Report Title: Risks, benefits and optimal management of recycled manure solids for use as bedding for dairy cattle

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DISCLAIMER

This report is the output of a study, the aim of which was to extend knowledge of the risks, benefits and optimal management of recycled manure solids for use as bedding for dairy cattle. This study is inevitably constrained by its short duration and the fact that sampling only occurred in the months of January to May. This report does NOT constitute a full risk assessment or "claim to be the definitive document of RMS use". Suggestions for modifications of existing guidance on use are based on current knowledge but cannot be expected to provide "fool proof advice". All users of RMS have to accept responsibility for their own decisions with respect to its use. The authors of this report cannot be held responsible for decisions made on the basis of the information contained herein.

Executive Summary

Disclaimer

This report is the output of a study, the aim of which was to extend knowledge of the risks, benefits and optimal management of recycled manure solids for use as bedding for dairy cattle. This study is inevitably constrained by its short duration and the fact that sampling only occurred in the months of January to May. This report does NOT constitute a full risk assessment or "claim to be the definitive document of RMS use". Suggestions for modifications of existing guidance on use are based on current knowledge but cannot be expected to provide "fool proof advice". All users of RMS have to accept responsibility for their own decisions with respect to its use. The authors of this report cannot be held responsible for decisions made on the basis of the information contained herein.

Background

The use of Recycled Manure Solids (RMS) as bedding for dairy cattle constitutes a "technical use" of a Category 2 Animal By-Product under the EU Animal By-Products (ABP) Regulation (EU Reg. 1069/2009). The regulation has provisions which permit "technical use" of animal by-products and derived products, provided these do not pose an unacceptable risk to public or animal health. At the time when UK farmers began to adopt the practice (attracted by perceptions that there would be benefits to farm economics and cow comfort), data on which to base an assessment of this risk under UK conditions was unavailable.

In 2013, DairyCo commissioned a desk top scoping study to collate the available evidence on the use of RMS. An executive summary of the study can be found at http://www.dairy.ahdb.org.uk/resources-library/technical-information/buildings/rms-bedding/ The study concluded that there were significant knowledge gaps limiting the ability to assess the risks to public and animal health in UK conditions.

In June 2014, Defra agreed to allow continued use of RMS in England under prescribed management conditions, while further data were collected. For cross-reference, these conditions can be downloaded from http://www.dairy.ahdb.org.uk/technical-information/buildings/housing/recycled-manure-solids/. In a similar manner, the Scottish Government also permitted use, whilst the Welsh Government held the opinion that the information to assess the risks to animal and human health was insufficient, and therefore the use of manure as bedding was not sanctioned in Wales at that point.

To address this gap in knowledge, research was commissioned to gather data on RMS use under UK conditions. The overall objective was to provide greater technical understanding to help inform the legal position with regard to the safe use of recycled manure solids as bedding, and in particular to investigate management and husbandry options to safely mitigate any potential risks to animal or human health.

Aims and Structure of the Project

The project aimed to:

- Assess the presence (and in some cases, levels) of selected pathogens and milk spoilage bacteria in cubicles bedded with RMS in comparison with other bedding materials.
- Assess the transfer of pathogens and milk spoilage bacteria from different bedding types to bulk milk and potential mitigating factors.
- Provide robust information on the relationships between bedding (including RMS) and udder health in dairy cows under UK conditions.
- Increase our understanding of factors influencing the success of use of RMS as a bedding material for UK dairy cows.
- Assess the potential to mitigate possible adverse impacts of the use of RMS as bedding.
- Assess specific aspects of welfare and comfort of cows on RMS and other bedding.
- Predict the likely levels of MAP and *Salmonella* spp in slurry and RMS bedding over time, in farms with different disease levels, by modelling literature based values of cow excretion patterns alongside the dynamics of slurry storage and removal.
- Provide information on antimicrobial resistance patterns in organisms isolated from farms using RMS and other bedding materials.
- Contribute to information on best practice for building and managing beds using RMS.
- Provide a cost calculator enabling farmers to evaluate a variety of bedding options.
- Enable exchange of information and experiences on RMS use between the UK and the Netherlands.

The work involved collection and analysis of data from:

- An epidemiological survey of 125 farms using RMS, sawdust and sand as bedding.
- A controlled study of different bedding materials and depths.
- An observational study of different methods of initial build-up of deep RMS beds.
- *In silico* modelling to predict the likely levels of MAP and *Salmonella* spp in slurry and RMS bedding over time, in farms with different disease levels.

The project also incorporated:

- Development of an economic cost calculator to allow evaluation of the cost of converting to, and subsequent use of RMS bedding.
- Analysis of bacterial isolates collected on the survey to assess implications for antimicrobial resistance.

Epidemiological Farm Survey

The survey included 40 farms using RMS bedding, 41 farms using sand and 44 farms using sawdust.

Bacterial counts in "used bedding"

- Across all the species and groups enumerated, with the exception of *Streptococcus* spp, bacterial counts in "used" bedding were significantly higher in RMS than either sand or sawdust. Numerically, mean and median counts were typically lowest on sand farms. However, it is important to note that there was often as much variation within bedding type as between bedding type.

Bacterial counts in bulk milk

- Despite the high levels of bacteria in "used" RMS bedding, bacterial counts in bulk milk did not differ between the groups of farms with different bedding types, and there was no association between bacterial count in "used" bedding and in bulk milk sampled on the same day, across all bedding types.
- Across all bedding types, fore-milking was associated with a lower total bacterial count (TBC) in bulk milk (2,503 *vs* 4,800 cfu/ml; p=0.047), but not with any other bacterial species/grouping.
- However, within the population of RMS farms, higher total bacterial count in "used" bedding was associated with higher total bacterial count in bulk milk.

Somatic cell counts in bulk milk

- Somatic cell counts were not significantly different between farms bedded on the different materials, though there was a trend for SCCs to be lower on the sawdust farms compared to the RMS farms (134 *vs* 171 $x10^3$ cells/ml; p=0.06).
- Within RMS farms pre-dipping was associated with a lower bulk milk SCC (137 *vs* 206 $x10^3$ cells/ml; p=0.037).

Specific zoonotic pathogens in bedding

- *Yersinia enterocolitica* was identified in the bedding on between 4.9% and 9.8% of farms, but the prevalence did not vary between bedding types.
- *Salmonella* spp were identified in used bedding on four farms (two sand and two RMS).
- *Listeria monocytogenes* was isolated from a significantly higher proportion of bedding from sand farms (58.5%) than RMS (15.0%) or sawdust farms (31.7%) (p<0.01), which did not differ.

Zoonotic pathogens in milk

- *Yersinia enterocolitica* was identified in the bulk milk on between 0% and 12.2% of farms, but the prevalence did not vary between bedding types.
- A *Salmonella* spp was identified in the bulk milk of one sawdust farm and was subsequently identified as *S. montivideo* (APHA).
- *Listeria monocytogenes* was isolated least frequently from bulk milk from sand farms and was isolated from between 2.4% and 12.5% of farms across the bedding groups. However, the prevalence in milk did not vary between bedding types.

Udder health

- No significant differences were identified between farms utilising the different bedding materials, in any of the measures of udder heath analysed, based on either SCC or clinical mastitis cases. No significant effects of changing to RMS from a different bedding material were identified.
- The ability to identify differences in clinical mastitis rates between cows housed on different bedding types was hampered by a lack of robust data.

Culling

- No significant difference in reasons for culling cows was identified between herds using different types of bedding.

Cow comfort and welfare

- RMS beds would appear to offer some advantages with respect to cow comfort and cleanliness:
    - based on measures of cleanliness and hock condition, deep RMS beds typically performed as well as sand beds.
    - when used on mats, RMS demonstrated clear advantages over sawdust.

The findings of this survey need to be interpreted in the light of the fact that the use of RMS as a bedding material is still in its infancy in the UK and Europe. Whilst early indications are that there need not be an adverse effect on udder and animal health, this will need to be monitored, as and if more herds adopt the practice.

Mitigation of risk through management practices to reduce bacterial levels in used RMS bedding

- With the exception of the use of RMS as deep or shallow beds, management had no consistent effect on bacterial levels in RMS bedding.
- TBCs, *Streptococcus* spp and psychrotrophic counts were higher in RMS managed in shallow beds. *Bacillus cereus* counts were higher in RMS managed as deep beds.
- There was no detectable impact of using conditioner on bacterial counts in used RMS bedding.
- No significant relationship between the frequency of bedding and bacterial counts in bedding was identified. There was a trend for *Streptococcus* spp counts to be lower in beds to which fresh RMS was applied daily (1.08×10^8 *vs* 2.80×10^8 cfu/g; p=0.057).
- No effect of separating RMS bedding under cover was found on i)dry matter of the fresh bedding on the day of production, ii) dry matter of used RMS or iii) bacterial counts in RMS. However, only a single sample of bedding from the day of the visit was analysed and farmers without 'cover' avoided separating RMS in wet weather.

Mitigation of risk of transfer of bacteria to bulk milk through milking and bed management practices for RMS, sand and sawdust bedding

- Across all bedding types (RMS, sand, and sawdust):
    - Fore-milking was associated with a lower TBC in bulk milk.
    - Pre-milking teat preparation that involved a pre-dip followed by wiping dry was associated with a lower *Streptococcus* spp count in bulk milk (and with a lower psychrotrophic count in the subpopulation of RMS farms).
    - Cluster disinfection was not found to be associated with lower bacterial counts in milk, with the exception of thermophilic spore counts and psychrotrophic counts.
    - No difference was detected between manual and automated cluster disinfection systems.
- Within RMS farms, there were no significant differences in any of the bulk milk bacterial counts or milk constituents between farms with deep and shallow beds.

Controlled Trial

This study represents one of the most comprehensive investigations of the impact of different bedding materials and managements on udder health and milk quality conducted to date. In a modified crossover design trial, four groups of 40 cows rotated twice around the four bed types (deep sand, deep RMS, shallow RMS and shallow sawdust). Bacterial loads on "unused" and "used" bedding, and in bulk milk, were assessed. Impact on udder health was evaluated by assessment of individual quarter somatic cell counts, specifically, the acquisition of new infections at the quarter level.

Bacterial counts in "unused" bedding

- Bacterial counts varied significantly between the three "unused" bedding materials, being highest in the RMS and lowest in sawdust.

Bacterial counts in "used" bedding

- RMS beds were replenished twice weekly, sawdust beds twice daily and sand once every two weeks. Counts in used bedding need to be interpreted in this context.
- TBCs of "used" bedding varied significantly between the four bedding materials, being highest in shallow RMS and lowest in sawdust.
- Coliform counts in RMS were higher than in sawdust, but not significantly different from sand.
- *Streptococcus* spp counts were highest in shallow beds, with shallow RMS and sawdust showing no significant difference.
- *Staphylocccus* spp counts were significantly higher in shallow RMS than in other used bedding materials.
- The laboratory pasteurised counts were highest in deep RMS and lowest in sand, whilst thermophilic spore counts were high in both deep and shallow RMS beds.
- Psychrotrophic counts were significantly lower in sawdust than in other bedding materials.

- *Bacillus cereus* counts were significantly higher in deep RMS beds, being 3 logs higher than in sand or shallow RMS beds; very little *Bacillus cereus* was identified in sawdust beds.

Bacterial counts in bulk milk

- With the exception of *Streptococcus* spp and *Staphylococcus* spp, there was no effect of bedding treatment group on the bacterial count of bulk milk. *Streptococcus* spp counts were significantly lower ($p<0.05$) in milk from cows on deep beds (regardless of bedding material) whilst variation was less predictable in *Staphylocccus* spp counts.
- With the exception of *Streptococcus* spp and *Listeria* spp there was no clear relationship between bacterial numbers in bedding and in bulk milk, although this may, in part, reflect the hygiene practices during milking.

Udder health

- In this study, sawdust, applied to mats twice daily, appeared to offer the best protection against new intramammary infection (as measured by SCC). New Quarter intramammary Infections were significantly less likely to occur in cows on sawdust beds than on deep RMS (47/961 *vs* 84/965; $p=0.012$) or sand beds (47/961 *vs* 78/965; $p=0.04$). No impact of bedding material on the likelihood of a quarter curing could be identified.
- Unlike the assessment of udder health using SCCs, the analysis of clinical mastitis suggests that RMS as a bedding material may increase the risk of clinical mastitis; there was a trend for cows bedded on RMS to be at higher risk of developing clinical mastitis than cows not bedded on RMS (7/73 *vs* 2/78; $p=0.086$). This is an area that warrants further research.
- No consistent, biologically plausible, repeatable correlations were found between bedding bacterial counts and udder health.

Cow comfort

- Deep beds offered the highest of cow comfort, regardless of bedding material. RMS was relatively protective when used on shallow beds, in comparison with sawdust.

Bed Building Study

Farmers have reported that RMS may not dry out optimally, and heating may occur if a large amount is initially applied during the process of establishing deep beds. Such conditions might be expected to encourage microbial growth and could have an effect on udder health. This farmer-inspired experiment was designed to test whether building beds gradually was associated with an increase in the dry matter content of the bedding and less heating. In addition the impact of the presence of cows during the bed building phase was assessed. The main findings were as follows:

- Rapid building of beds elevated temperatures of bedding in the building phase, and the effect appeared to persist to some extent for four weeks. Temperature was affected more by speed of fill than by the presence of cows.
- The effect of building speed on dry matter content at the surface during the first week and four weeks later was inconsistent.

- Dry matter content of the surface material appears to be influenced by factors in addition to the speed of bed building. These may include the presence of cows, but also environmental conditions. There appears to be a complex interaction between environmental conditions, including temperature and relative humidity, and the temperature and dry matter of RMS bedding.
- Rapid building of beds and the consequent increases in temperature and decreases in dry matter may be associated with a higher coliform count in the bedding material in the early stages of bed establishment.
- Slow building will by definition limit the depth of beds and thus the comfort provided if cows are present during the building phase.
- Further research is required to understand the behaviour of this material in different environmental conditions, as this may be a key to its optimal use.

In silico modelling of Levels of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and *Salmonella* spp in Cattle Slurry and RMS

Individual cow excretion patterns of MAP and *Salmonella* spp were obtained from peer reviewed literature and the potential load in slurry was modelled, using assumptions with respect to number of cows affected within a herd and factors relating to slurry storage and removal. Different scenarios of herd disease prevalence and slurry handling methods were evaluated. The estimated levels of organisms present in RMS were considered alongside potential infective doses to assess the degree of risk posed by each pathogen and scenario.

- The infective dose of MAP suggests that fairly large quantities of RMS (of the order of 100-1000 g) would need to be ingested by cattle to reach the published values for the infective dose. Furthermore, this figure is probably representative of an infective dose for calves, and for adult cows may be substantially higher. Therefore in terms of MAP, the models constructed in this research suggest that whilst bedding of youngstock using RMS should be avoided, the risk to adults may be minimal.
- The infective dose of *Salmonella* spp suggests that in a very severe outbreak, when levels in RMS may become high, only small quantities (of the order of <1g) of RMS would need to be ingested to cause disease in cattle. Clearly this depends on many factors and there will be other transmission routes, which in fact may be more significant, including, but not limited to, feed and water, wildlife (especially birds), and fomites.

Economics and Bedding Cost Calculator

A Cost Calculator spreadsheet has been created which can be used to cost individual scenarios of conversion from an existing bedding material to RMS.

Antimicrobial Resistance

Coliform organisms and *Enterococcus* spp were isolated from bedding and milk collected at the time of the visit to farms participating in the epidemiological farm survey. In addition data was collated on antibiotic use on farm. An in-depth analysis of the impact of bedding type and management on antimicrobial resistance was not envisaged as part of the research outlined in the original tender prior to this study and for that reason any findings need to be interpreted with care.

- Differences in the MICs for antimicrobials were identified between the different bedding types.
- No one bedding type was associated with higher MICs overall, with each bedding type being associated with the highest MICs for at least one antibiotic class.
- No clear evidence was identified to suggest that the short term use of recycled manure solids as bedding, as compared to sawdust and sand, is associated with a general increase in MICs of the major classes of antibiotics when considering coliforms and *Enterococcus* spp.
- Further research, using the dataset generated by this study and elsewhere, is needed to further our understanding of any potential interactions between bedding type and management and antimicrobial resistance in the environment.

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1 Introduction

1.1 Background

The concept of using recycled manures solids as bedding has been developed in the US, and is currently being applied in several northern European countries. The technique has already been adopted by a number of GB dairy farmers and there is growing interest by others, investigating potential sources of bedding for dairy cows which are sustainable, cost effective, and safeguard comfort and cow health.

However, livestock manure is classified as a Category 2 animal by-product under the EU Animal By-Products (ABP) Regulation (EU Reg. 1069/2009) which regulates the use of animal by-products. The Regulation does not directly permit uses other than for land application, without further consideration of whether such use may pose a risk to public or animal health. As such, the ABP regulation does not directly permit the use of manure as bedding, without further transformation. The regulation has provisions which permit animal by-products and derived products to go for technical uses, provided these do not pose an unacceptable risk to public or animal health.

Currently, there is insufficient data available under British conditions to make a definitive decision on whether this practice poses unacceptable risks. However, the Regulation does provide scope for processing ABPs, including livestock manures, for use as technical products. In theory, this could include use for animal bedding, provided it can be demonstrated that any risks to animal or to public health have been effectively mitigated.

In 2013, DairyCo commissioned a desk top scoping study to collate the available evidence on the use of RMS. An executive summary from the study can be found at http://www.dairy.ahdb.org.uk/resources-library/technical-information/buildings/rms-bedding/

Drawing from sources worldwide, the study found very few peer reviewed publications on the subject. Much of the information available is taken from case studies and from anecdotal evidence. The report listed gaps in current knowledge, which could be addressed through further research.

In June 2014, Defra agreed to allow continued use of RMS in England under prescribed management conditions for a further period, while further data were collected. For cross-reference, these conditions can be downloaded from http://www.dairy.ahdb.org.uk/technical-information/buildings/housing/recycled-manure-solids/. The Scottish Government has also permitted use for the time being.

The Welsh Government currently holds the opinion that the information to assess the risks to animal and human health is insufficient, and therefore the use of manure as bedding is not sanctioned in Wales at present.

To tackle this gap in knowledge, further research was commissioned to gather data in circumstances relevant to Welsh conditions. The requirement was to deliver scientifically robust evidence on the risks, benefits and optimal management surrounding the use of recycled manure solids as bedding. The overall objective was to provide greater technical understanding to help inform the legal position with regard to the safe use of recycled manure solids as bedding, and in particular to investigate management and husbandry options to safely mitigate any potential risks to animal or human health.

The project was administered by AHDB Dairy (formerly DairyCo).

The funding was obtained through the 'Improving the Welsh Dairy Supply Chain' project, via the Rural Development Plan for Wales 2007-2013, which is in turn funded by the Welsh Government and the European Agricultural Fund for Rural Development.

1.2 Existing Information

A scoping study (Bradley *et al*, 2014) has indicated gaps in knowledge, and highlighted the lack of objective information available on performance, management and best practice for use of RMS under UK conditions. The only peer reviewed publications addressing the topic of RMS use, management, and its implications for animal health are based on data gathered in the US where climatic and management conditions differ from those in the UK (Hogan *et al*, 1999; Husfeldt *et al*, 2012; Sorter *et al*, 2014). Case studies of three farms converting to RMS (Feiken and van Laarhoven, 2012) and a survey of 96 RMS users (Driehuis, Feiken, van Laarhoven - ongoing research) have been conducted in the Netherlands. However, even these European studies have been undertaken in climatic conditions under continental influence; conditions will be drier than in many parts of the UK, particularly Wales. Most recently, the Danish Knowledge Centre for Agriculture (SEGES Videncentret for Landbrug) produced a report of an observational study and farmer experiences with RMS, (referred to as "Fibre-bedding") on 11 Danish farms (Marcher Holm and Pedersen, 2015). Other work in the Netherlands including farms with RMS bedding has concentrated on food spoilage organisms, rather than herd health or pathogenic organisms (Driehuis *et al*, 2013, 2014). Therefore there is a need for scientific work to inform the assessment of risk to animal and human health from RMS use, in the UK, to identify benefits the material may offer, and inform best practice.

