ (81% DM), and sawdust (81% DM), at equal depth (Keys et al., 1976). Cows have also shown preference for cubicles bedded with ‘manure separates’ compared to straw, sand and sawdust (Adamski, 2011). Longer lying times were recorded on three commercial farms following a change from mats to deep beds of RMS (Feiken and van Laarhoven, 2012).

RMS has advantages for hocks over mats with or without sawdust or straw (Zähner et al., 2009), or dolomitic limestone (Hippen et al., 2007). However, hock lesion prevalences when on RMS of 40-53% for deep beds (Zähner et al., 2009; Husfeldt and Endres, 2012), and 63-72% for mattresses (Husfeldt and Endres, 2012) have been reported. From a survey of 297 dairies, Lombard et al. (2010) reported a higher prevalence of severe hock lesions in cows bedded on dry or composted RMS compared with sand, straw and sawdust. The main advantage may be that farmers are willing to use more generous amounts of RMS (Leach et al., 2014); deeper layers of bedding have been associated with lower prevalence of hock (Brenninkmeyer et al., 2013) and claw lesions (Barker et al., 2009).

In support of farmer perception of cow cleanliness (Leach et al., 2014), Hippen et al. (2007) reported a trend for cleaner cows on RMS than on dolomitic limestone, and Timms (2008c) an ‘improvement’ in cleanliness on RMS from a previous, unspecified bedding material. Feiken and van Laarhoven (2012) found cows on RMS to be dirtier than those on sawdust or wheat straw, but cleaner than those on compost. However, visual cleanliness does not necessarily mean absence of pathogens, and, in view of the bacterial load of the bedding, close attention should still be given to pre-milking teat preparation (Endres and Husfeldt, 2012).

The lower dust levels reported with RMS compared with chopped straw or sawdust (Leach et al., 2014) or oat hulls (Meyer, 2007) may have benefits in terms of respiratory health for both animals and humans, and reduced transmission of pathogens via dust particles, but there is no information on the transmission of pathogens by aerosols related to this material.

Risks posed by RMS used as bedding on dairy farms

The main potential risks of RMS bedding are to animal health, human health, product quality, and consumer perception. From the financial perspective of the farmer, there is also the risk of future prohibition if threats to animal or human health are deemed to be too high.

Based upon literature review and input by Defra (the UK ‘Competent Authority’) to a scoping study (Bradley et al., 2014), key micro-organisms that should be considered are shown in Table 1. Lungworm and most intestinal parasites have not been included since these would be unlikely to complete their full life cycle in the manure, and experience with other farm species indicates that total confinement systems are not associated with high parasite burdens. Information to evaluate risk for viruses is extremely limited.

Tables 2 and 3 summarise the data available on pathogen load in RMS before use, after separation only, and after further processing, respectively. Table 4 summarises data on pathogen load for various used bedding materials, including RMS. These data illustrate the fact that, although bacterial counts in RMS as a raw material are high, counts in many other materials can reach similar levels once in use as bedding.

Any increased potential for development and perpetuation of antimicrobial resistance caused by recycling manure would have implications for both animal and human health. There is one report of an association between use of RMS and presence of antimicrobial resistant strains of *Salmonella* in cattle faeces (Habing et al., 2012).

Animal health risks

No studies were found that directly related RMS use to clinical incidence or prevalence of any infectious disease other than mastitis. The three health conditions for which there is any more than a theoretical basis for consideration of the risks associated with RMS bedding are discussed below.

Udder health

In view of work that has linked risk of mastitis to pathogen numbers in bedding (Bramley and Neave, 1975; Carroll and Jasper, 1978; Hogan et al., 1989), RMS must be considered as at least a theoretical risk, based on the pathogen levels reported in the literature. However, evidence to quantify the risk of actual clinical outcomes compared with other bedding materials is limited, particularly from climates comparable to the UK.