1.3 Rationale

This study comprised an investigation into specific aspects of the risks, benefits and optimal management of RMS bedding in conditions relevant to Welsh farms (and many other areas of GB), within the constraints of the timescale available (nine months, data collection covering winter and spring). A longer period would be necessary to advise fully on best practice and longer term risks associated with RMS use. The study concentrated on use of RMS for bedding adult dairy cattle, since use is prohibited, by Defra, for cattle under six months, and not advised for cattle under twelve months of age. Comparison was made with sand and sawdust, representing an inorganic, and an organic bedding material currently used in the UK.

The timescale and budget of the project did not permit effective study of the implications for viral disease. Digestion as a pre-use treatment of RMS was not included as the number of variables is large and control over 'feedstock' will remain an issue. Composted material was not included as its use is not currently accepted by Defra. The main question that needs to be addressed with composting, is whether a solution can be found to the conflict that composting is likely to reduce mastitis pathogens, but encourage spore forming food spoilage organisms, but this was considered beyond the scope of this

project. Moreover, it was considered that research linked to any particular technology should be the responsibility of the manufacturer.

The key knowledge gaps that this project seeks to address (of those identified in the scoping study (Bradley *et al*, 2014)) are:

- The presence (and in some cases, levels) of selected endemic and zoonotic pathogens in RMS bedding in UK dairy herds (in comparison with sand and sawdust) and their impact on milk quality.
- The influence of bedding type on udder health as measured by individual cow and quarter somatic cell count (SCC) dynamics and clinical mastitis.
- Specific aspects of construction and management of RMS beds.
- Antibiotic sensitivity of microorganisms on farms using RMS and other bedding materials.
- Objective comparison of hock lesions in cows on RMS and other bedding.
- Presence of key endemic, zoonotic and milk spoilage bacteria in bulk milk from herds using RMS, sawdust and sand so as to assess the potential risk to milk quality as well as assessing the ability of different milking routines to mitigate any risk.
- A prediction of the approximate levels of *Salmonella* spp and *Mycobacterim avium* ssp. *paratuberculosis* (MAP) in slurry using a theoretical approach to modelling cow excretion patterns.

The report includes interpretation of results within the risk assessment framework laid out in the Scoping Study (Bradley *et al*, 1014), and indicates where the results of this further study have filled knowledge gaps, reduced uncertainty or indicated a qualitative alteration to our understanding of level of risk. However, the study was not designed to deliver a full quantitative risk assessment for RMS use.

1.4 Aims

The project aimed to:

- Assess the presence (and in some cases, levels) of selected pathogens and milk spoilage bacteria in cubicles bedded with RMS in comparison with other bedding materials.
- Assess the transfer of pathogens and milk spoilage bacteria from different bedding types to bulk milk and potential mitigating factors.
- Provide robust information on the relationships between bedding (including RMS) and udder health in dairy cows under UK conditions.
- Increase our understanding of factors influencing the success of use of RMS as a bedding material for UK dairy cows.
- Assess the potential to mitigate possible adverse impacts of the use of RMS as bedding.
- Assess specific aspects of welfare and comfort of cows on RMS and other bedding.

- Predict the likely levels of MAP and *Salmonella* spp in slurry and RMS bedding over time, in farms with different disease levels, by modelling literature based values of cow excretion patterns alongside the dynamics of slurry storage and removal.
- Provide information on antimicrobial resistance patterns in organisms isolated from farms using RMS and other bedding materials.
- Contribute to information on best practice for building and managing beds using RMS.
- Provide a cost calculator enabling farmers to evaluate a variety of bedding options.
- Enable exchange of information and experiences on RMS use between the UK and the Netherlands.

1.5 Structure of Project

The research involved six parallel activities as outlined below:

1: An epidemiological survey of 120 farms using RMS, sawdust and sand to allow analysis of the impact of different ways of managing RMS as well as comparison between RMS and other types of bedding - see Chapter 2.

2: A controlled study of different bedding materials to allow an assessment of the impact on pathogen load of bedding on milk quality and udder health - see Chapter 3.

3: An observational study of different methods of initial build-up of deep RMS beds - see Chapter 4.

4: In silico modelling to predict the likely levels of MAP and *Salmonella* spp in herd slurry stores over time, in farms with different disease levels - see Chapter 5.

5: Development of an economic cost calculator to allow evaluation of the cost of converting to RMS bedding - see Chapter 6.

6: Analysis of bacterial isolates collected during the survey to assess implications for antimicrobial resistance - see Chapter 7.

At the start of the project, a workshop was held with stakeholders to give an opportunity to discuss and direct certain aspects of the work. Researchers from the Netherlands were invited to this workshop, to discuss past and current research projects. Ongoing work in the Netherlands is currently concentrating on understanding the conditions under which high levels of heat resistant spore-forming organisms are found. This is generally associated with composting or composted bedding materials; however, occasionally high numbers of these organisms are found in various types of bedding materials which have not been deliberately subjected to a composting process, including RMS.

2 Epidemiological Farm Survey

2.1 Introduction

The five main objectives of the farm survey, as outlined in the original tender document, were:

1. To assess bacterial load of bedding and bulk tank milk from farms using RMS compared with other bedding.
2. To monitor health status in RMS herds and "control herds" using other types of cubicle bedding, as far as farm records allow.
3. To gather further information about the ways in which RMS is currently being managed in the UK and the costs associated with its use compared to more conventional bedding materials.
4. To provide data for analysis of relationships between management factors, including bedding type and management, and herd health parameters, in particular, rates of new intramammary infection as indicated by individual cow somatic cell counts.
5. Based on the above, to increase understanding of the reasons why udder health varies between farms using RMS.

To assist and inform this process a number of specific study hypotheses were developed and tested. These included, but were not limited to:

"Used" RMS bedding has higher bacterial counts than "used" sand or sawdust. To be tested for the total bacterial count and specific bacterial groups of interest with respect to animal and human health and food quality.

The number of bacteria in bulk milk from cows bedded on RMS differs from counts in milk from cows on sand or sawdust. To be tested for the total bacterial count and specific bacterial groups of interest with respect to animal and human health and food quality.

There is a direct relationship between bacterial load of bedding (at its "dirtiest", before replenishment) and bacterial load of bulk tank milk. To be tested for the total bacterial count and specific bacterial groups of interest with respect to animal and human health and food quality.

The risk of new intramammary infection during lactation and the prevalence of persistent intramammary infection is increased by bedding on RMS compared with sand or sawdust.

The use of RMS as a bedding material results in cleaner cows and less hock damage than other bedding materials.

In addition, it was proposed to explore the data collected during the survey to improve our understanding of how to best manage and mitigate any risks associated with the use of RMS (or other bedding materials).

2.2 Methods

2.2.1 Farm Recruitment

The aim was to recruit 40 farms into each of three groups, bedding at least 85% of the milking herd in cubicles on RMS, sand and sawdust respectively (up to 15% of animals could be housed differently to allow for a "hospital" and/or freshly calved group). It was calculated that this number of farms would give sufficient power to detect a clinically important 2 log score difference in bacterial counts between the treatment groups.

Once the location of RMS farms was known, matching sand and sawdust farms were recruited, on the basis of approximate herd size (<150 cows, 150 – 500 cows, >500 cows) and geographical location (East/West); matching on a North/South basis was not possible due to the limited number of farms using sand in the North. Milking method (parlour or automated milking system (AMS)) was also used in matching. In the case of sand and sawdust farms, those carrying out regular recording of milk yields and individual cow cell counts were preferentially selected.

Farms using RMS were recruited via contacts made during the Scoping Study, distributors of separation machinery, veterinary surgeons and agricultural consultants. Sand and sawdust farms were identified by a combination of contacts of the Dairy Group, participating RMS farmers, Veterinary Surgeons and agricultural consultants and through the QMMS database.

2.2.2 Data Collection

Farm visits were carried out by five members of The Dairy Group, between 9th December 2014 and 31st March 2015 (though >97% (122/125) of visits were conducted in January, February and March). Each farm was visited once by one consultant. Information was collected using a combination of a questionnaire and observations. The key observations made and their interpretations are outlined in Table 2.1. Further details of methodology are available in Appendix 1 at the end of this report

Prior to the first visit the survey team met to agree and standardise scoring and data capture methods; a training session was held and two separate assessments of agreement of scoring made.

2.2.3 Sample Collection

Bedding: Fresh bedding was sampled from the stock currently in use. In the case of RMS, samples were taken during or immediately after separation. All farm visits were arranged so that "Used" bedding samples could be taken on a day when bedding would normally be replenished, and were collected just prior to the addition of fresh bedding. At least 10 cubicles were sampled on each farm, proportionally distributed across different passageways and sheds using a previously agreed method. Approximately 100g of bedding material was collected from the top 2.5 cm layer from a standardised position at the rear of each cubicle (an A4 frame was placed in landscape format, 15 cm in from the centre of the rear of the cubicle bed (See Appendix 1)) - if fresh faeces were present in the sampling position a new cubicle was selected. These samples were then comingled and thoroughly mixed. Sufficient cubicles were sampled to provide at least 750 ml of bedding. If there were two distinct types of bed design then these were sampled separately; subsamples from the two types were later comingled proportionally according to the relative numbers of cubicles of the different designs.

Bulk milk: A sample of 500 ml of thoroughly agitated milk was collected from the bulk tank ensuring that a full 24 or 48 hours of milking had been accumulated and cooled. If more than one bulk tank was in use, both were sampled and subsamples were later comingled in proportionally according to the relative volumes in the separate tanks.

All samples were packed in insulated boxes with icepacks and immediately shipped to the laboratory for bacteriological analysis.

Table 2.1: Key observations and interpretations made on farm survey visits (also see Appendix 1).

Parameter	Assessment Method	Assessment Unit	Farm Level Descriptor
General Shed Observations (recorded just prior to application of fresh bedding)			
Ventilation	Subjective ordered categorical scale 1: Excellent 2: Good 3: Poor 4: Inadequate	Each shed scored	Overall score allocated in proportion to number of cubicles in each shed
Dust in sheds	Descriptive comment	Each shed scored	Categorised to 0,1,2 (minimal, some, a lot) and then averaged across sheds
Bed Observations (recorded just prior to application of fresh bedding)			
Bedding depth	Measured at front and back of cubicle	At least 10 cubicles across all sheds – unless distinctly different bed types in which case 10 of each type	Proportional mean across all sheds for each bed type
Cow Observations			
Cleanliness of udder, lower leg and upper leg with flank	1-4 scale Cook (2002)	Random selection of 30 cows proportionally distributed across milking groups, excluding straw yard groups. Both sides scored, highest score reported	Total count across all farms for each bedding type used for analysis (sample too small to give reliable measure at farm level)
Hock swelling	0-3 scale, maps to AHDB scale* and Potterton *et al* (2011)		
Hock hairloss and lesions	0-3 scale, maps to AHDB scale* and Potterton *et al* (2011)		

*http://dairy.ahdb.org.uk/technical-information/animal-health-welfare/welfare-assessment/#.VZQ9JmfbKUk accessed 30/6/15

2.2.4 Laboratory Methods

Bacteriological analyses: Thirty grams of thoroughly mixed bedding material was added to 270 ml of maximum recovery diluent (MRD) and mixed in a stomacher for 1 minute at 100rpm prior to aliquoting for preparation of serial dilutions. Serial dilutions of milk and the bedding aliquots were then made in MRD to encompass the 2 or 3 dilutions anticipated to reflect likely counts. When necessary and where appropriate, further dilutions were undertaken to allow an accurate enumeration of colony forming units (cfu) to be determined.

Growth was evaluated and enumerated on selective media 'pour plates', with the aim of allowing counts of a number of 'putative' bacterial populations to be made - the media used and the bacterial species enumerated are outlined below. Positive and negative controls were also utilised to demonstrated profuse growth and 'no growth' respectively.

Total Bacterial Count (TBC): Samples incubated in milk agar for 66-72 hours at 30^{o}C (±2^{o}C).

Coliform Count (CC): Samples incubated in VRB (MUG) agar for 66-72 hours at 37^{o}C (±2^{o}C).

Laboratory Pasteurised Count (LPC): Samples heated to 63.5^{o}C (±0.5^{o}C) for 35 minutes prior to being incubated in milk agar for 66-72 hours at 30^{o}C (±2^{o}C).

***Streptococcus* spp Count (StrC):** Samples incubated in Edwards agar for 66-72 hours at 37^{o}C (±2^{o}C).

***Staphylococcus* spp Count (StaC):** Samples incubated in Baird Parker agar for 48 hours at 37^{o}C (±2^{o}C). Colonies demonstrating morphology typical of *S. aureus* were then enumerated.

Thermophilic Spore Count (TSC): Samples heated to 80^{o}C (±1^{o}C) for 10 minutes prior to being incubated in milk agar for 24-48 hours at 55^{o}C (±2^{o}C).

Psychrotrophic Count (PsyC): Samples incubated in milk agar for 6 days at 5^{o}C (±2^{o}C).

***Bacillus cereus* Count (BCerC):** Samples heated to 80^{o}C (±1^{o}C) for 10 minutes prior to being incubated in *Bacillus cereus* agar for 18-24 hours at 35^{o}C (±2^{o}C). Plates were re-examined after a further 18-24 hours at room temperature.

In addition, specific enrichment and plating techniques to facilitate detection of additional pathogens of interest were undertaken as outlined below:

***Salmonella* spp:** 25 g of bedding or 25 ml of milk was inoculated into 225 ml of Buffered Peptone Water (BPW) and incubated at 37^{o}C (±2^{o}C) for 18-24 hours. Following incubation, 100 ul of the BPW was inoculated into 10 ml of Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42^{o}C (±2^{o}C) for 24-48 hours. Following this second incubation 10ul of the RV broth was inoculated in duplicate onto Brilliant Green Agar and XLD Agar plates and incubated at 35^{o}C (±2^{o}C) for 18-24 hours. Suspicious colonies were identified by MALDI-TOF MS (matrix assisted laser desorption/ionization time-of-flight mass spectrometry) (MALDI Biotyper, Bruker Daltonics) and submitted for typing to the AHVLA.

***Listeria* spp:** 25 g of bedding or 25 ml of milk was inoculated into 225 ml of Listeria Enrichment Broth (LEB) and incubated at 30^{o}C (±2^{o}C) for 7 days. LEBs were then sub-cultured at 1, 2 and 7 days onto *Listeria* Selective Agar (LSA) and incubated at 35^{o}C (±2^{o}C) for up to 48 hours. Suspicious colonies were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

***Yersinia enterocolitica*:** 100 ul of the 10^{-1} dilution of milk or bedding was inoculated on *Yersinia* selective agar and incubated for 18-24 hours at 32^{o}C (±2^{o}C). Suspicious colonies were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

A direct plating onto sheep blood agar, Edwards agar and MacConkey agar was also undertaken to assist the identification and recovery of key pathogens. Where necessary the identity of micro-organisms was confirmed by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

Somatic cell count determination: SCCs were determined using the Fossomatic method (Delta CombiScope - Model FTIR 400, Drachten, The Netherlands), according to the FIL . International Dairy Federation 141 C: 2000(Infrared).

Milk compositional analysis: Milk constituents were determined by near infrared analysis (Delta CombiScope - Model FTIR 400, Drachten, The Netherlands), according to the FIL . International Dairy Federation 148 A: 95 norm.

Dry matter determination: Dry matter content and bulk density of fresh and used bedding were determined. Two subsamples of 50 g sand, 20 g sawdust or 20 g RMS were taken for determination of dry matter content, by drying to constant weight in an oven. Bulk density was determined by determining the weight of material in a 150 ml container filled in a standard manner (NRAES, 1992).

2.2.5 Evaluation of Udder Health

Herds that took part in the bedding survey were asked, where available, to send in electronic herd management, milk recording and health data to be used for analysis, assessment and anonymous benchmarking. Herds were then selected for analysis based on data available from the monthly milk recordings. Herds reporting no clinical mastitis cases in March 2015 were excluded from that comparative analysis, as were herds reporting less than 10 cases / 100 cows / year for the quarter ending March 2015. Herds reporting clinical mastitis rates below this threshold in any analysis period were also excluded from analysis in that period, as data from such herds was unlikely to be robust.

All herds were benchmarked using the TotalVet© software (Version 2.6.016) (QMMS/SUM-IT; www.total-vet.co.uk). Parameters selected were those that encompassed udder health based on somatic cell count (SCC) and clinical mastitis data. In addition, data on herd size, average days in milk, calving index and 305-day milk yield were also collated. A summary of the benchmarking parameters and their definitions are outlined in Table 2.2. Benchmarks were collated for the 12 months ending in March each year for the period March 2012 to March 2015. Benchmarks were calculated for the month, quarter and year-ending to March in each 12-month period.

2.2.6 Data Collation and Statistical Analysis

Data were collated and initially analysed using Excel and Access 2003 (Microsoft Corp) and Minitab 15.1 (Minitab Inc). Descriptive and graphical analyses were carried out to explore the data. Where appropriate groups were compared using ANOVA or the Kruskal-Wallis Test if data were not normally distributed. Pairwise comparisons were made using either the Two Sample T-test or Mann-Whitney U test as appropriate. Univariable analysis of treatment efficacy was performed using the Chi-Square test to investigate differences in proportions between groups; a layered Bonferroni correction was used to allow for multiple comparisons where appropriate (Darlington, 1990).

When analysing cow hygiene and hock scores, for each parameter the total count for each score level across all farms was used for analysis. For measures with several categories, the groups were compared statistically in terms of the percentage of the “best” and “worst” categories for each indicator, unless numbers in a category were below 11 for any of the groups, in which case the two “best” or “worst” categories were amalgamated. The Chi-squared test was first used to test for a difference across the

four bedding/bed type groups. If the null hypothesis was disproved, individual pair-wise tests with layered Bonferroni correction were carried out.

Table 2.2: A summary of parameters used to benchmark udder health and performance.

Parameter	Definition
Lactation new infection rate (SCC)	Proportion of cows moving from below to above a 200,000 cells/ml threshold between milk recording dates in lactation
Dry period new infection rate (SCC)	Proportion of cows moving from below to above a 200,000 cells/ml threshold between drying-off and 1st test-day recording post-calving (including maiden heifers)
Dry period cure rate (SCC)	Proportion of cows moving from above to below a 200,000 cells/ml threshold between drying-off and 1st test-day recording post-calving
Fresh Calver Rate (SCC)	The rate of fresh calver infections as measured by the proportion of cows and heifers above 200,000 cells/ml at the first milk recording date in lactation
Chronic cows (SCC)	The proportion of the herd chronically infected (*ie* more than one of the last three SCC >200,000 cells/ml)
Cows >200,000 cells/ml (SCC)	The proportion of the herd infected (*ie* >200,000 cells/ml)
Clinical mastitis rate (cow cases per 100 cows/year)	All incidence of cow cases of clinical mastitis per 100 cow years using a 7-day lag period
Apparent dry period origin clinical mastitis rate	Proportion of cows reported with a 1st clinical case of mastitis less than 31 days in lactation (target <1 in 12 cows affected)
Apparent lactating period origin clinical mastitis rate	Proportion of cows reported with a 1st case of clinical mastitis between 31 and 305 days in lactation (target <2 in 12 cows affected)
305-day milk yield for cows and heifers	305-day adjusted milk yield for cows and heifers at the latest test-day
Total animals in herd	Total animals in herd at latest milk recording
Calving index (mean)	Median historic calving index (days)
Average days in milk	Average days in milk at latest recording

2.3 Results

2.3.1 Descriptive Statistics across all Bedding Types

Average herd size and milk sales per cow per year on the day of the visit are summarised in Table 2.3. The three groups did not differ significantly in herd size. Mean milk sales per cow were significantly higher for sand farms (9446 l) than for RMS farms (8803 l) or sawdust farms (8491 l) ($p<0.005$). There was no difference between groups in cubicle stocking rate for the milking cows on the day of the visit.

Table 2.3: A summary of key farm descriptors of survey farms.

Variable	Bedding	n	Mean	Median	SD	Min	Max	25th Percentile	75th Percentile
Average herd size									
	RMS	40	374	290	217.0	135	1000	220	480
	Sand	41	370	300	248.7	120	1550	228	435
	Sawdust	44	336	265	191.7	110	1020	205	425
Milk sales l/cow/year									
	RMS	40	8803[a]	8663	1140	6500	10833	7895	9766
	Sand	41	9446[b]	9524	1367	6567	12115	8473	10419
	Sawdust	43	8491[a]	8333	1090	5902	10435	7800	9308
Stocking rate (cows/100/cubicles) on day of visit									
	RMS	40	96.1	97.7	9.70	69.4	116.4	92	101
	Sand	41	99.3	98.4	11.51	75.3	137.2	94	104
	Sawdust	44	94.4	95.3	7.86	72.9	111.4	91	99

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

The mean length of time of use of RMS at the time of the visit was 13.6 months (median 13.6, range 1-35 months).

Other management descriptors are summarised in Table 2.4. The majority of herds were Holstein-Friesian, and calved all year round. The RMS group had the highest proportion of herds where all the milking cows were housed all year round (70% *vs* 54% of sand farms and 39% of sawdust farms ($p=0.012$)). Forty percent of farms in each group had had staff changes in the past year. Thirty-five percent of RMS farms, 34% of sand farms and 23% of sawdust farms had had infrastructure changes in the past year (other than changes associated with introduction of RMS bedding). Automatic scrapers were used in at least one shed on 40% of RMS farms and 44% of sawdust farms, but on only one sand farm. Twenty-two percent of RMS farms, 51% of sand farms and 27% of sawdust farms housed the milking cows in a single shed; compared to other bedding materials, cows on farms using sand as a bedding material were significantly more likely to be housed in a single shed ($p<0.05$).

Table 2.4: Key management descriptors of survey farms.

	RMS	Sand	Sawdust	Total
Breed				
All Holstein Friesian	35	34	37	106
Other breed or mixed	5	7	7	19
Calving pattern				
All year round	35	35	36	106
Other	5	6	8	19
Milkers housed all year				
None	6	11	13	30
Some	6	8	14	28
All	28	22	17	67
Milkers housed in multiple sheds				
No	9	21	12	42
Yes	31	20	32	83
Alleyway cleaning				
All automatic scrapers	16	1	23	40
All tractor scraped	16	35	13	64
At least one shed with slats (with additional scraping)	3	1	3	7
Other variation in scraping methods between sheds	5	3	5	13
Floodwash	0	1	0	1

Key descriptors relating to the design and management of beds are summarised in Table 2.5. Sand was predominantly used in deep beds (*ie* within an enclosed structure which contained a measurable depth of at least 4 cm of bedding material and reduced loss of bedding into the passageway). Sawdust was used predominantly on mats or mattresses. No distinction has been made between mats and mattresses in this study. On four RMS farms and two sand farms, the deep beds had been created by the addition of a wooden or metal "bedding retainer" on top of a mat or mattress. RMS was used both in deep beds and on mattresses; nine RMS farms had some beds of each type. The mean depth of 'deep' beds did not differ significantly between sand and RMS beds (14.8 cm (SD=5.70) and 14.6 cm (SD=6.67) respectively).

Table 2.5: A summary of counts of types of bed for different bedding materials.

Bed structure	RMS	Sand	Sawdust	Total
All mats or mattresses with a covering of bedding material	20	1	42	63
All "deep" beds (*ie* enclosed structure containing bedding material)	11	36	1	48
Mixed types	9	4	1	14

Milking frequency, cluster disinfection and teat preparation practices on the farms are summarised in Table 2.6. Twice daily milking was more common on sawdust farms than in the other two groups. Automatic cluster disinfection was used on 47.5% of the RMS farms, 46.3% of sand farms and 32% of sawdust farms. Sixty-five percent of RMS farms, 68% of sand farms and 50% of sawdust farms used a pre-dip.

Table 2.6: A summary of milking practices across the survey farms.

Parameter	RMS	Sand	Sawdust	All
n	40	41	44	125
Milking frequency				
x2	22	24	37	83
x3	13	14	3	30
Some x 3	0	1	2	3
All AMS	2	2	2	6
Some AMS	3	0	0	3
Cluster disinfection				
Automatic	19	19	14	52
Manual after all cows	0	1	0	1
Manual after some cows	11	14	24	49
Mixed	1	0	0	1
None	9	7	6	22
Teat preparation				
Dry wipe	4	5	7	16
Medicated wipe	0	3	4	7
Predip	26	28	22	76
Brush (including on AMS)	8	5	5	18
AMS wash and dry	1	0	0	1
Wash only	0	0	1	1
Unknown	1	0	5	6

AMS = Automated milking system (robot)

Reasons for culling of the last 10 cows culled are summarised in Table 2.7. There was no significant effect of bedding type on the proportion of cows culled for any of the reasons outlined. There was no significant difference in the proportion of herds that had culled a cow for Johne's disease in the previous 12 months (62%, 56% and 59% of RMS, sand and sawdust farms respectively).

Table 2.7: A summary of the reasons for culling of the last 10 cows by bedding type.

Reason	Bedding	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Infertility	RMS	39	3.5	4	0	10	2	5
	Sand	41	3.4	3	0	7	2	5
	Sawdust	44	3.1	3	0	8	1	5
Mastitis	RMS	39	1.4	1	0	5	0	2
	Sand	41	1.6	1	0	5	0	3
	Sawdust	44	1.4	1	0	4	0	2
Legs/Feet	RMS	39	1.6	2	0	5	0	3
	Sand	41	1.0	1	0	3	0	2
	Sawdust	44	1.5	1.5	0	6	0	2
Age	RMS	39	0.6	0	0	5	0	1
	Sand	41	0.9	0	0	5	0	1
	Sawdust	44	1.3	0	0	10	0	2
TB	RMS	39	0.0	0	0	1	0	0
	Sand	41	0.2	0	0	5	0	0
	Sawdust	44	0.1	0	0	3	0	0
Casualty	RMS	39	0.7	0	0	6	0	1
	Sand	41	1.1	1	0	5	0	2
	Sawdust	44	1.2	1	0	9	0	1.75
Johne's Disease	RMS	39	0.5	0	0	5	0	1
	Sand	41	0.4	0	0	4	0	0.5
	Sawdust	44	0.3	0	0	2	0	0
Udder shape	RMS	39	0.3	0	0	3	0	0
	Sand	41	0.1	0	0	4	0	0
	Sawdust	44	0.3	0	0	4	0	0

Descriptors of the environment in sheds at the time of sampling bedding are summarised in Table 2.8. There were no significant differences in external or internal temperature on the day of the visits between farm types. The ventilation score was significantly lower (better) on sand farms than sawdust or RMS farms (1.9 *vs* 2.1 and 2.3 respectively; $p<0.05$). Unsurprisingly, the subjective 'dust score' for sawdust was higher for sawdust than for either RMS or sand farms, which did not differ significantly.

Table 2.8: A summary of environment descriptors across the study farms.

Parameter	Bedding	n	Mean	Median	StDev	Min	Max	25th Percentile	75th Percentile
External temperature (°C)									
	RMS	39	6.1	5.5	2.96	1.0	11.0	3.9	8.2
	Sand	38	6.2	6.1	2.52	0.5	11.0	4.0	8.0
	Sawdust	39	5.6	5.5	2.88	-2.0	12.5	3.4	7.5
Internal shed temperature (°C)									
	RMS	39	7.4	6.6	2.75	2.0	12.8	5.9	9.0
	Sand	41	7.4	7.4	2.33	3.5	12.0	5.9	9.4
	Sawdust	43	7.0	7.0	2.59	1.0	12.2	5.0	9.1
Ventilation score (lower is better)									
	RMS	40	2.1[b]	2.2	0.71	1.0	3.2	1.8	2.8
	Sand	41	1.9[a]	2.0	0.65	0.8	3.0	1.2	2.1
	Sawdust	43	2.3[b]	2.2	0.64	0.5	4.0	2.0	2.7
Dust score (lower is better)									
	RMS	40	1.1	0.0[a]	1.66	0.0	6.0	0.0	2.0
	Sand	41	0.3	0.0[a]	0.72	0.0	3.0	0.0	0.0
	Sawdust	44	3.4	4.0[b]	2.42	0.0	6.0	1.0	6.0

[a,b] Values with different superscripts within columns, within parameters, differ (p<= 0.05).

The mean percentage bedding coverage of mats was 74% and 70% for RMS and sawdust respectively, with the rear 1/3 of the mattress covered 68% and 69% respectively. As would be expected, all deep beds were completely covered irrespective of bedding material. The mean and SD of depth at the front and rear of the cubicle ("knee" area and "udder" area) are outlined in Table 2.9. The depth of RMS on mats was significantly greater than that for sawdust (p<0.05).

Table 2.9: A summary of bedding depth (cm) in cubicles measured immediately prior to re-bedding.

	Front		Rear	
Bedding	Mean	SD	Mean	SD
Deep RMS	15[a]	6.8	13[a]	6.5
RMS on mats	4[b]	3.6	2[b]	2.9
Deep sand	12[a]	6.2	10[a]	5.9
Sawdust on mats	1[c]	0.8	0.8[c]	0.5

[a,b] Values with different superscripts within columns differ (p<= 0.05).

The mean temperature, measured at 5 cm depth in the rear 1/3 of the bed was not significantly different between deep RMS (17.6 °C ; SD 5.73, n=24) and deep sand beds (16.9 °C; SD 3.14, n=38).

Key aspects of management of bedding materials are summarised in Table 2.10. All sawdust users stored material under cover, whilst only a minority of sand users did so. The majority of RMS users separated / stored solids under cover. Almost half of sawdust users bedded cows more than once daily

and more than three quarters bedded at least daily. Less than 50% of RMS users bedded daily. Sand users bedded least frequently. There was a significant difference in the frequency of bedding between the different bedding types; sand users were significantly less likely to bed 'daily or more frequently' than either RMS users who were in turn less likely to bed 'daily or more frequently' than sawdust users (sand 1/39, RMS 16/24, sawdust 34/10; p<0.0001). RMS users bedding deep beds bedded no less frequently than users employing mats. Sawdust users were significantly more likely to be using a conditioner than RMS or sand users, who did not differ (39/5, 14/26 and 9/31 respectively (p<0.001).

Table 2.10: A summary of key bedding and bed management practices.

Parameter	RMS	Sand	Sawdust	All
n	40	41	44	125
Stored/Separated under cover*				
Yes	30	12	44	86
No	9	29	0	38
Frequency of bedding+				
More than once daily	0	0	21	21
Daily	16	1	13	30
Less than daily but more than weekly	24	23	10	57
Less than weekly	0	16	0	16
Bedding conditioner used				
All	11	8	37	56
None	26[a]	31[a]	5[b]	62
Some	3	1	2	6

[a,b] Values with superscripts within rows differ (p<= 0.05)
* One RMS user separated material in the open, but then stored it under a tarpaulin.
+ One sand farmer bedded erratically and had no fixed system.

2.3.2 Preparation, Use and Management of RMS.

2.3.2.1 Preparation of RMS.

During the survey four different types of machine were identified that were suitable for separating slurry to produce a high enough dry matter material to allow use as bedding. The most common was the FAN Screw Press separator. This was used on 31 of the survey farms. Five used the EYS Screw Press separator, two the Visscher Sep-Com and two the G-Bed separator.

Materials present in slurry that was separated to create bedding material are summarised in Table 2.11. These included slurry from other cattle groups, the output of washing the milking plant, waste milk, sometimes including that from cows under antibiotic treatment, the contents of footbaths, containing copper sulphate and/or formalin as well as silage effluent. The proportion of the liquor removed by the separator which was returned to the reception tank was variable.

The capacity of the reception pits, the percentage of slurry produced that is separated, and an estimate of the percentage of slurry removed from the reception pit each time bedding is made are summarised in Table 2.12. Fifteen farms separated all the slurry that was produced; on the remainder, only part of the slurry produced was separated, and used for bedding.

2.3.2.2 Product Monitoring

Only four farms had ever taken any measurements of DM content of the separated material. Two monitored it regularly, one using equipment provided by the machinery supplier, the other using a grain moisture meter (not calibrated for this material or range of DM contents). The rest considered that DM content of the product was important but judged this by feel and/or sight.

Table 2.11: Materials present in slurry, in addition to slurry from milking cows, which was used for producing RMS for use as bedding.

Material	Number of Farms	Percentage of Farms
Slurry from dry cows	21	52.5
Slurry from youngstock (12-24 months)	11	58.8
Slurry from youngstock (under 12 months)	2	5.0
Parlour washings	29	72.5
Waste milk	15	37.5
Milk from cows under antibiotic treatment	8	20.0
Footbath contents	33	82.5
Silage effluent	7	19.6
All liquid separated off returns to reception tank	3	7.5
Some liquid separated off returns to reception tank	13	32.5
No liquid separated off returns to reception tank	20	50.0
Fate of liquid separated off unknown	4	10.0

Table 2.12: Capacity and details of emptying of the reception pit used for producing RMS for bedding.

	Capacity of reception vessel (m^3)	% of slurry produced that is separated	% of slurry removed from reception vessel each time bedding is made
Mean	378	88	66
SD	1068	20	31
Median	54	100	60
Min	4.5	25	10
Max	5909	100	100

2.3.2.3 Previous Bedding Material

A variety of bedding materials had been used by farmers prior to the use of RMS, most commonly sawdust. These are listed in Table 2.13.

Table 2.13: A summary of bedding materials previously used by farmers before employing RMS.

Material	Number of Farms	Percentage of Farms
Sawdust	23	57.5
Paper product	7	17.5
Sand	3	7.5
Straw*	3	7.5
Gypsum	2	5.0
Oat husks	1	2.5
New unit	1	2.5

*One had previously used straw in yards

A number of farms used RMS for cattle groups other than milking cows. Seven farms used RMS for youngstock; one from six months old and the remainder starting use with bulling heifers. Seven farms used RMS for dry cows in cubicles.

2.3.2.4 Bed Establishment

Eighteen farmers had experience of creating deep beds of RMS. With four exceptions they had filled the beds slowly, adding more material once or twice daily and taking ten days to three weeks to reach the full depth desired. Three farmers were content with the rapid building method, the fourth considered that slow building would have been preferable. On three farms the beds were built up gradually while cows were still at grass. One of these farmers commented that six to eight inches depth of bedding was reduced to two inches as soon as the cows had access, another considered that building beds in summer might have contributed to heating of the material. RMS bedding was sometimes added on top of an existing layer of bedding, including sand, sawdust, straw and paper products. Two farmers who had constructed beds by adding RMS on top of oathusks subsequently regretted the decision; one suggested that the RMS and oathusk combined to form hard balls of material; in addition slurry was harder to separate when oathusk was present. Two farmers mentioned intentionally using RMS with a lower dry matter initially, as they considered that this compacted to make a better base. Two farmers mentioned that RMS did not remain in place as well on rubber mats as on a concrete base. One farmer mentioned mastitis problems during the initial period of use.

2.3.2.5 Factors Influencing the Nature of Bedding Material

On ten farms the separation equipment was not covered. These farmers generally reported that they did not prepare bedding material if the weather was very wet, delaying bedding until a drier day, as the weather conditions affected the DM of the material. Three farmers mentioned that, in wet weather, with more water entering the reception pit, a drier separated material was produced.

Six farmers mentioned effects of diet on the product, reporting variously that feeding drier silage gave drier material, feeding more straw gave a “fluffier” material, and reducing fibre in the diet reduced the amount of RMS produced. The nature of the input material was said to affect the nature of the product, with thicker slurry resulting in wet material due to blockage of the screen preventing water extraction.

2.3.3 Bacteriology of Bedding and Milk - All Farms

The findings of the bacteriological analysis of bedding and milk samples is summarised in Tables 2.14 and 2.15 and Tables 2.16 and 2.17 and in Figures 2.1 to 2.9 and 2.10 to 2.19 respectively.

Across all the species and groups enumerated, with the exception of *Streptococcus* spp, bacterial counts in bedding were significantly higher in RMS than either sand or sawdust ($p<0.05$). Numerically, mean and median counts were typically lowest on sand farms. However, it is important to note that there was often as much variation within bedding type as between bedding type.

Total bacterial counts in bedding were not significantly different between sand and sawdust farms. Only one RMS farm had a TBC in the bottom quartile of all those measured whilst only one sand farm was in the upper quartile. Whilst 50% (22/44) of the sawdust farms were in the lowest quartile of TBCs, 18% were to be found in the upper quartile.

Coliform counts in bedding were lowest on sawdust farms ($p<0.05$) with the median count (2.9×10^4 cfu/g) being a $\log^{10}$ less than on sand farms (1.75×10^5 cfu/g) and 2 $\log^{10}$ less than on RMS farms (1.9×10^6cfu/g). Twenty five of the lowest 30 coliform counts were recorded on sand farms. The highest coliform count was reported on a sawdust farm (1.0×10^8 cfu/g).

Streptococcus spp counts were lowest on sand farms ($p<0.05$) but were not significantly different between sawdust and RMS farms. Again there was a large amount of variation between groups. The highest (2.27×10^9 cfu/g) and lowest count (2×10^5 cfu/g) were reported on sawdust farms. RMS farms were more evenly distributed than for other counts.

Staphylococcus spp counts varied dramatically between farms varying between 0 and 8×10^6 cfu/g. Two thirds of the upper quartile was made up of RMS farms, thought the highest count was recorded on a sand farm.

LPCs were highest on RMS farms ($p<0.05$) with no RMS farms being represented in the lowest 33% of counts. Sand and sawdust farms did not differ and were evenly distributed, with less than 10% appearing in the upper quartile of counts.

Thermophilic spore counts were significantly different between all bedding types ($p<0.05$) being lowest on sand farms and highest on RMS farms. Half the RMS farms were in the upper quartile of all farms; however, three of the lowest six counts were recorded on RMS farms.

Bacillus cereus counts were significantly higher in RMS beds ($p<0.05$) being 2 $\log^{10}$ higher on RMS farms than on either sand or sawdust farms which did not differ from each other. On one RMS farm in excess of 4×10^6 organisms were present per gram of bedding.

Yersinia enterocolitica was identified in the bedding on between 4.9% and 9.8% of farms, but the prevalence did not vary between bedding types.

Salmonella spp were identified in used bedding on four farms (two sand and two RMS). No isolations were made from bedding collected before the 9th March. The isolate from both RMS farms and one sand farm was identified as *S. mbandaka*, the isolate from the second sand farm was identified as *S. montevideo*. Freshly generated RMS was subsequently sampled on one RMS farm 22 days and 82 days later and on the other 70 days later - on all three occasions *Salmonella* spp were isolated from the freshly separated solids. All isolates were sensitive to all antibiotics tested (APHA).