Some case studies reported udder health problems, and others demonstrated no detrimental effects arising from changing to RMS bedding. Case studies in Italy (Locatelli et al., 2008) and the USA (New York State; Ostrum et al., 2008), have linked increases in environmental mastitis caused by *Escherichia coli* or *Klebsiella* spp. with separated manure solids that were stored before use. In three Dutch herds converting to RMS, no increased incidence of *Klebsiella* spp.-related mastitis or total cases of clinical mastitis was identified, although the concentration of *Klebsiella* spp. was higher in the RMS than in sawdust (Feiken and Van Laarhoven, 2012).

On two American farms, Buelow (2008) failed to find a correlation between bacterial counts in RMS bedding and clinical or subclinical mastitis. Husfeldt and Endres (2012) reported a range of mastitis incidence of 9 - 109 cases per 100 cows per year on 34 farms in the American mid-West using RMS bedding. Cows were culled more frequently for mastitis on the study farms than in the national population, with mastitis being given as the most common cause of culling, compared with infertility for the national population.

Harrison et al. (2008) retrieved mastitis records and individual cow somatic cell count (ICSCC) data for six farms using different types of RMS bedding, but although mastitis incidence differed between ‘experimental units’ (farm/bedding strategy combinations), neither bacteria levels nor physical properties of bedding affected mastitis incidence. Prevalence of elevated SCC (>200,000 cells/mL for cows and >100,000 cells/mL for heifers) did not differ between three groups of animals kept on sand, separated and composted RMS on one of these farms. No detailed analysis has been made of ICSCC dynamics as cows are introduced to RMS bedding.

The widespread use of RMS in the US could be taken to suggest that success is common but it should be remembered that the requirements for bulk milk somatic cell counts (bmSCC) are less stringent in the US than in the UK (US, 750,000 cells/mL; EU, 400,000 cells/mL). A telephone survey of 38 farmers in the upper mid-west States indicated that those using digested manure solids were able to keep bmSCC consistently below 250,000 cells/mL, while for those using separated solids bmSCC exceeded 450,000 cells/mL (Endres, 2008). On 34 farms, (9 using raw solids, 21 digestate, and 4 composted material), average bmSCC was 274,000 cells/mL (± SD 98.000 cells/mL) (Husfeldt and Endres, 2012). When Harrison et al. (2008) followed the bmSCC patterns of nine farms that converted to RMS (including fresh, composted and digested), some increased and some decreased after conversion. An attempt was made to compare the change in bmSCC over a 7 year period on these farms with the whole state population; this unpublished analysis indicated that a linear score for bmSCC increased more rapidly on the RMS farms than in the whole state population, but, since the bedding types in the whole state were not known, the authors were reluctant to draw conclusions.

Early experiences in Europe suggest that acceptable bmSCC levels can be achieved on RMS, but variation between farms is wide. Feiken and van Laarhoven (2012) monitored three farms in The Netherlands for 2 years after changing to RMS. With a previous annual mean bmSCC range of from 147,000 to 272,000 cells/mL, two of the three farms reduced bmSCC. Only the farm with the lowest cell count increased (to 183,000 cells/mL) in the second year. The authors considered that success with RMS was associated with high quality management of the bedding. One year after introduction of RMS bedding on 11 Danish farms, annual average bmSCC was lower on four farms, and higher on seven, than in the previous year (Marcher Holm and Petersen, 2015).

The overall conclusion from studies and data collated to date is that there is no consistent impact on SCC of the use of RMS, and any effect on clinical mastitis has not been clearly demonstrated. Case studies illustrate the fact that mastitis problems can be experienced, but cannot give definitive information on the likelihood, reasons or mitigation strategies.

Johne's disease

Survival of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in slurry is temperature dependent. MAP may survive for 250 days at low temperatures, but <1 day if heat treated at ~50 ºC. These figures relate to storage in a tank or pit where conditions are largely anaerobic (Elliott et al., 2015). Harrison et al. (2012) tested 15 - 36 samples of unused RMS bedding from each of nine types of bedding from six farms – including composted and digested material. Both composting (Bonhotal et al., 2011) and anaerobic digestion (Timms, 2008b; Pronto and Gooch, 2009) significantly reduced MAP levels. However, on at least one occasion, MAP was found in all but one of the materials, albeit at low levels, indicating that neither composting nor digestion can guarantee elimination of this pathogen. The highest prevalence was positive results from 12/24 samples of freshly separated material from one farm, with a mean load of 174 cfu/g. For this reason, and because of the high risk of MAP transmission in early life, it is recommended that RMS is not used to bed any areas where cows are kept for the late dry period or calving, or housing for calves or young stock.

Lameness

The only peer reviewed figures for lameness on RMS bedding (of various types) report a 95% confidence interval of 13-16% prevalence for deep beds, and 18-22% for mats, based on locomotion scoring on a single visit (Husfeldt and Endres, 2012). These figures are similar to those reported in Minnesota, USA, by Wells et al. (1993) and lower than those reported in high production groups of cows in a number of American states by von Keyserlingk et al. (2012).

Timms (2008c) commented that 'foot and leg health improved' with the introduction of composted RMS but gave no specific information on either the previous bedding material or the absolute levels of lameness. Adamski (2011) remarked that the hooves of cattle housed on RMS were dry, which is likely to be beneficial for foot health.

Two anecdotal reports have suggested that alleyways can be more slippery when using RMS bedding than when sand is used (Ostrum et al., 2008; M. Endres, unpublished data) the former linking this finding with more leg injuries.

Pathogens in general

As distinct from other bedding materials (except recycled sand), RMS is used in a 'closed cycle', in the housing environment in close contact with livestock and humans. This contrasts with the traditional fate of manure and slurry (which are spread on the fields) and could result in selection for organisms, including pathogens, that thrive in these specific conditions, rather than being restricted or destroyed by exposure to outdoor conditions. However, there is little or no information on the influence that such a ‘closed cycle’ will have, or on the virulence of pathogens or (of particular current concern) on the genetic material conveying antimicrobial resistance. One US study of antimicrobial resistant *Salmonella* spp. found that those dairy herds with at least one resistant strain of *Salmonella* isolated from faeces were more likely to be using composted or dried manure as bedding than those with no resistant strains (Habing et al., 2012).

Impact on human health

There is very little evidence available to evaluate the risks but, in general, it would be expected that personal hygiene and protective equipment, along with pasteurisation of milk, would be the main risk mitigation strategies for farm workers and consumers, respectively. The reported reduction in dust could be beneficial. Key pathogens (amongst others) to consider with respect to food safety would be *Salmonella* spp. and *E. coli* (especially O157). The risk of increased levels of these organisms in RMS is not well defined, but mitigation is relatively straightforward if milk is pasteurised.

The main exception is the food borne zoonotic pathogen *Bacillus cereus*, whose spores are able to survive heat treatment. Levels of 1.1 – 1.4 log 10 cfu/g *B. cereus* spores were found in fresh RMS by Driehuis et al. (2013), meaning this pathogen cannot be ignored. However, the authors did not find that levels of spores in either bedding or bulk tank milk were any higher in farms using RMS bedding than in those using straw or sawdust. Further work on RMS and zoonotic pathogens is ongoing in The Netherlands, but has not yet been published.