Listeria monocytogenes was isolated from a significantly higher proportion of sand farms (58.5%) than RMS (15.0%) or sawdust farms (31.7%) ($p<0.01$), which did not differ.

Table 2.14: A summary of bacterial counts in bedding from survey farms (all bacterial counts are cfu/g wet weight).

Parameter	Bedding Type	n	Mean	Median	Minimum	Maximum	25th Percentile	75th Percentile
Total Bacterial Count	RMS	40	8,863,500,000	7,450,000,000[a]	805,000,000	21,050,000,000	5,322,500,000	12,413,000,000
	Sand	41	1,899,280,488	1,505,000,000[b]	153,000,000	7,700,000,000	992,500,000	2,450,000,000
	Sawdust	44	3,072,306,818	792,500,000[b]	3,500,000	18,900,000,000	101,375,000	3,027,500,000
Coliform Count	RMS	40	3,191,350	1,900,000[a]	9,000	37,500,000	241,250	3,637,500
	Sand	41	321,829	175,000[b]	10,000	1,900,000	70,000	337,500
	Sawdust	44	2,541,927	29,000[c]	0	100,000,000	2,300	138,750
***Streptococcus* spp Count**	RMS	40	300,950,000	167,500,000[a]	6,500,000	1,650,000,000	53,000,000	446,250,000
	Sand	41	84,929,268	38,000,000[b]	2,850,000	850,000,000	26,500,000	97,500,000
	Sawdust	44	321,937,500	114,750,000[a]	200,000	2,270,000,000	11,950,000	315,000,000
***Staphylococcus* spp Count**	RMS	40	636,875	300,000[a]	0	5,000,000	156,250	837,500
	Sand	41	237,293	15,000[b]	0	8,000,000	5,000	32,000
	Sawdust	44	139,773	15,000[b]	0	1,000,000	0	118,750
Laboratory Pasteurised Count	RMS	40	5,263,375	3,675,000[a]	700,000	23,250,000	1,931,250	6,400,000
	Sand	41	1,301,076	635,000[b]	45,500	10,250,000	282,750	1,350,000
	Sawdust	44	1,326,199	762,500[b]	10,400	10,350,000	239,500	1,502,500
Thermophilic Spore Count	RMS	40	2,249,579	1,647,500[a]	3,900	14,000,000	555,000	2,887,500
	Sand	41	418,204	250,000[b]	3,850	2,150,000	73,750	477,500
	Sawdust	44	939,727	555,000[c]	6,200	6,500,000	132,500	1,137,500
Psychrotrophic Count	RMS	40	715,160,000	595,000,000[a]	12,400,000	2,550,000,000	195,000,000	840,000,000
	Sand	41	24,900,122	11,300,000[b]	250,000	180,000,000	4,025,000	27,125,000
	Sawdust	44	113,619,295	5,050,000[b]	4,500	1,495,000,000	586,250	40,375,000
***Bacillus cereus* Count**	RMS	40	281,881	48,250[a]	130	4,130,000	4,375	276,250
	Sand	41	2,509	900[b]	95	35,000	450	1,898
	Sawdust	44	3,526	553[b]	0	56,000	168	1,788
Fresh Bedding Dry Matter (%)	RMS	40	33.1	32.6	26.6	40.4	31.2	35.6
	Sand	41	91.9	92.4	79.4	96.8	90.6	93.9
	Sawdust	44	83.4	86.8	46.5	94.6	80.1	92.3
Used Bedding Dry Matter (%)	RMS	40	44.5	43.2	33.7	69.6	40.0	47.9
	Sand	41	94.4	94.9	88.6	96.9	93.6	95.9
	Sawdust	44	76.2	78.5	58.2	90.2	72.0	81.8

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

Figure 2.1: An illustration of the total bacterial counts in used bedding across the survey farms.

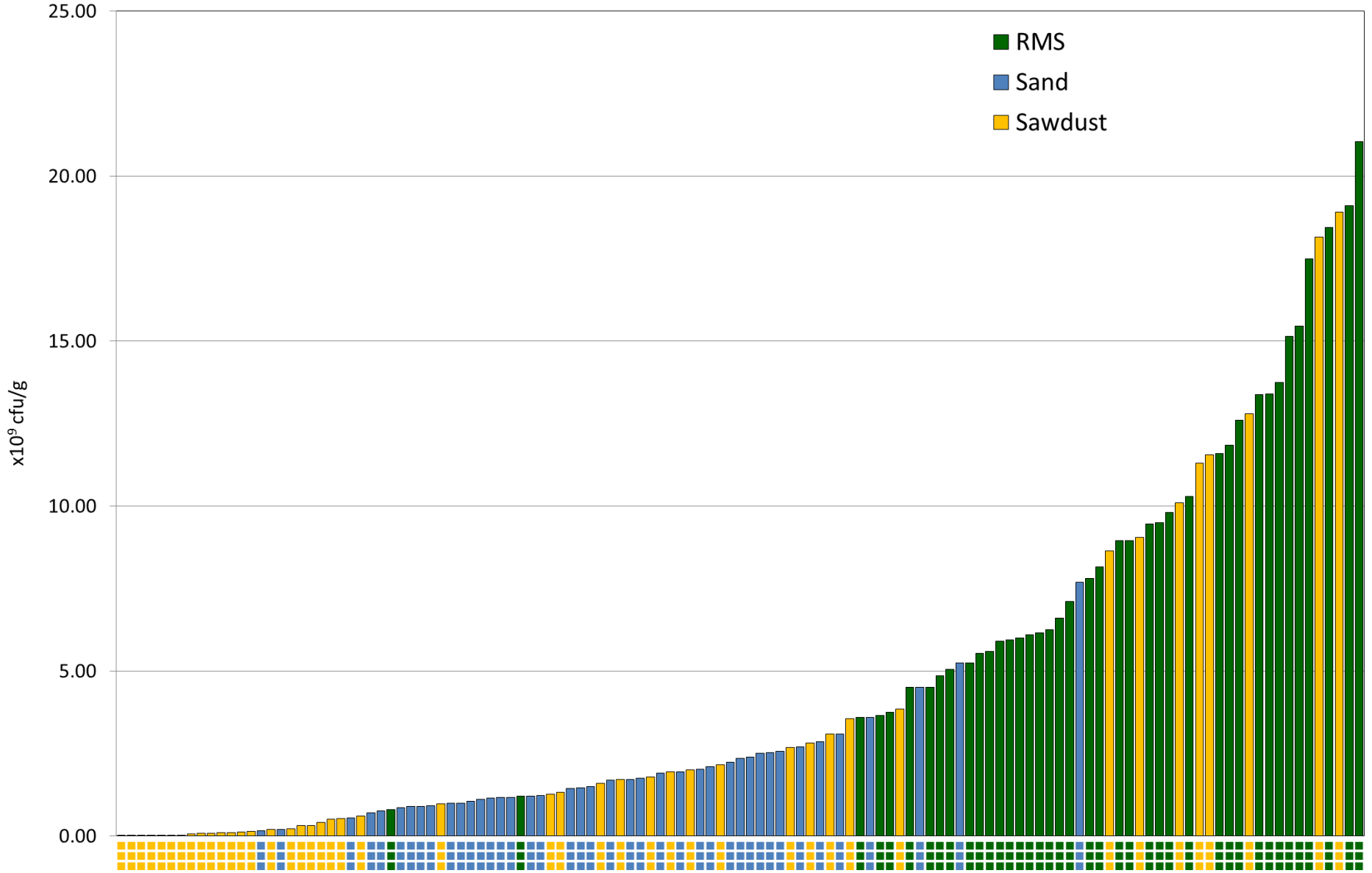


Figure 2.2: An illustration of the coliform counts in used bedding across the survey farms.

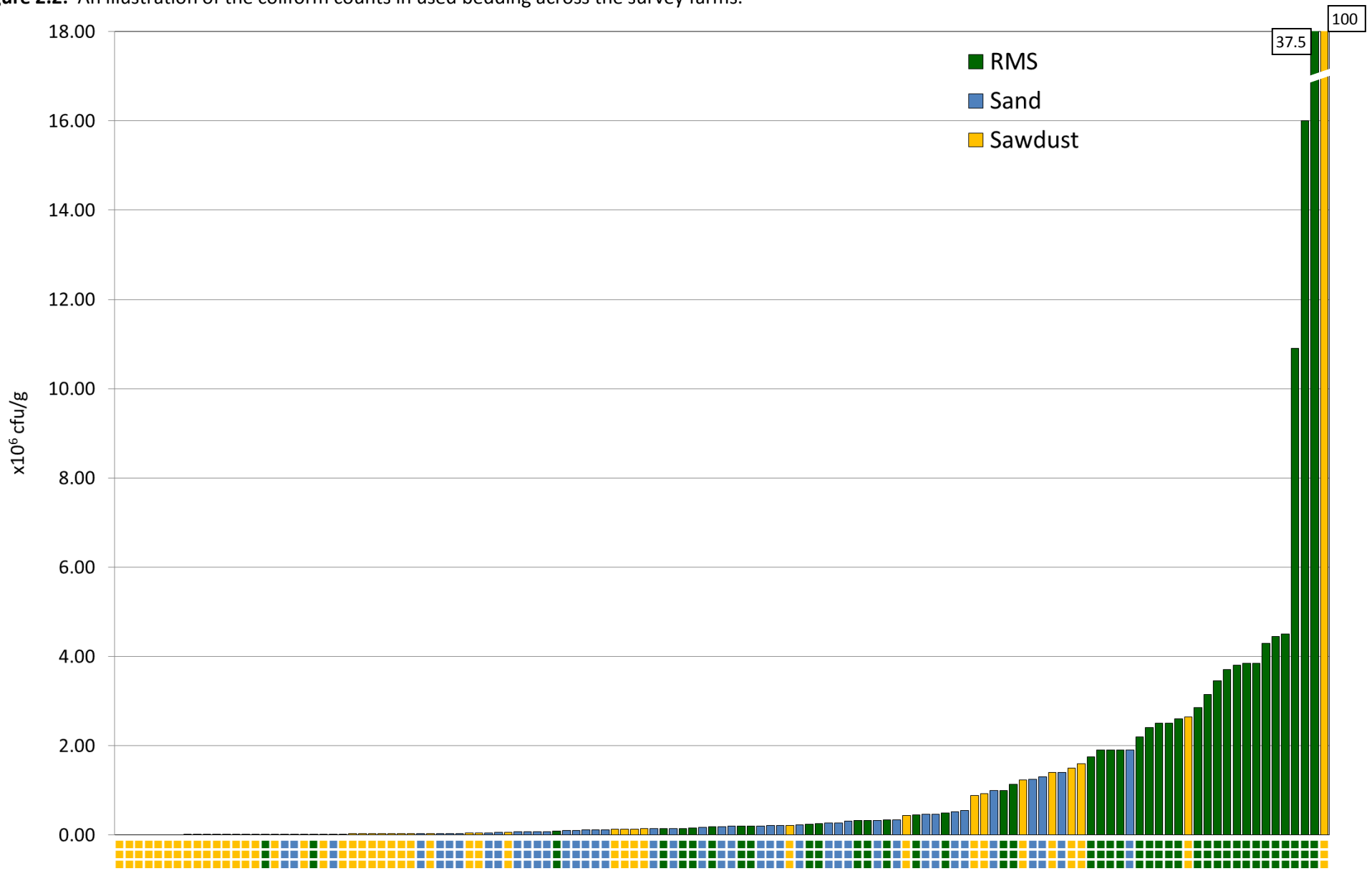


Figure 2.3: An illustration of the *Streptococcus* spp counts in used bedding across the survey farms.

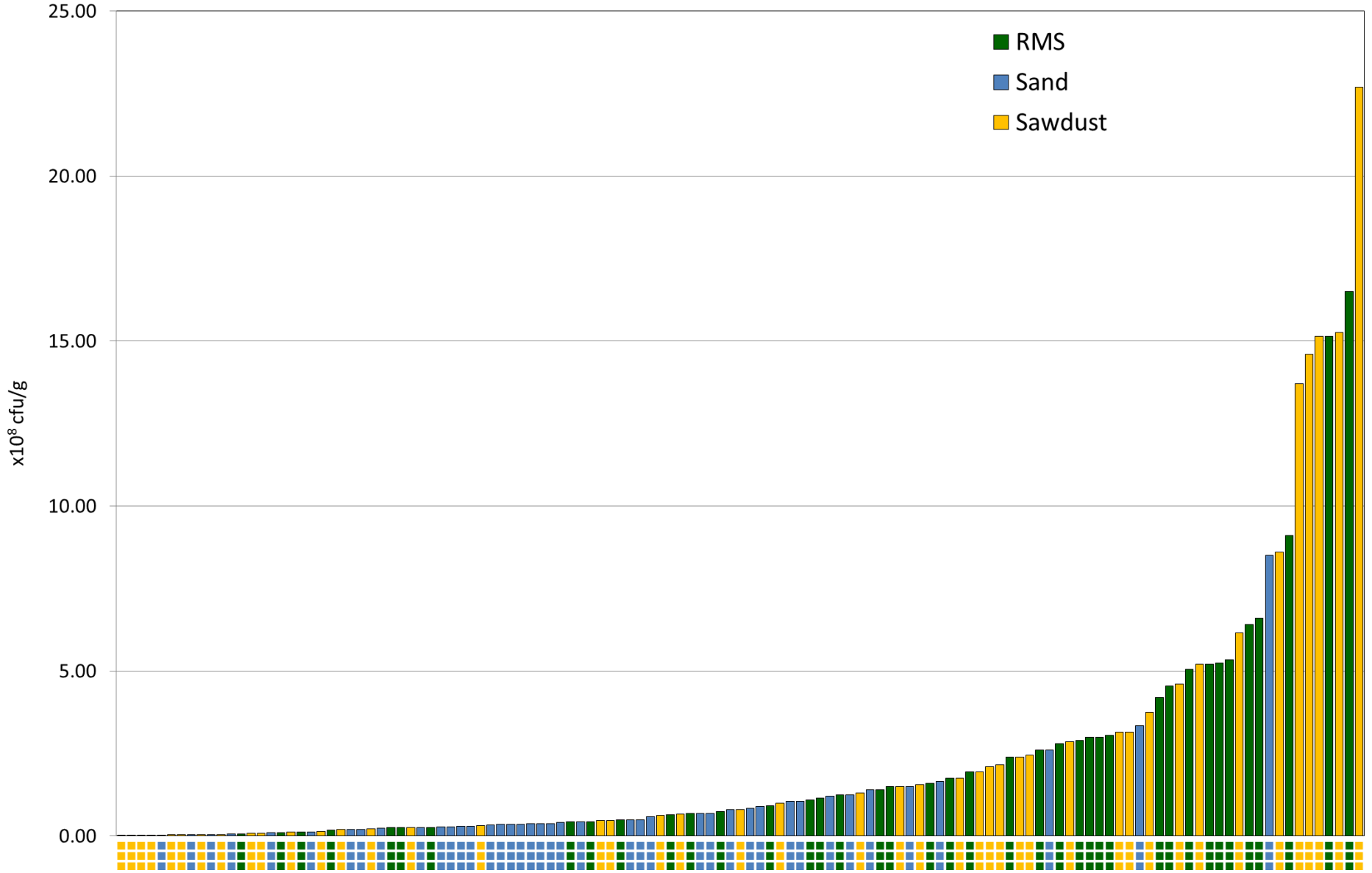


Figure 2.4: An illustration of the *Staphylococcus* spp counts in used bedding across the survey farms.

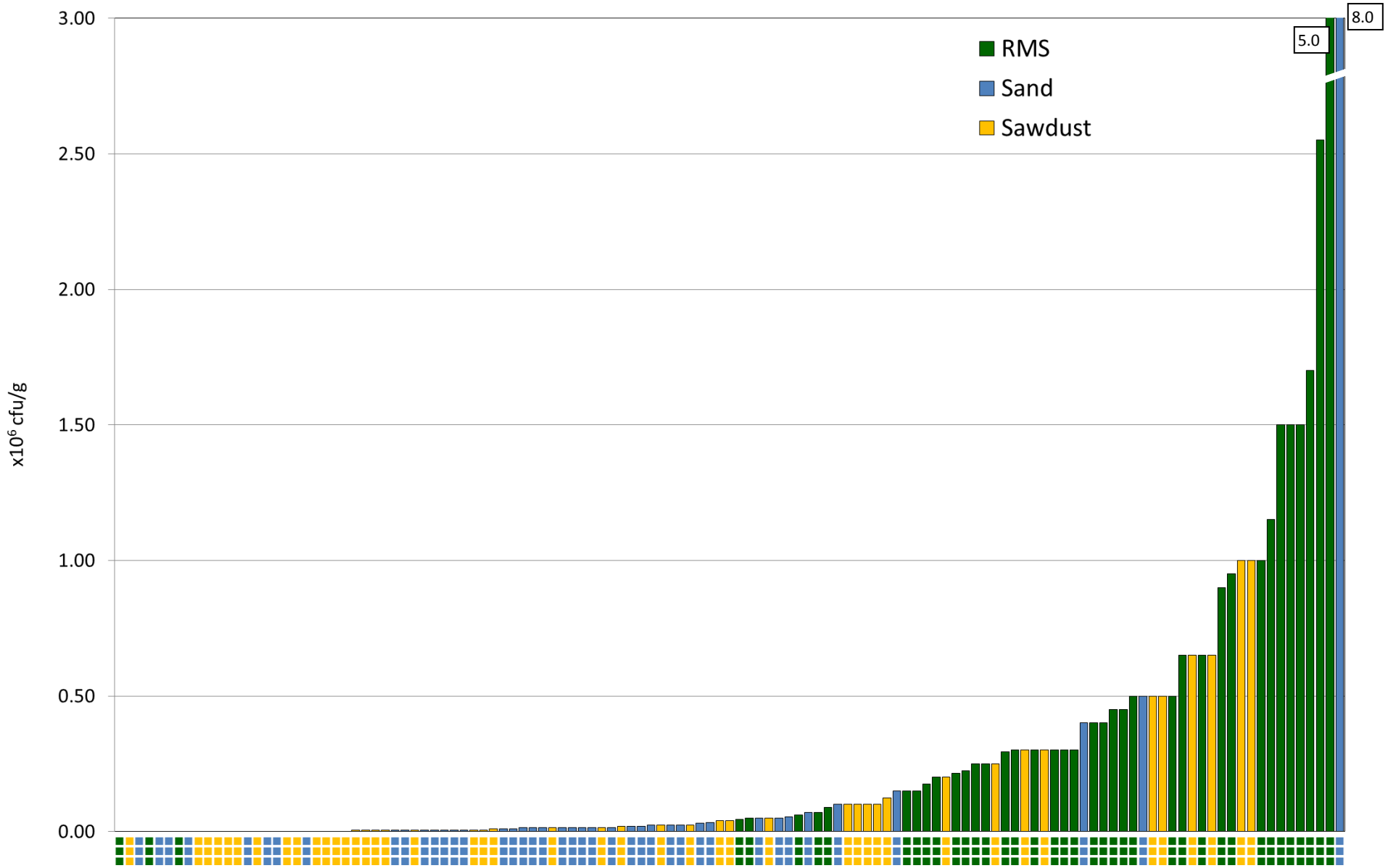


Figure 2.5: An illustration of the laboratory pasteurised counts in used bedding across the survey farms.