Impact on food quality

Micro-organisms transferred from bedding to milk may affect the keeping properties of the milk if they survive pasteurisation. Recent work in The Netherlands has focussed on this aspect of food quality. Mesophilic, thermophilic (Driehuis et al., 2012), and extremely-heat resistant (Driehuis et al., 2014), aerobic spore formers were studied, and freshly separated manure solids was one of the bedding materials evaluated. On average, freshly separated manure solids did not show elevated levels of these spores, but all composted materials (which in this trial did not include composted RMS) did. The elevated levels in composted bedding were translated to farm bulk milk, with spore concentrations of the mesophilic group being six times higher and the thermophilic group being 100 times higher in milk from farms using composted materials. Although composted RMS was not included in that trial, the implication is that similar patterns would be likely for this material also. Several Dutch milk buyers discourage or prohibit the use of composted bedding materials to protect the long-life storage qualities of milk products.

Public perception

There is a risk that the concept of bedding animals on manure based products would be unattractive to consumers. However, public perception of the practice has not been formally gauged.

Practical questions: How should RMS be prepared and managed?

Additional processing

Methods for reducing pathogens in whole manure and slurry (see review by Heinonen-Tanski et al., 2006), include composting of solid material, either in the open or in a reactor, aeration of slurry, anaerobic treatment (digestion), addition of lime or peracetic acid, and heat treatment.

Only digestion and composting have been widely employed in converting slurry to bedding material. Bishop et al. (1981) found bacterial counts decreased in RMS composted over 14 days and considered the material suitable for bedding. Reductions in coliform counts to below levels of detection by culture have been reported after composting manure waste, either in windrows or in enclosed mechanical units (Carrol and Jasper, 1978; Husfeldt et al., 2012). However, on beds, levels rapidly increase again (see, for example, Carrol and Jasper, 1978; Harrison et al., 2008; Feiken and van Laarhoven, 2012); whether this is through multiplication of surviving organisms or re-contamination is unknown. Composting will be conducive to food spoilage bacteria and the pathogenic *B. cereus,* whose spores will survive pasteurisation. Some jurisdictions (including England and Scotland, in June 2014), and milk buyers, have therefore prohibited use of composted materials for bedding.

Pathogen populations in digestate depend on the feedstock and temperature in the digester (Meyer et al., 2007; Timms et al., 2008b; Tulloch et al., 2009). In general, bacterial levels are considerably reduced and coliforms often undetectable by culture after digestion (Meyer et al., 2007; Tulloch et al., 2009). However, the temperature in the digester is critical; mesophilic digesters running at temperatures of

30 ^{o}C – 38 ^{o}C can increase bacterial numbers (J. Tulloch, personal communication). With mesophilic anaerobic digestion of beef cattle slurry, the time taken for *E. coli*, *Salmonella enterica* serotype Typhimurium and *Yersinia enterocolitica*, to reduce by 90% (T90) ranged from 0.7 to 0.9 days during batch digestion and 1.1 to 2.5 days during semi-continuous digestion. *Listeria monocytogenes* took longer to reduce (T90 = 37 days during semi-continuous digestion and 12 days with batch digestion). Anaerobic digestion had little effect on viable numbers of *Campylobacter jejuni* (Kearney et al., 1993). MAP has been shown to be reduced (Timms, 2008b; Pronto and Gooch, 2009), but not necessarily eliminated (Harrison et al., 2008) by digestion.

Practical management

The scientific basis for appropriate practical management of RMS bedding is limited. Both laboratory based studies (Zehner et al., 1986) and farm comparisons (Harrison et al., 2008) suggest that management of bedding has greater influence on bacterial load than the type of material. However, RMS has specific properties of high initial bacterial load, and large capacity for water uptake and release (Misselbrook and Powell, 2005), of which users need to be aware. Patterns of microbial growth in maritime climates may differ from those in continental climates; transferability of management practices is not guaranteed. The hygroscopic nature of RMS (Misselbrook and Powell, 2005) means it should be prepared under cover and used only in well ventilated buildings.

Although the general advice is that RMS should not be stored, with a Dutch method of storage in a compacted, covered heap, total bacterial count, *E. coli* and *Klebsiella* spp. were not significantly increased after 6 weeks (Feiken and van

LaarHoven, 2012). The material was largely unaltered physically and chemically as a lack of rapidly metabolisable carbohydrate prevented fermentation and anaerobic conditions prevented composting activity.