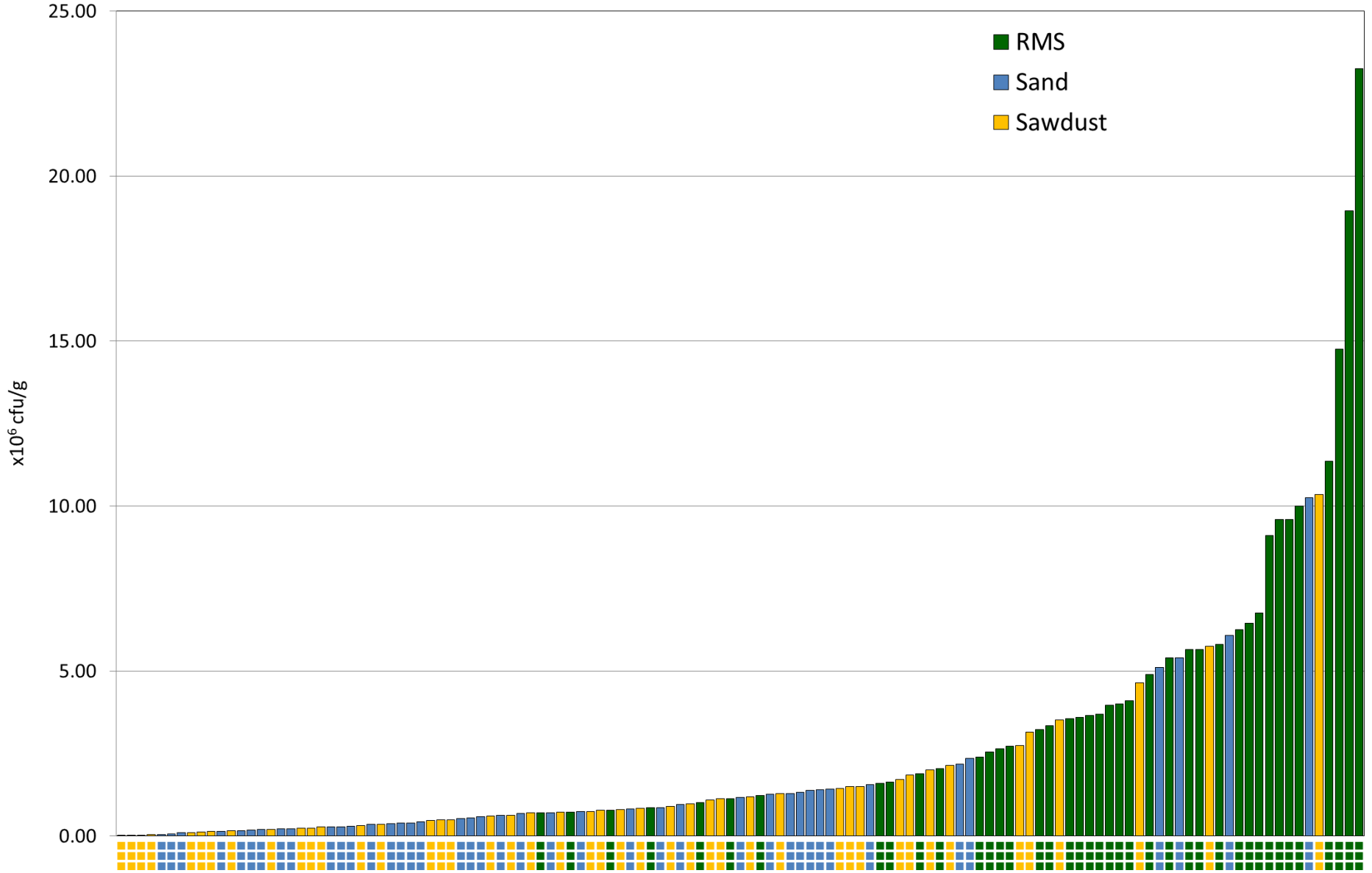


Figure 2.6: An illustration of the thermophilic counts in used bedding across the survey farms.

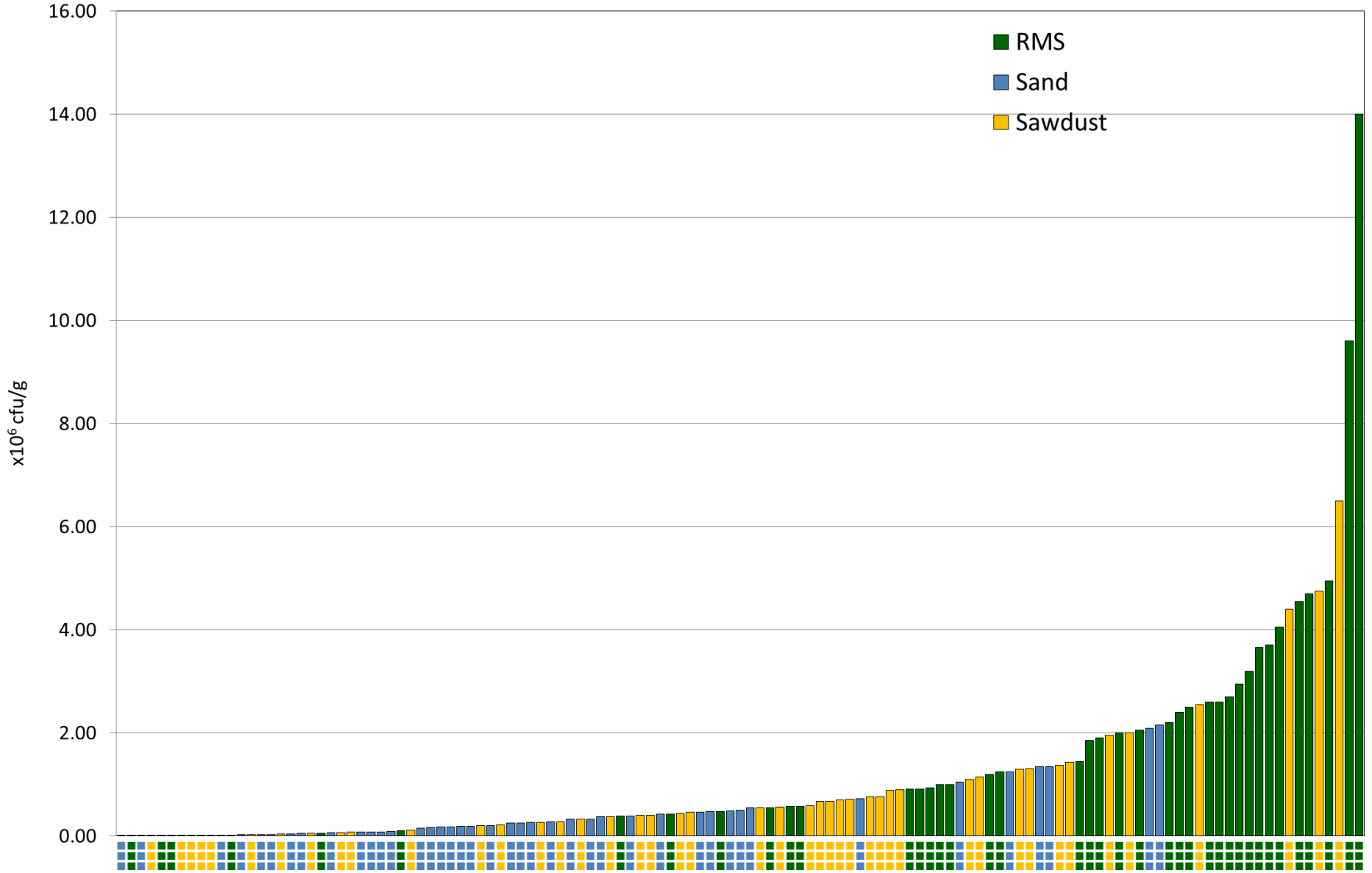


Figure 2.7: An illustration of the psychrotrophic counts in used bedding across the survey farms.

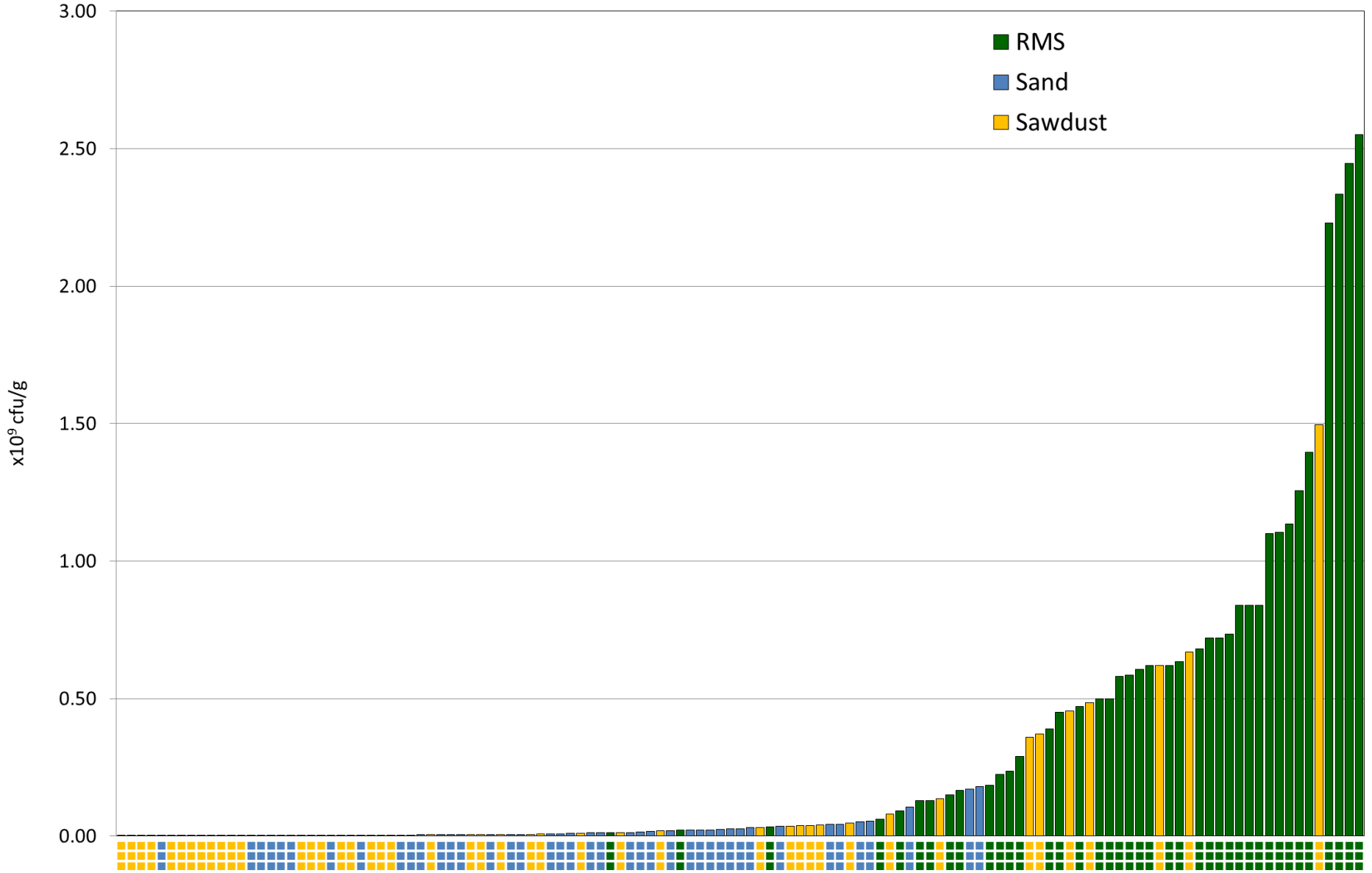


Figure 2.8: An illustration of the *Bacillus cereus* counts in used bedding across the survey farms.

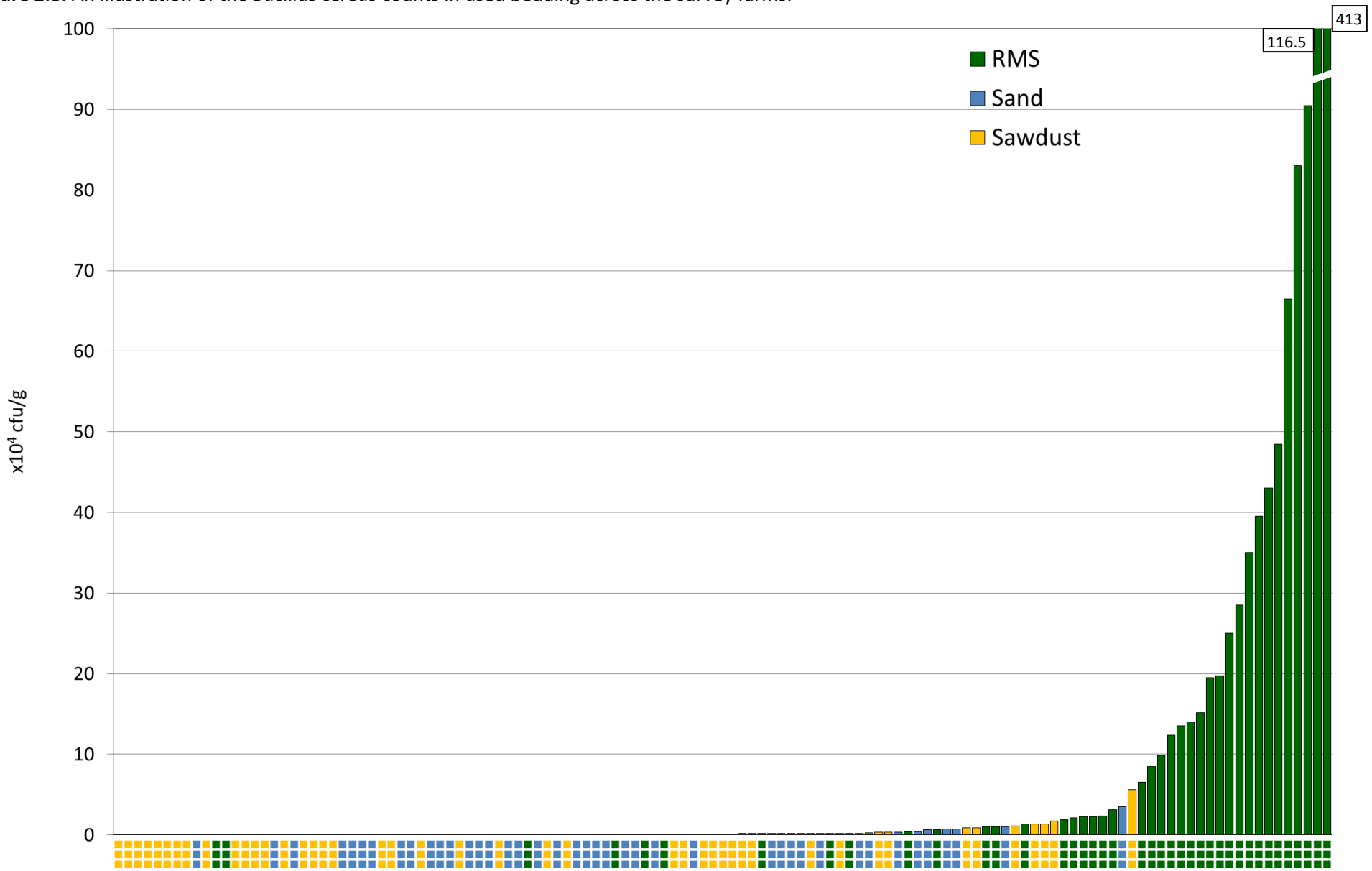
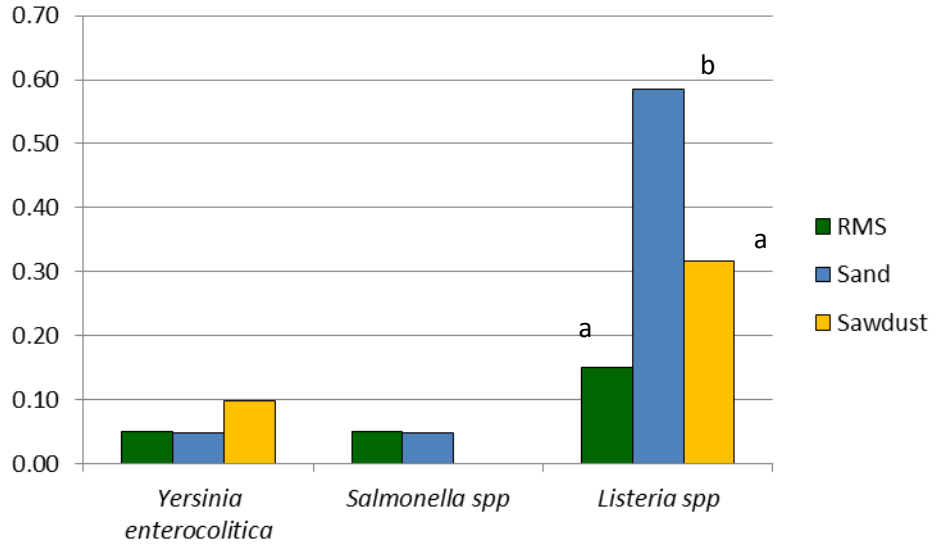


Table 2.15: A summary of the proportion of farms from which *Yersinia enterocolitic, Salmonella* spp, and *Listeria* spp respectively were isolated from bedding (rows with different superscripts differ p<0.05).

Organism	RMS (n=40)		Sand (n=41)		Sawdust (n=44)	
	n	%	n	%	n	%
Yersinia enterocolitica	2	5.0	2	4.9	4	9.8
***Salmonella* spp**	2	5.0	2	4.9	0	0.0
***Listeria* spp**	6	15.0[a]	24	58.5[b]	13	31.7[a]

Figure 2.9: An illustration of the proportion of farms from which *Yersinia enterocolitica*, *Salmonella* spp, and *Listeria* spp respectively were isolated from bedding (columns within organism with different superscripts differ p<0.05).



Across all the species and groups enumerated bacterial counts in bulk milk did not differ across the farms bedding on different materials. Somatic cell counts were not significantly different between farms bedded on the different materials, though there was a trend for SCCs to be lower on the sawdust farms compared to the RMS farms (134 *vs* 171 x10^3 cells/ml; p=0.06).

Bacillus cereus was only identified in milk on five farms, three RMS, one sand and one sawdust – in all cases there were <= 10cfu/ml of milk.

Yersinia enterocolitica was identified in the bulk milk on between 0% and 12.2% of farms, but the prevalence did not vary between bedding types.

A Salmonella spp was identified in the bulk milk of one sawdust farm and was subsequently identified as *S. montivideo* (APHA).

Listeria monocytogenes was isolated least frequently from bulk milk from sand farms and was isolated from between 2.4% and 12.5% of farms across the bedding groups. However, the prevalence in milk did not vary significantly between bedding types.

Table 2.16: A summary of bacterial counts (cfu/ml) somatic cell counts and milk constituents in bulk milk from survey farms.

Parameter	Bedding Type	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Total Bacterial Count	RMS	40	103,460	4,048	495	3,849,000	1,763	9,888
	Sand	41	16,746	4,700	375	371,000	1,665	7,650
	Sawdust	44	6,504	4,195	770	42,500	2,125	6,500
Coliform Count	RMS	40	3,164	13	1	119,000	6	36
	Sand	41	273	10	0	10,000	3	27
	Sawdust	44	44	11	0	435	3	31
***Streptococcus* spp Count**	RMS	40	9,666	440	25	327,000	203	1,213
	Sand	41	1,546	270	20	38,000	133	850
	Sawdust	44	1,113	598	95	10,500	183	1,150
***Staphylococcus* spp Count**	RMS	40	170	80	0	2,650	50	136
	Sand	41	92	40	0	540	20	150
	Sawdust	44	118	50	0	2,550	30	93
Laboratory Pasteurised Count	RMS	40	34,646	240	0	1,366,000	120	443
	Sand	41	452	195	25	2,965	68	533
	Sawdust	44	838	225	10	10,100	86	433
Thermophilic Spore Count	RMS	40	84	45	0	980	20	70
	Sand	41	84	30	0	945	5	58
	Sawdust	44	149	50	0	2,200	21	123
Psychrotrophic Count	RMS	39	751	140	3	7,750	90	515
	Sand	41	765	130	0	21,000	58	318
	Sawdust	44	1,054	118	5	35,000	61	298
***Bacillus cereus* Count**	RMS	40	0.38	0	0	5	0	0
	Sand	41	0.24	0	0	10	0	0
	Sawdust	44	0.23	0	0	10	0	0
Fat (%)	RMS	40	4.00	3.98	3.31	4.60	3.87	4.12
	Sand	41	3.98	4.00	2.55	6.08	3.75	4.11
	Sawdust	44	4.08	4.04	3.44	5.77	3.88	4.18
Protein (%)	RMS	40	3.35	3.35	3.07	3.60	3.27	3.43
	Sand	41	3.36	3.33	3.13	3.88	3.28	3.46
	Sawdust	44	3.35	3.35	3.12	4.00	3.26	3.39
Lactose (%)	RMS	40	4.81	4.81	4.60	4.94	4.78	4.86
	Sand	41	4.80	4.82	4.58	4.92	4.76	4.85
	Sawdust	44	4.80	4.81	4.65	4.96	4.75	4.83
Total Solids (%)	RMS	40	12.92	12.89	12.17	13.71	12.74	13.10
	Sand	41	12.87	12.86	11.54	14.86	12.55	13.05
	Sawdust	44	12.97	12.94	12.40	15.41	12.69	13.10
SCC (x10^3 cells/ml)	RMS	40	187	171	42	629	125	221
	Sand	41	147	145	26	298	107	183
	Sawdust	44	144	134	68	325	106	171

[a,b] Values with different superscripts within columns, within parameters, differ (p<= 0.05).

Figure 2.10: An illustration of the total bacterial counts in bulk milk across the survey farms.

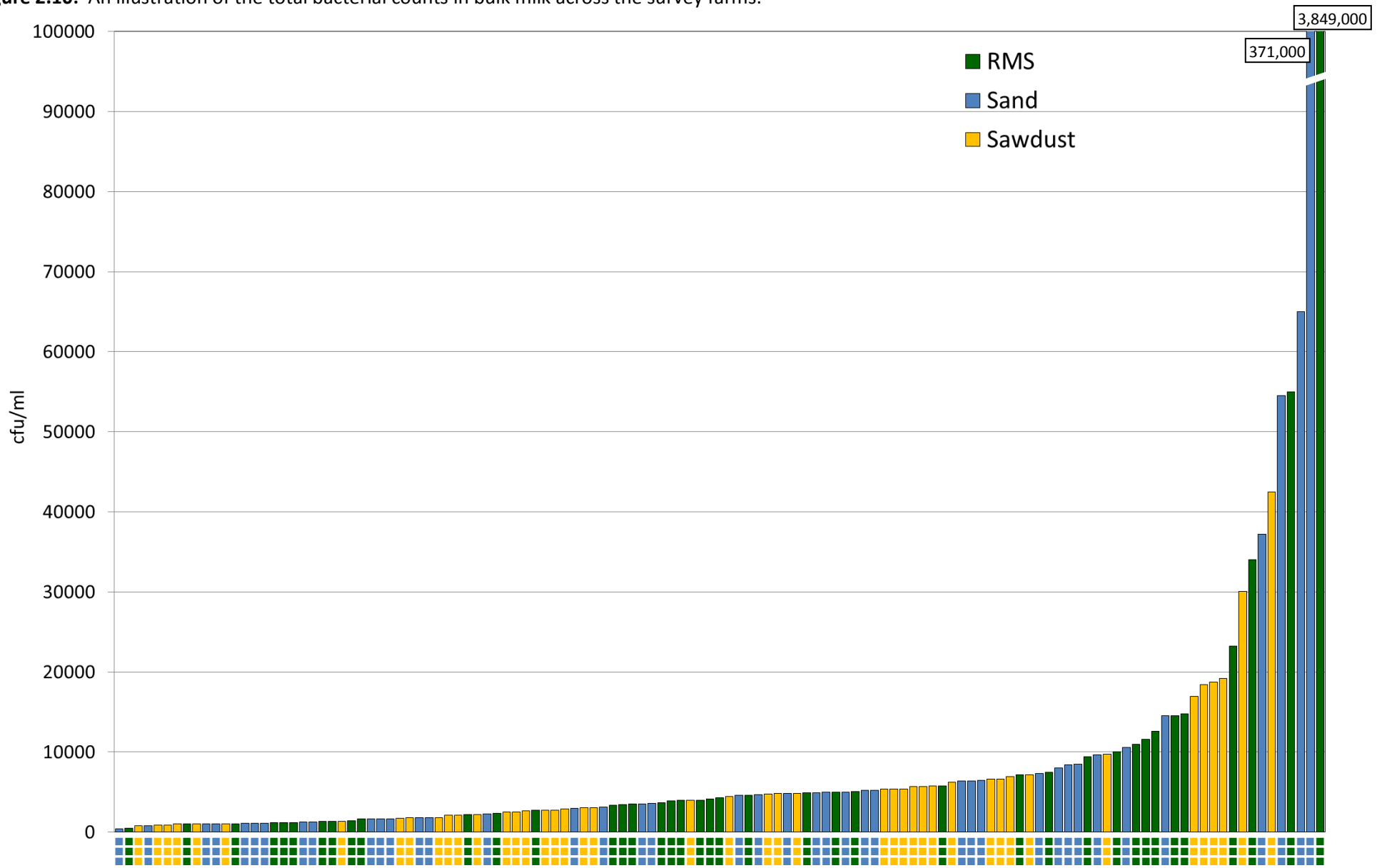


Figure 2.11: An illustration of the coliform counts in bulk milk across the survey farms.

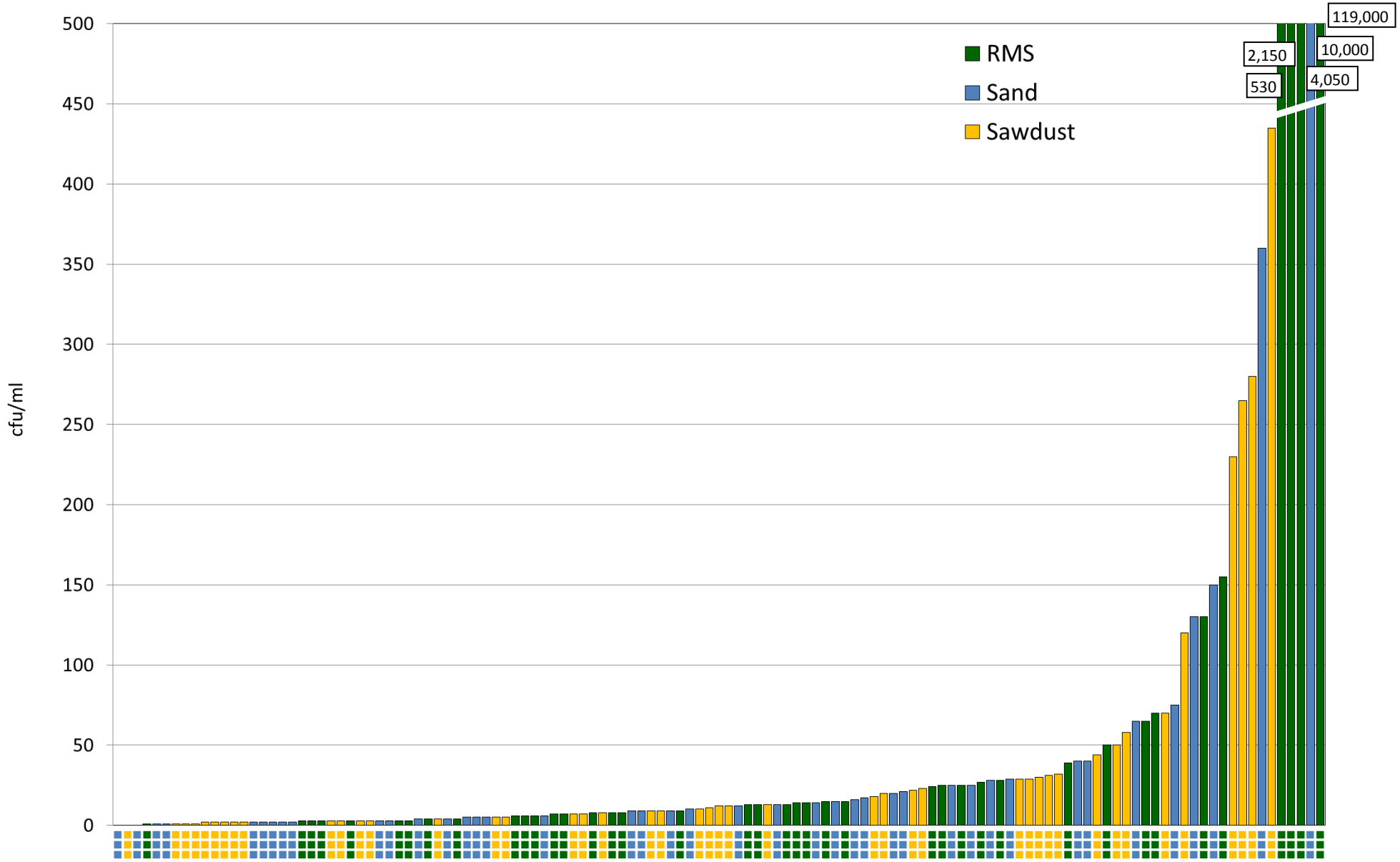


Figure 2.12: An illustration of the *Streptococcus* spp counts in bulk milk across the survey farms.

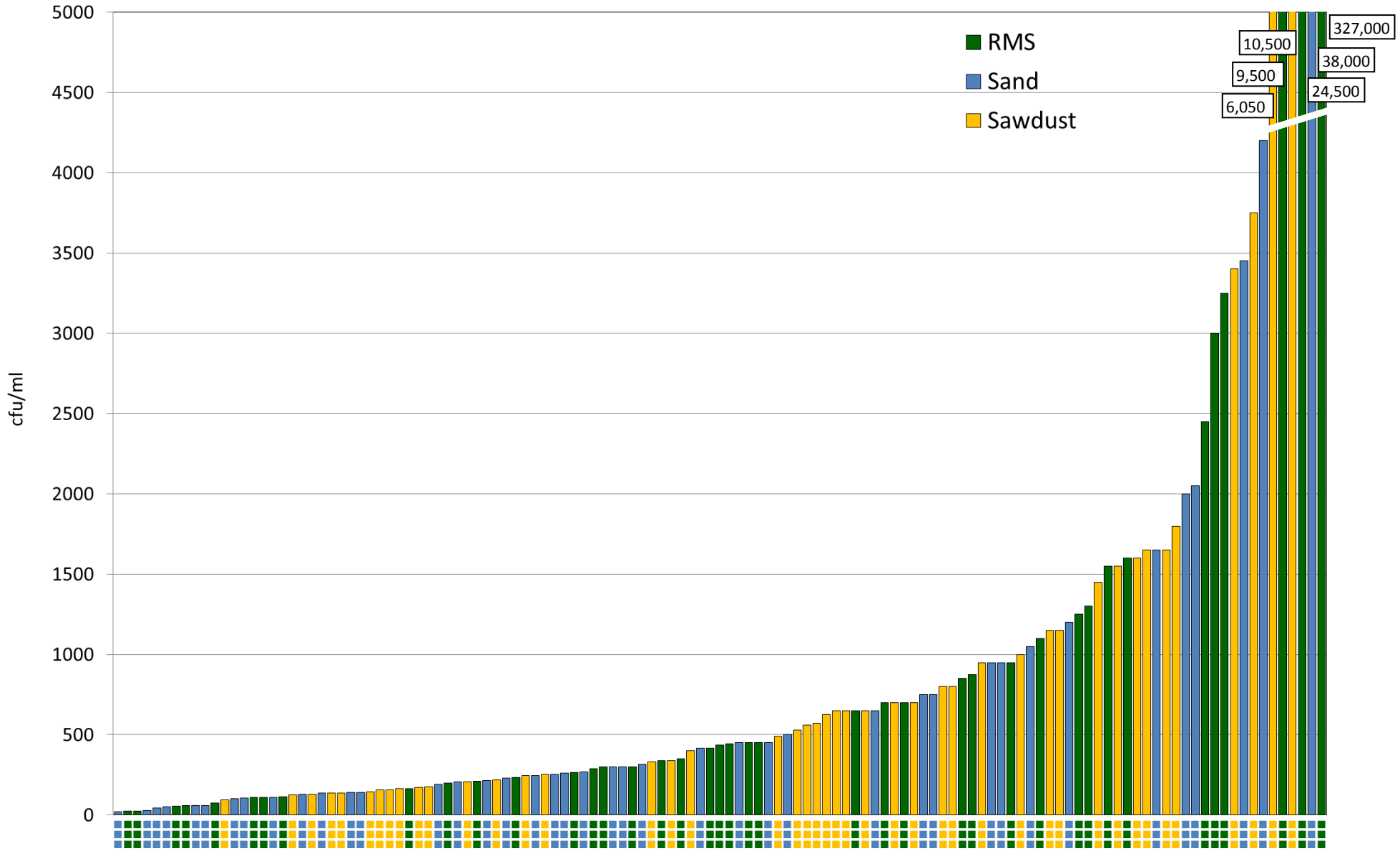


Figure 2.13: An illustration of the *Staphylococcus* spp counts in bulk milk across the survey farms.

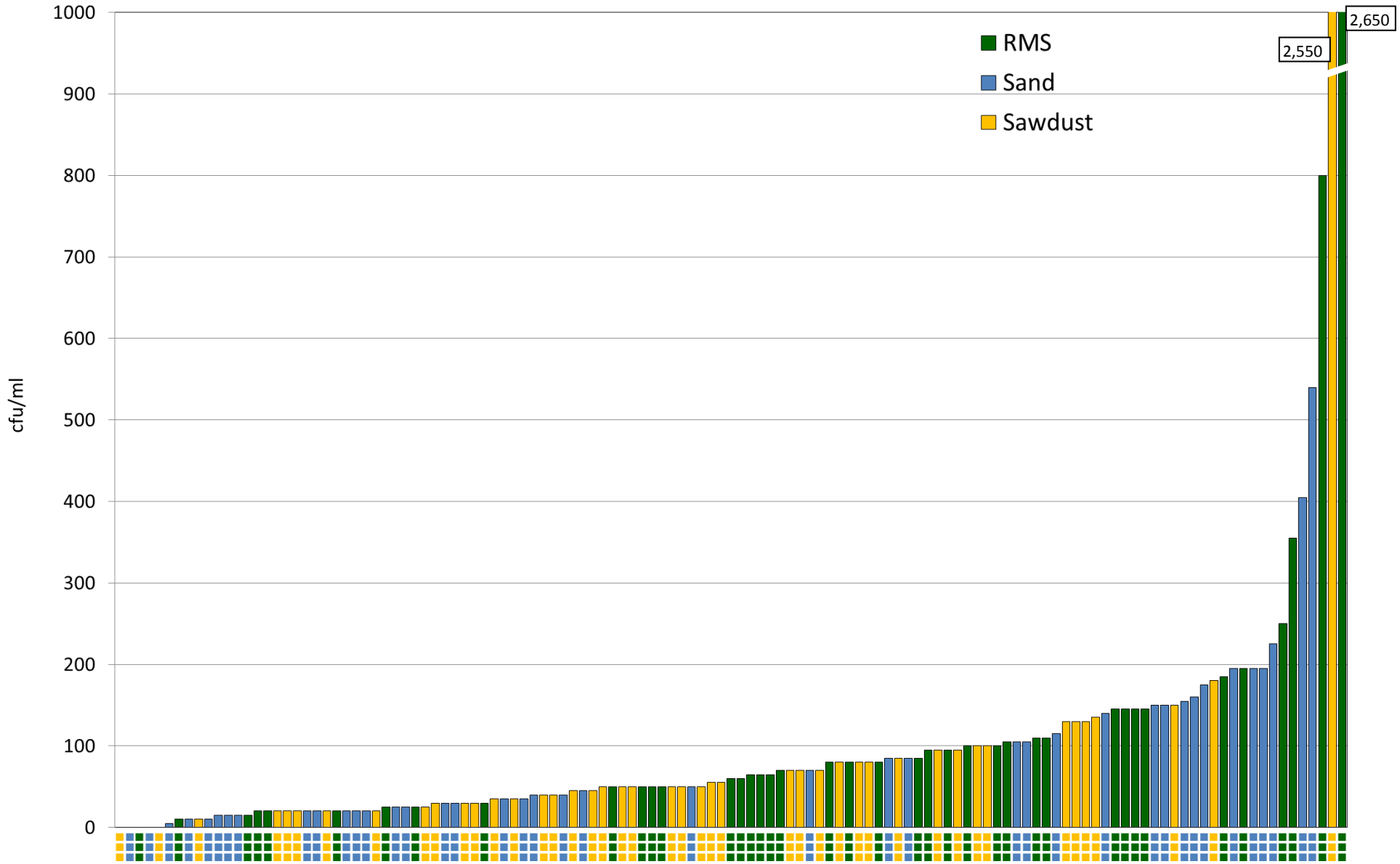


Figure 2.14: An illustration of the laboratory pasteurised counts in bulk milk across the survey farms.

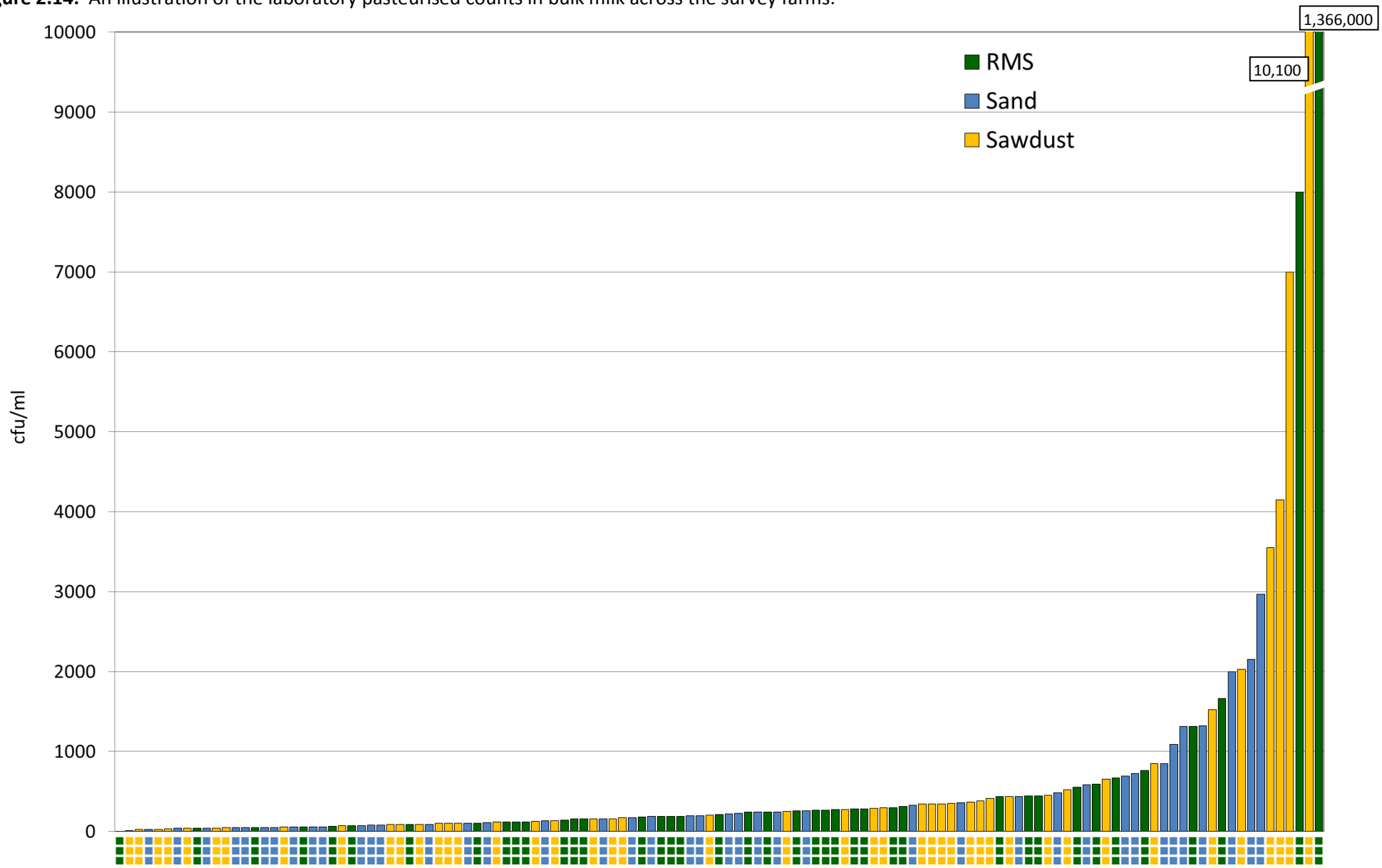


Figure 2.15: An illustration of the thermophilic spore counts in bulk milk across the survey farms.