One decision for farmers considering RMS as cubicle bedding is whether to use it on mats or mattresses, or in deep beds. Deep beds per se are likely to improve physical cow comfort, but depth will affect the environment for bacteria. Shallow beds and frequent replacement are likely to give better control of coliforms, particularly *Klebsiella* spp., than can be achieved in deep beds that are infrequently replenished (Sorter et al., 2014), but streptococcal counts are likely to be higher in shallow beds (Husfeldt et al., 2012; Sorter et al., 2014). Sorter et al. (2014) suggested this might stem from the more frequent addition of material, because high initial levels of streptococci were high, although in this trial the effects of bedding depth and frequency of replenishment cannot be separated.

Schwarz et al. (2010, 2011) compared daily and weekly addition of RMS to deep bedded stalls, on two commercial farms, and found that season had a greater effect on bacterial numbers than frequency of bedding; the authors concluded that daily bedding did not necessarily improve bacterial levels, milk quality or mastitis, compared with weekly bedding.

'Conditioners' to alter the pH of bedding materials are sometimes recommended for control of microbial populations. Effects are usually short-lived, in the range of 24 - 48 h (Hippen et al., 2007). Hogan et al. (1999) included RMS as a substrate in an experiment testing the effect of 'bedding conditioners' on bacterial load. Specifically for ‘raw’ RMS, these authors reported that, although both acid and alkali conditioners reduced bacterial populations in unused material, only the alkali conditioner and hydrated lime inhibited bacteria in used bedding, and only for 1 day; use of an acid conditioner had little effect on bacteria in bedding. Sharkey et al. (2011) reported a more rapid and greater decline in *Klebsiella* counts in composted RMS stored in a pile, as a result of application of a proprietary conditioner (SOP-C COW[1]), but there was no effect on streptococci. Feiken and van Laarhoven (2012) added lime and a proprietary alkali to RMS cubicles but found that the resulting pH change was insufficient to reduce most bacteria effectively, although there was a significant reduction in *B. cereus* with the proprietary conditioner.

Scientific evidence for optimum management (for example in terms of bed design, bedding frequency, aeration and replacement) is limited and sometimes conflicting. Since practical experience indicates that there can be udder health problems with wetter ‘fresh’ bedding, or damp climatic conditions, this area is in need of further research.

Conclusions

Recycling manure solids as bedding material can present advantages for farmers in terms of availability, convenience and, in some cases, economics. UK farmers also perceive benefits for cow comfort and cleanliness, likely to be dependent on the previous bedding material used for comparison. The literature gives less evidence for the scale of absolute welfare benefits but there are definitely advantages of comfort compared with abrasive materials on mattresses. There are challenges and risks associated with the practice, not least in view of the dearth of information on many of the long term implications. Anecdotal reports of difficulties of maintaining udder health on RMS exist, but no large scale, long term studies of effects on clinical and subclinical mastitis have been published; nor is there any information on clinical implications for other diseases. Very little is known about the influence of maintaining the material in a 'closed cycle', the effects of its use on pathogen virulence and antimicrobial resistance, or the risk of airborne pathogens arising from it. Should farmers choose to adopt RMS bedding, they are advised to do so with caution, apply the required strategies for risk mitigation, maintain strict hygiene of bed management and milking practices and monitor the effects on herd health closely. With current understanding, important factors in risk management on-farm are good machine maintenance and product monitoring, use in well-designed housing, and avoiding use of RMS in or from calving areas or for housing calves or youngstock. Care should be taken in transferring management approaches from hot dry climates to wetter, cooler areas.

Acknowledgements

The scoping study on which this paper is based was funded by DairyCo, a division of the Agriculture and Horticulture Development Board (now AHDB Dairy).