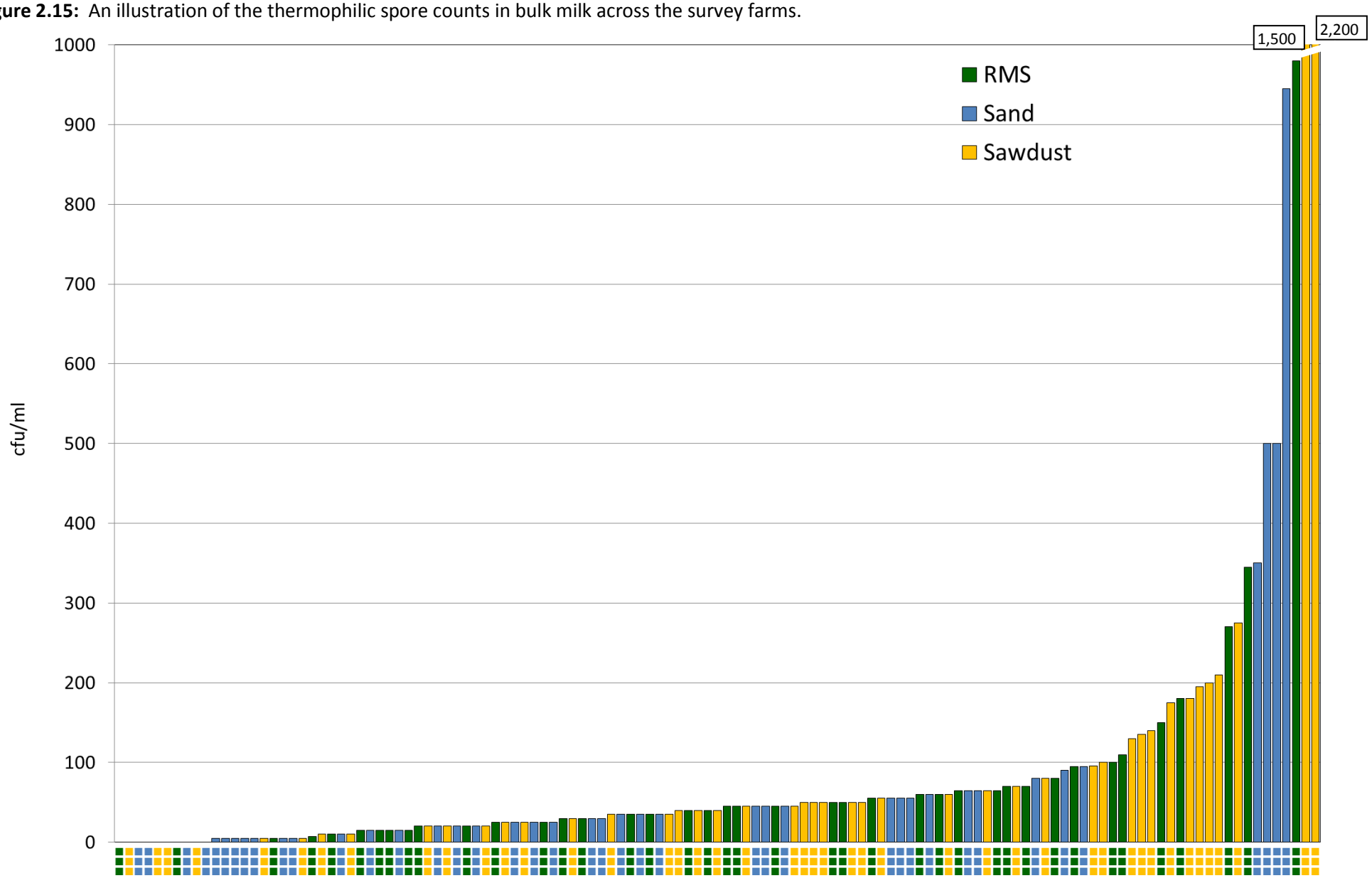


Figure 2.16: An illustration of the psychrotrophic counts in bulk milk across the survey farms.

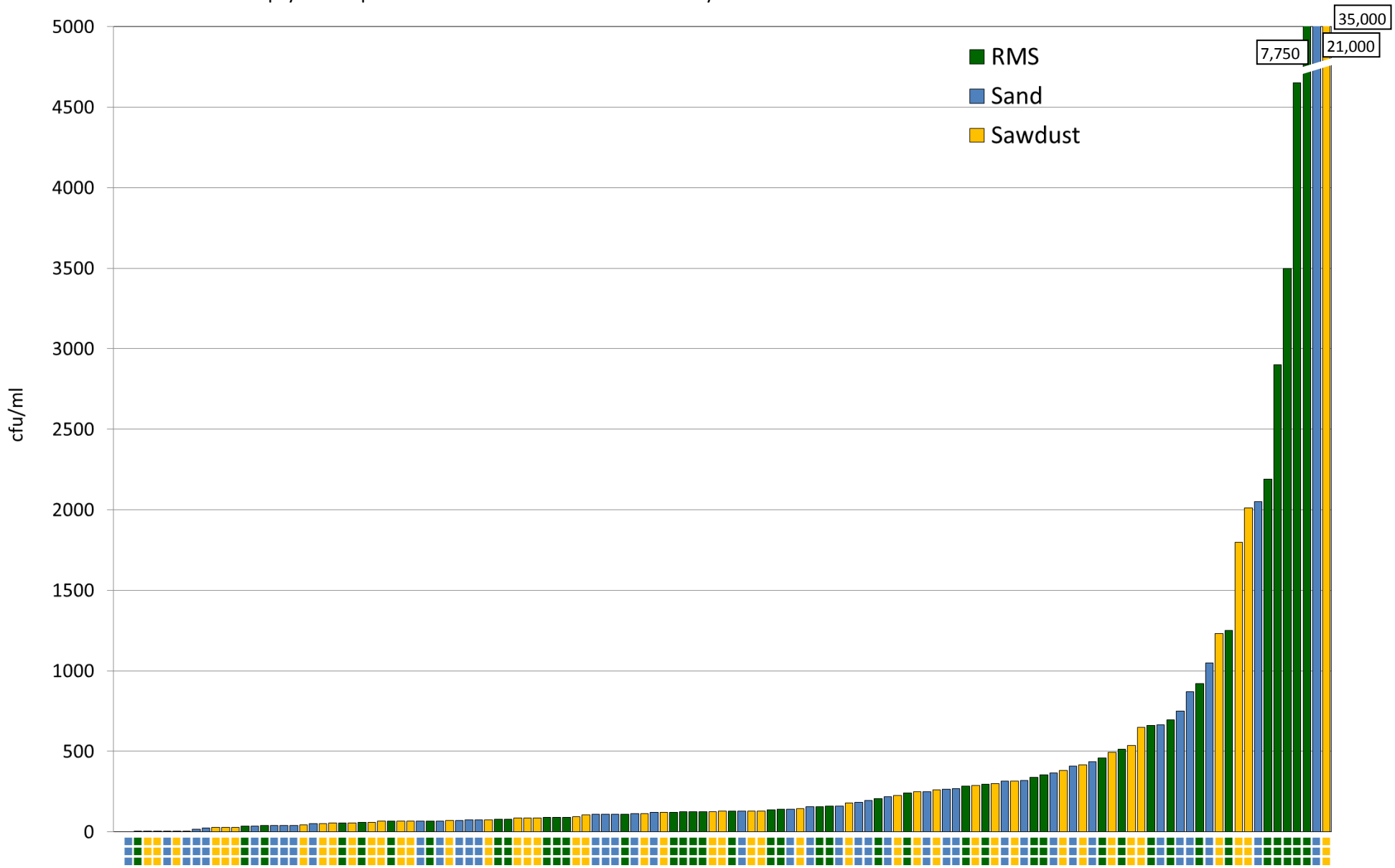


Figure 2.17: An illustration of the *Bacillus cereus* counts in bulk milk across the survey farms.

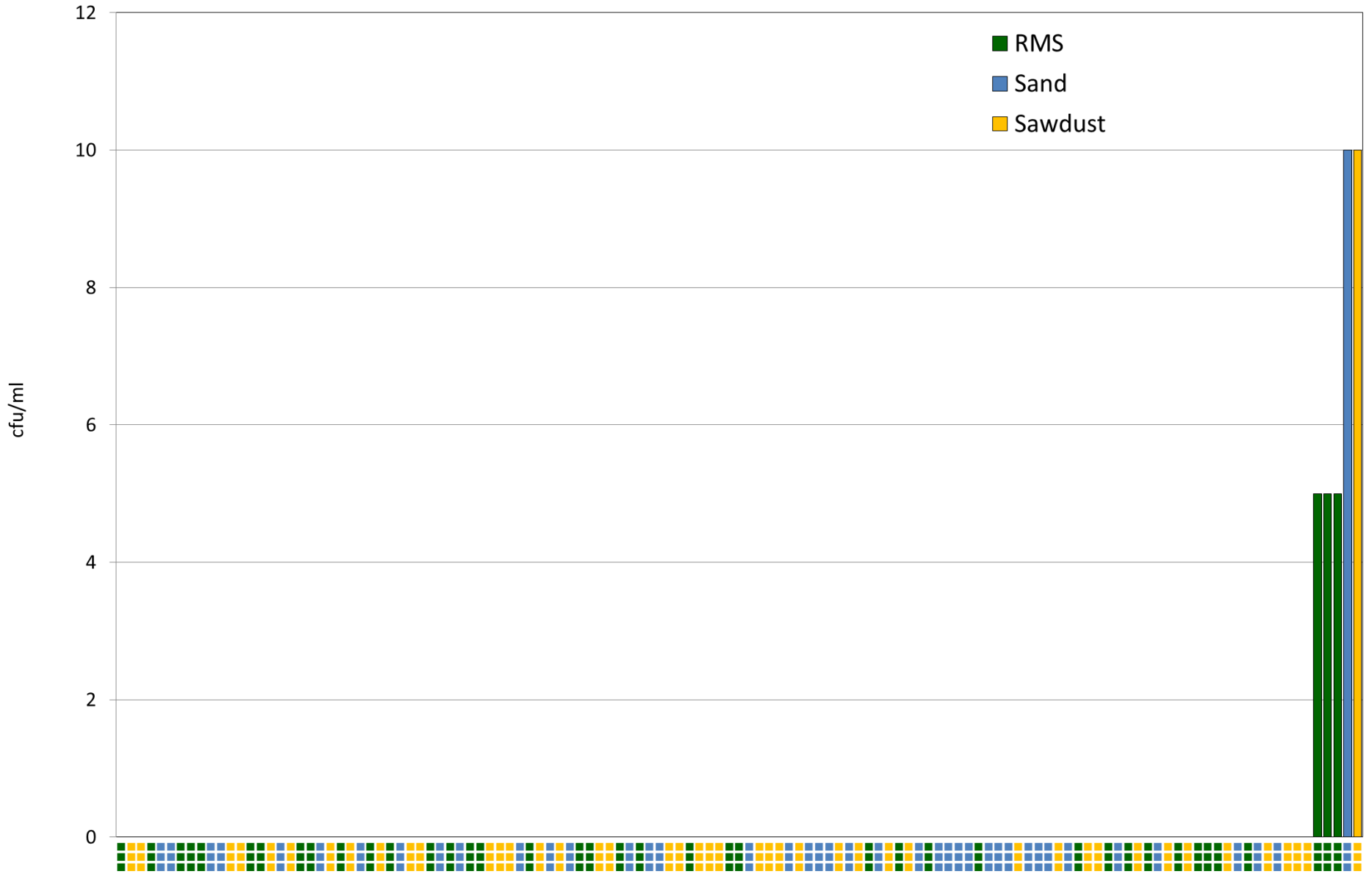


Figure 2.18: An illustration of somatic cell counts in bulk milk across the survey farms.

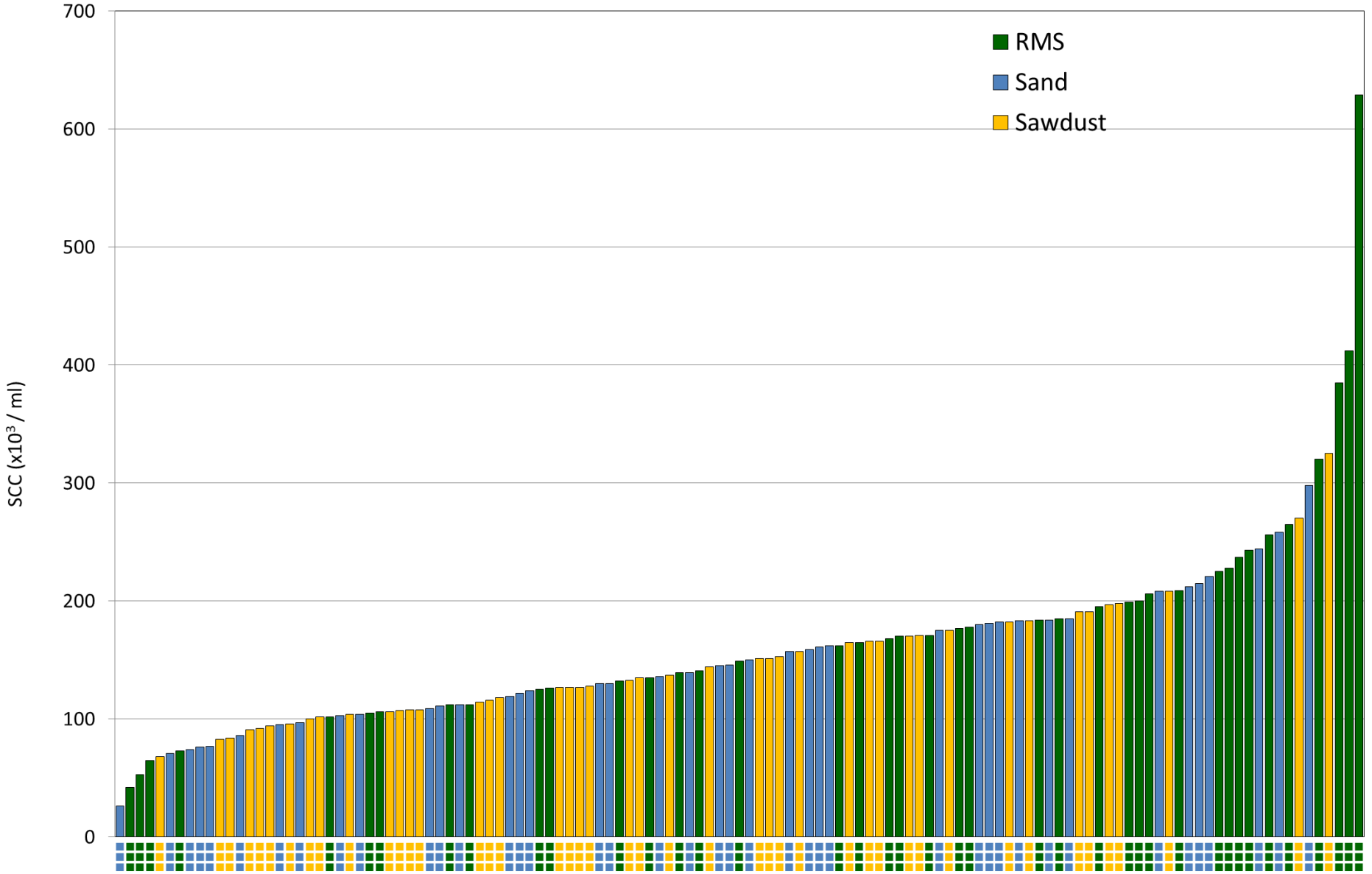
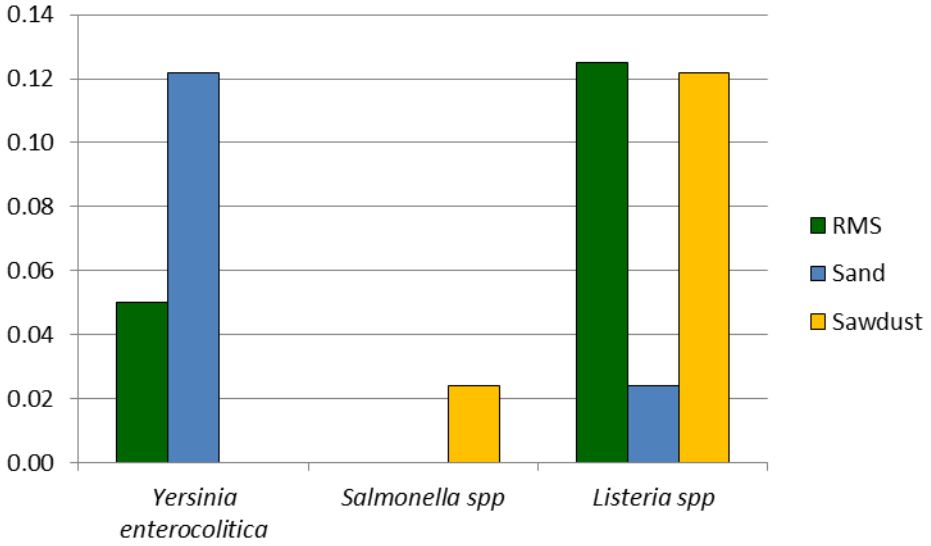


Table 2.17: A summary of the proportion of farms from which *Yersinia enterocolitica*, *Salmonella* spp, and *Listeria* spp respectively were isolated from bulk milk.

Organism	RMS (n=40)		Sand (n=41)		Sawdust (n=44)	
	n	%	n	%	n	%
Yersinia enterocolitica	2	5.0	5	12.2	0	0.0
***Salmonella* spp**	0	0.0	0	0.0	1	2.4
***Listeria* spp**	5	12.5	1	2.4	5	12.2

Figure 2.19: An illustration of the proportion of farms from which *Yersinia enterocolitica*, *Salmonella* spp, and *Listeria* spp respectively were isolated from bulk milk.



Data was explored in an attempt to identify any correlations between the number of bacteria in bedding and in bulk milk. No relationships were identified between bacterial numbers in bedding and in bulk milk when farms using all types of bedding were considered together.