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1 Key micro-organisms in consideration of potential risks associated with use of recycled manure solids as bedding, and the availability of evidence of load

Pathogen	Area of concern	Potential for high load in slurry	Other factors in assessment of relevance	Data sources on RMS load
Bacteria				
Bacillus cereus	A,H,F	Y		Driehuis et al. (2012, 2013) (spores); Feiken and van Laarhoven (2012)
Campylobacter spp.	A,H	Y		
Coxiella burnetii	A,H		Very low minimum infective dose	
Enterococcus spp.	A,H	Y	Particularly likely to perpetuate antimicrobial resistance	
Escherichia coli	A,H	Y		Bishop et al. (1981)* (composted RMS); Harrison et al. (2008); Zehner et al. (1986)*
E. coli 0157	A,H	Y		
Listeria spp.	A,H	Y		
Mycobacterium avium subsp. *paratuberculosis*	A,H	Y		Harrison et al. (2008); Timms (2008b); Pronto and Gooch (2009)
Mycobacterium bovis	A,H	Uncertain but unlikely with regular TB testing	Major UK animal health issue	
Salmonella spp.	A,H	Y	Reported association between use of composted or dried RMS and resistant strains (Habing et al. 2012)	Meyer et al. (2007); Timms (2008b) - presence/absence
Klebsiella spp.	A	Y	Reports of links between RMS and *Klebsiella* mastitis	Feiken and van Laarhoven (2012); Harrison et al. (2008); Hogan et al. (1999)*; Sorter et al. (2014)*
Streptococcus uberis	A	Y		Zehner et al. (1986)*
Yersinia enterocolitica	H	Y		
Mesophilic spore formers	F		High levels in **other composted**	Driehuis et al. (2012, 2013) (spores)

Pathogen	Area of concern	Potential for high load in slurry	Other factors in assessment of relevance	Data sources on RMS load
			materials	
Thermophilic spore formers	F		High levels in **other composted** materials	Driehuis et al. (2012, 2014) (spores)
Extremely heat resistant spore formers	F		High levels in **other composted** materials	Driehuis et al. (2014)
Spirochaetes				
Leptospira spp.	A,H	Y		
Treponemes	A	Uncertain	Implicated in digital dermatitis	
Viruses [1]				
Rotavirus	A,H	Less likely from adult population		
FMDV	A	Only in outbreak	Notifiable disease in UK	
Bovine coronavirus	A	Less likely from adult population		
Parasites and protozoa [2]				
Cryptosporidium spp.	A,H	Y		
Giardia spp.	A,H	Y		
Coccidia spp.	A	Large contribution from adult population unlikely		
Prototheca				
Prototheca spp.	A	Y		

A - Animal health, H - Human health, F - Food quality * Peer reviewed paper

[1] For the majority of viruses (e.g. Bovine Coronavirus, Rotavirus), there is no quantitative information on the levels likely to be in RMS or even levels in slurry.

[2] Other gut parasites and lungworm have not been included since these would be unlikely to complete their full life cycle in the manure and experience with other species indicates that total confinement systems are not associated with high parasite burdens.

Table 2 Examples of bacterial counts in separated manure solids

Units (log 10 colony forming units)	**Total bacterial count**	**Coliforms**	**Gram -ve bacteria**	***Bacillus* spp.**	**Environmental Streptococci**	**Staphylococci**	***E. coli***	***Klebsiella***	***Bacillus cereus* spores**	**MAS**	**Reference**
per g	6-8	2-4			5-8						Timms (2008a)
per g		2-3	4-5		4-5						Timms (2008b)
per g	8.3 - 9.1				6.6		4.4-5.5	3.1 - 4.2			Feiken and van Laarhoven (2012)
per g									2.3	6.7	Driehuis et al. (2013)
per mL		4.1		6.5	6.4	3.0					Husfeldt and Endres (2012)*
per mL			4.5 - 4.7		4.3 – 5.4	0 - 0.3	0.3 – 1.7	1.7 – 2.0			Harrison et al. (2008)

MAS – mesophilic aerobic spore formers * Peer reviewed paper

Less frequently found: *Bacillus* spp. (Husfeldt et al., 2012), enterococci (Zehner et al., 2009*), Enterobacteriaceae (Carrol and Jasper 1978*; Zehner et al., 2009*), propionic acid bacteria (Zehner et al., 2009*), and *Proteus* spp. (Harrison et al., 2008).

Table 3. Examples of bacterial counts in separated manure solids after composting or digestion

Processing	**Units (log 10 cfu)**	**Coliforms**	**Gram –ve bacteria**	***Bacillus* spp.**	**Environmental Streptococci**	**Staphylococci**	***E. coli***	***Klebsiella***	**Reference**
Separated, compacted, covered and stored 5 weeks	per g	9.4							Feiken and van Laarhoven (2012)
Composted	per mL	0		3.9	4.0	1.0			Husfeldt and Endres (2012)
Composted	per g	< 2	2-6		4-6				Timms (2008c)
Composted (and stored)	per g	4-6							Timms (2008c)
Composted	per mL		2.9 – 5.1		2.6 – 3.1	0	0	0 – 2.0	Harrison et al. (2008)
Digested	per g	0	4-5						Timms (2008b)
Digested	per mL	1.73		4.6	4.1	1.5			Husfeldt and Endres (2012)
Digested	per mL		4.6		5.2	0.2	0.2	0.5	Harrison et al. (2008)

Table 4. Examples of bacterial counts in used bedding – in cubicles unless otherwise specified

Material	Units (log 10 cfu)	Total bacterial count	Coliforms	Gram –ve bacteria	Streptococci	Staphylococci	*E. coli*	*Klebsiella*	Reference
Straw in loose yards	per g		7.2 - 7.6		7.9 - 8.4				Ward (2002) *
Straw in loose yards (mean of four seasons)	per g DM		6.4		7.4			4	Hogan et al. (1989) *
Straw	per g		6.5		7.7	8.9		4.8	Rendos (1975) *
Chopped straw (mean of four seasons)	per g DM		6.3		7.8			3.7	Hogan et al. (1989) *
Straw	per g	9.6			7.7		5.5	4.6	Feiken and van Laarhoven (2012)
Sawdust	per g		7.7		7	8.5		6.6	Rendos (1975) *
Sawdust	per g	9.9			3.1		< 2	1.9	Driehuis et al. (2012)
Sawdust	per mL				7.3	3.0	4.9	0.2	Harrison et al. (2008)
Sawdust on cubicles after 1 week	per g		7.1					6.4	Fairchild et al. (1982) *
Sawdust and lime after 1 week	per g		7					6.9	Fairchild et al. (1982) *
Sand	per mL				7.6	1.6	2.4	4.5	Harrison et al. (2008)
Sand after 1 day	per g		6		6.5			4.1	Zdanowicz et al. (2004) *
Sand after 2 days	per g		6.1		6.9			4.3	Zdanowicz et al. (2004) *
Sand after 6 days	per g		5.8		7.2			4.1	Zdanowicz et al. (2004) *
Sand (mean of four seasons)	per g DM		5.7		7			3.2	Hogan et al. (1989) *
Separated RMS	per mL	3.1	2.1		2.9	2.2			Husfeldt and Endres (2012) *
Digested RMS	per mL	2.9	2.0		2.6	2.3			Husfeldt and Endres (2012) *
Drum composted RMS	per mL	3.2	2.0		2.9	2.45			Husfeldt and Endres (2012) *
Composted RMS	per mL		8.7		8.2	8.2			Bishop et al. (1981) *
Drum composted RMS	per mL				7.2	2.0	1.6	5.9	Harrison et al. (2008)
Windrow composted RMS	per mL				7.3	0.3	1.4	4.3	Harrison et al. (2008)

Material	Units (log 10 cfu)	Total bacterial count	Coliforms	Gram –ve bacteria	Streptococci	Staphylococci	*E. coli*	*Klebsiella*	Reference
Digested RMS	per mL				7.2	1.5	2.9	3.2	Harrison et al. (2008)
Separated RMS	per mL				7.2	1.1	1.3	5.6	Harrison et al. (2008)
RMS dried by forced air	per mL				7.2	5.4	5.3	4.0	Harrison et al. (2008)
Partially composted RMS	per mL				7.7	2.1	3.6	2.7	Harrison et al. (2008)
Mature composted RMS	per mL				7.6	2.4	5.3	2.6	Harrison et al. (2008)
Separated RMS	per g	10.1			7.5		5.5	6.2	Feiken and van Laarhoven (2012)
RMS 30% DM	per g	10			6.6		4.2	3.1	Driehuis et al. (2012)
RMS on back of mattress replaced daily from pile at front	per g DM							5.7	Sorter et al. (2014) *
RMS on deep bed after 1 day	per g DM							6.2	Sorter et al. (2014)*
RMS on deep bed after 2 days	per g DM							6.6	Sorter et al. (2014)*
RMS on deep bed after 6 days	per g DM							6.5	Sorter et al. (2014)*
RMS after 1 day	per mL		6	8.2	8			6.5	Hogan et al. (1999)*
RMS after 2 days	per mL		6.8	8.2	7.8			6.5	Hogan et al. (1999)*
RMS after 6 days	per mL		6.4	7.9	7.8			6.3	Hogan et al. (1999)*
RMS with lime after 1 day	per mL		5.7	7	7.7			5	Hogan et al. (1999)*
RMS with lime after 2 days	per mL		6.7	8	8			6	Hogan et al. (1999)*
RMS with lime after 6 days	per mL		6.2	7.8	8			6.2	Hogan et al. (1999)*

RMS, recycled manure solids. * Peer reviewed

14-01009

Highlights

- Information on recycled manure solids (RMS) bedding is mainly from dry US climates
- Bacterial counts in fresh material are high; other bedding types can reach similar levels with use
- Well evidenced reports of effects of RMS on udder health are few and do not show consistent patterns
- Information on impact of RMS on other diseases is lacking
- Should non-US farmers adopt RMS, caution is advised; monitor herd health closely

***Revision Note**

Final revision note - Ms. No. YTVJL-D-14-01009R3
Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms

Please find below our response to the comments of the Editor in Chief:

"I have slightly changed the focus from 'UK farmers' to cover not only UK but other farmers in climates unlike the USA. As an international journal I feel we can do this without distracting in any way from the importance of the review to those in UK. Please check carefully to ensure you are content."

This is a good idea. The edited highlights written by the Editor in Chief did exceed the character limit, so I have provided a shorter version which retains the meaning of the alterations suggested.

"I also inserted a footnote URL at line 404 to describe the product. You may wish to change this."

I have consulted with co- authors and we feel that to provide a direct link to a commercial product in a review would not be appropriate, as it might compromise the impression of impartiality. "SOP-C cow" can be easily found with an internet search if the reader wishes for more details. Therefore I have removed the footnote - though I cannot remove a line that belongs to it.

"Finally, some pages are missing in the references (see my notes in red)"

Page numbers (and URL's where available) have been provided where requested. The papers by Timms are rather unconventional, being referred to as leaflets rather than having page numbers. A "suggested form of reference" is provided on their title pages, which I have followed; I hope this is acceptable for The Veterinary Journal. e.g.Timms, L., 2008a. Preliminary evaluation of separated manure solids characteristics at the new ISU dairy. Iowa State University Animal Industry Report AS654, ASL R2318.

"I also modified the title of the article in line 513."

A good idea to provide the link to the translation, thank you.

Katharine Leach 4 August 2015