In addition, the impact of milking practices on bacterial counts in bulk milk was investigated, across all bedding types.

Across all bedding types, fore-milking was associated with a lower TBC in bulk milk (2,503 *vs* 4,800 cfu/ml; p=0.047), but not with any other bacterial species/grouping.

Pre-milking teat preparation that involved a pre-dip followed by wiping dry was associated with a lower *Streptococcus* spp count in bulk milk (340 *vs* 650 cfu/ml; p=0.023), but not with difference in any other bacterial species/grouping.

Cluster disinfection was not found to be associated with lower bacterial counts in milk, with the exception of thermophilic spore counts and psychrotrophic counts. Thermophilic spore counts were significantly lower in the bulk milk of farms employing any cluster disinfection than those not employing cluster disinfection (35 *vs* 62.5 cfu/ml; p=0.01). Similarly psychrotrophic counts were significantly lower in the bulk milk of farms employing any cluster disinfection than those not employing cluster

disinfection (125 *vs* 245 cfu/ml; p=0.04). No difference was detected between manual and automated systems.

There was a trend for a hot wash after every milking to be associated with a reduction in the laboratory pasteurised count in milk (272.5 *vs* 190 cfu/ml; p=0.144), but not with any other bacterial species/grouping.

2.3.4 Bacteriology of Bedding and Milk - RMS Farms

The findings of the bacteriological analysis of bedding and milk samples for RMS herds that bedded all cows on either deep (n=11) or shallow (n=20) beds is summarised in Tables 2.18 and 2.19 and in Figures 2.19 to 2.26 and 2.27 to 2.38 respectively.

Both total bacterial counts (9.65 $x10^9$ *vs* 4.51 $x10^9$ cfu/g; $p<0.05$) and *Streptococcus* spp counts (2.85 $x10^8$ *vs* 4.4 $x10^7$ cfu/g; $p<0.05$) were significantly higher in shallow rather than deep RMS beds. Coliform counts, *Staphylococcus* spp, laboratory pasteurised counts and thermophilic spore counts were not significantly different between shallow and deep beds. Psychrotrophic counts were significantly higher in shallow rather than deep RMS beds (7.27 $x10^8$ *vs* 2.35 $x10^8$ cfu/g; $p<0.05$). In contrast, *Bacillus cereus* counts were higher in deep than shallow RMS beds (2.5 $x10^5$ *vs* 8.3 $x10^3$ cfu/g; $p<0.05$). Dry matter of the used bedding did not differ between the two groups.

There were no significant differences in any of the bulk milk bacterial counts or milk constituents between farms with deep and shallow beds.

Table 2.18: A summary of bacterial counts in bedding from farms using RMS as bedding in deep or shallow beds (all bacterial counts are cfu/g wet weight).

Parameter	Bedding Type	n	Mean	Median	Minimum	Maximum	25th Percentile	75th Percentile
Total Bacterial Count	Deep RMS	11	4,638,181,818	4,510,000,000[a]	805,000,000	8,950,000,000	3,590,000,000	5,950,000,000
	Shallow RMS	20	10,739,000,000	9,650,000,000[b]	3,650,000,000	21,050,000,000	6,725,000,000	13,663,000,000
Coliform Count	Deep RMS	11	2,859,091	1,900,000	15,000	16,000,000	320,000	3,700,000
	Shallow RMS	20	3,223,700	1,070,000	9000	37,500,000	166,250	2,762,500
***Streptococcus* spp Count**	Deep RMS	11	68,272,727	44,000,000[a]	6,500,000	175,000,000	11,500,000	140,000,000
	Shallow RMS	20	436,725,000	285,000,000[b]	25,500,000	1,650,000,000	113750000	523,750,000
***Staphylococcus* spp Count**	Deep RMS	11	467,727	400,000	200,000	1,150,000	300,000	500,000
	Shallow RMS	20	790,000	275,000	0	5,000,000	150,000	987,500
Laboratory Pasteurised Count	Deep RMS	11	7,610,909	4,100,000	700,000	23,250,000	2,400,000	10,000,000
	Shallow RMS	20	3,797,750	2,892,500	720,000	14,750,000	1,158,750	5,575,000
Thermophilic Spore Count	Deep RMS	11	2,500,318	2,200,000	58,500	9,600,000	910,000	3,200,000
	Shallow RMS	20	2,292,173	1,647,500	6,950	14,000,000	572,500	2,600,000
Psychrotrophic Count	Deep RMS	11	329,863,636	235,000,000[a]	21,000,000	1,105,000,000	61,500,000	500,000,000
	Shallow RMS	20	1,007,475,000	727,500,000[b]	129,500,000	2,550,000,000	581,250,000	1,225,000,000
***Bacillus cereus* Count**	Deep RMS	11	700,809	250,000[a]	9,900	4,130,000	135,000	830,000
	Shallow RMS	20	54,085	8,300[b]	130	430,000	1188	56,875
Unused bedding Dry Matter (%)	Deep RMS	11	33.6	32.6	30.6	38.2	31.2	35.8
	Shallow RMS	20	32.8	32.6	26.6	37.5	29.5	35.4
Used Bedding Dry Matter (%)	Deep RMS	11	44.6	41.5	33.7	69.6	38.2	48.0
	Shallow RMS	20	44.3	43.2	34.6	58.7	38.5	48.9

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

Figure 2.19: An illustration of the total bacterial counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

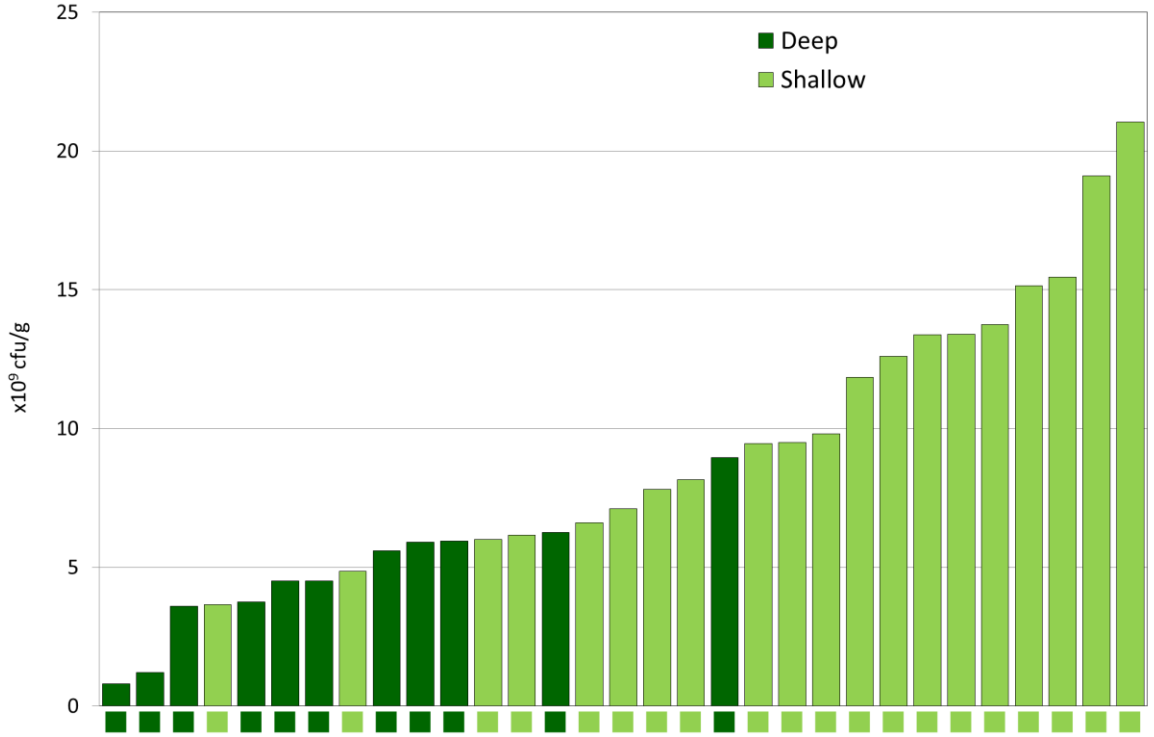


Figure 2.20: An illustration of the coliform counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

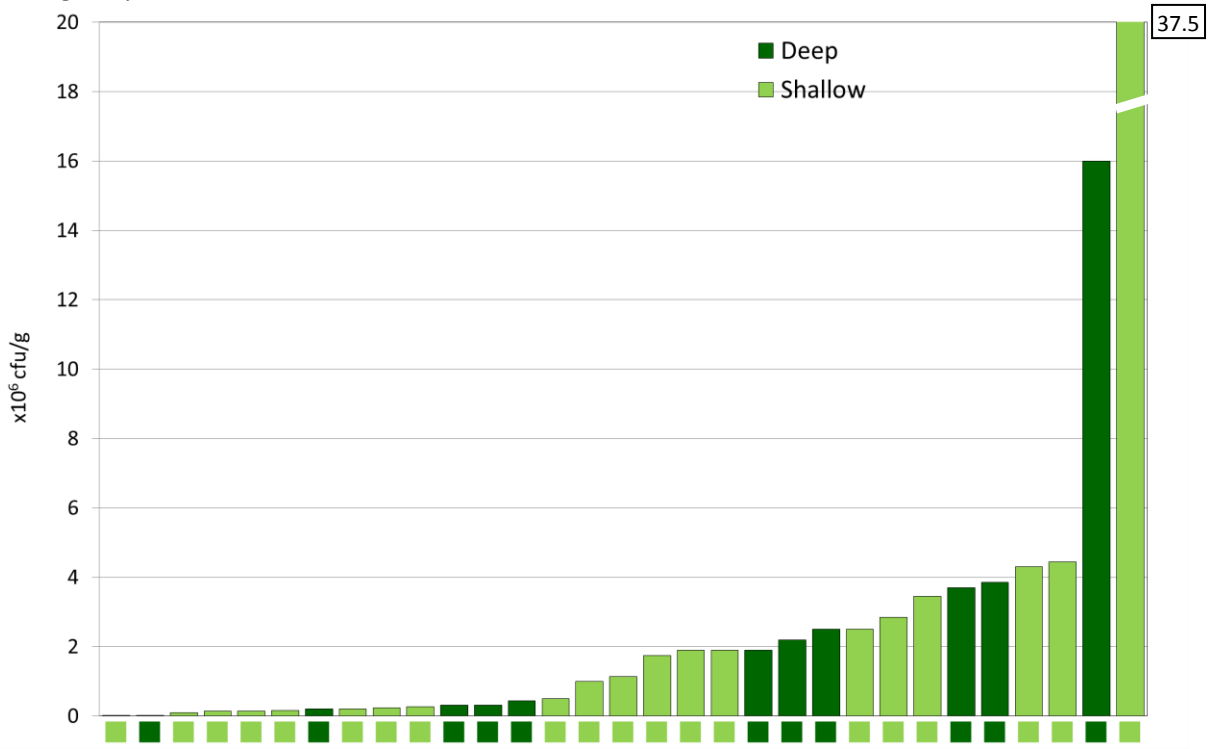


Figure 2.21: An illustration of the *Streptococcus* spp counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

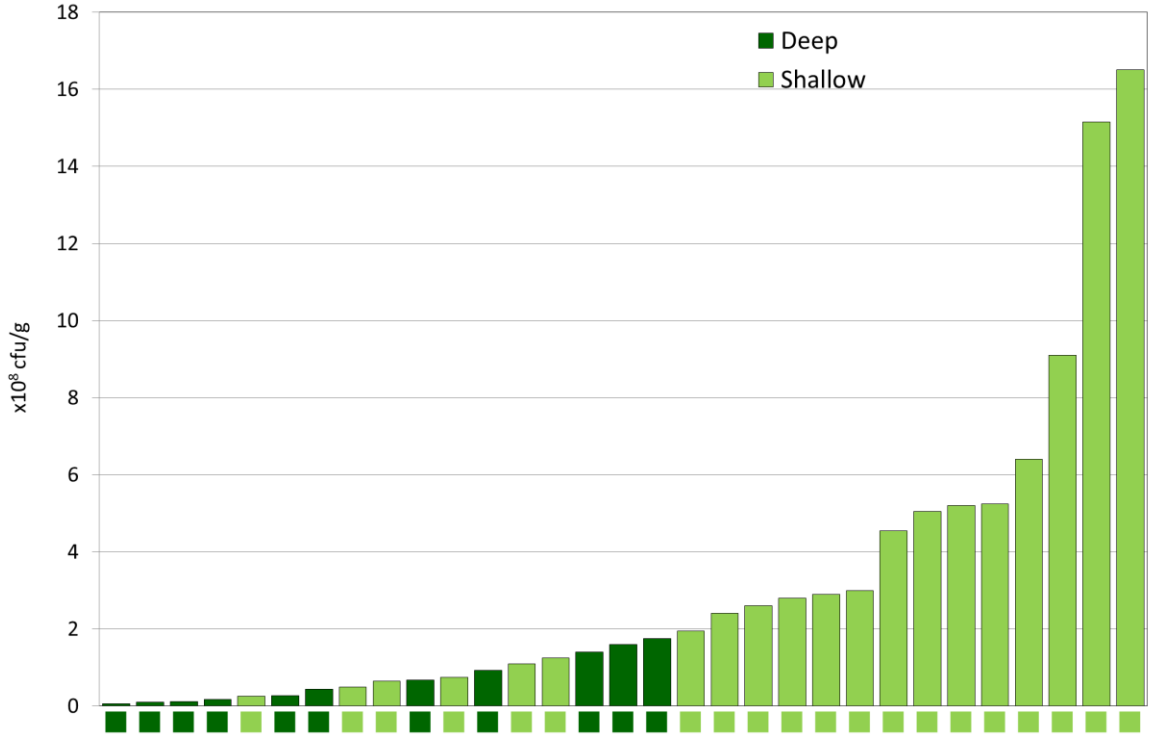


Figure 2.22: An illustration of the *Staphylococcus* spp counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

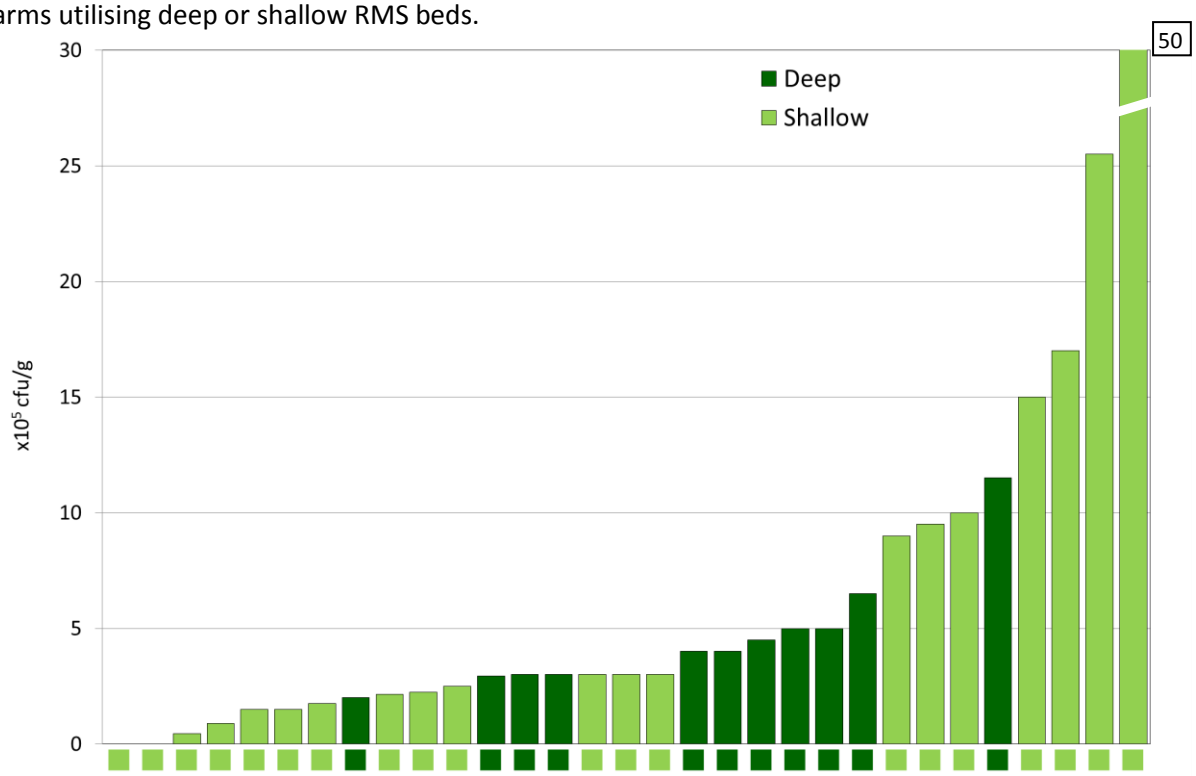


Figure 2.23: An illustration of the laboratory pasteurised counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

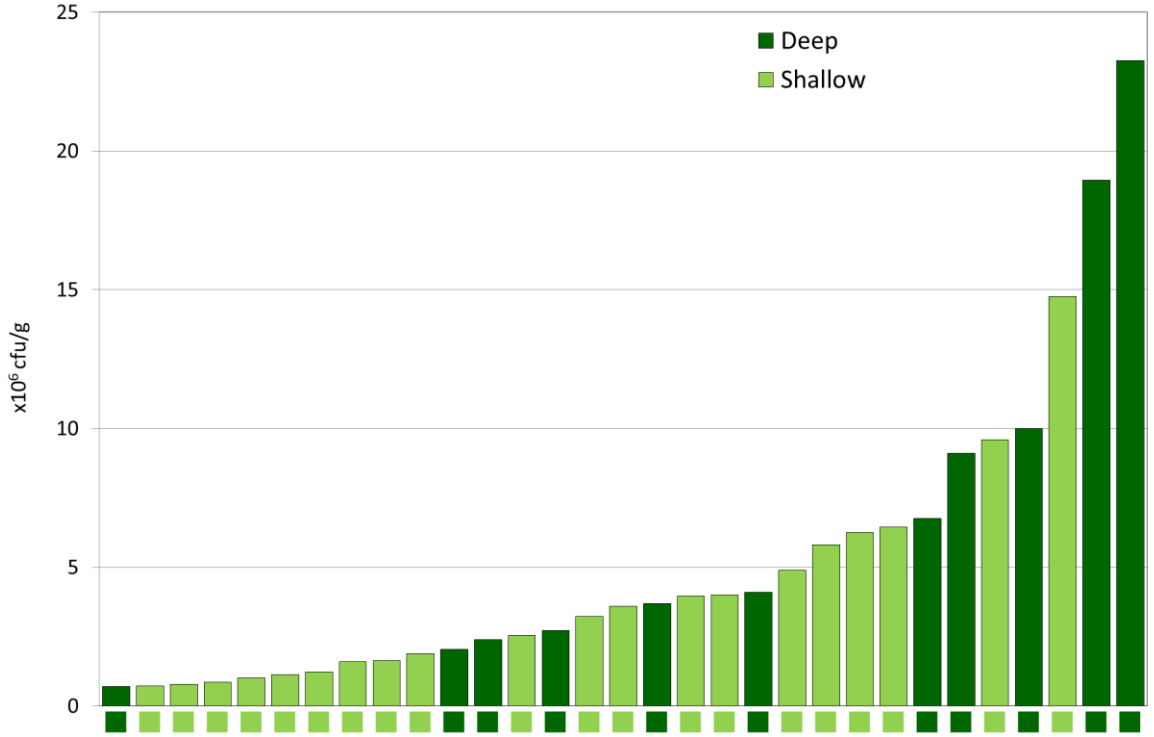


Figure 2.24: An illustration of the thermophilic spore counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

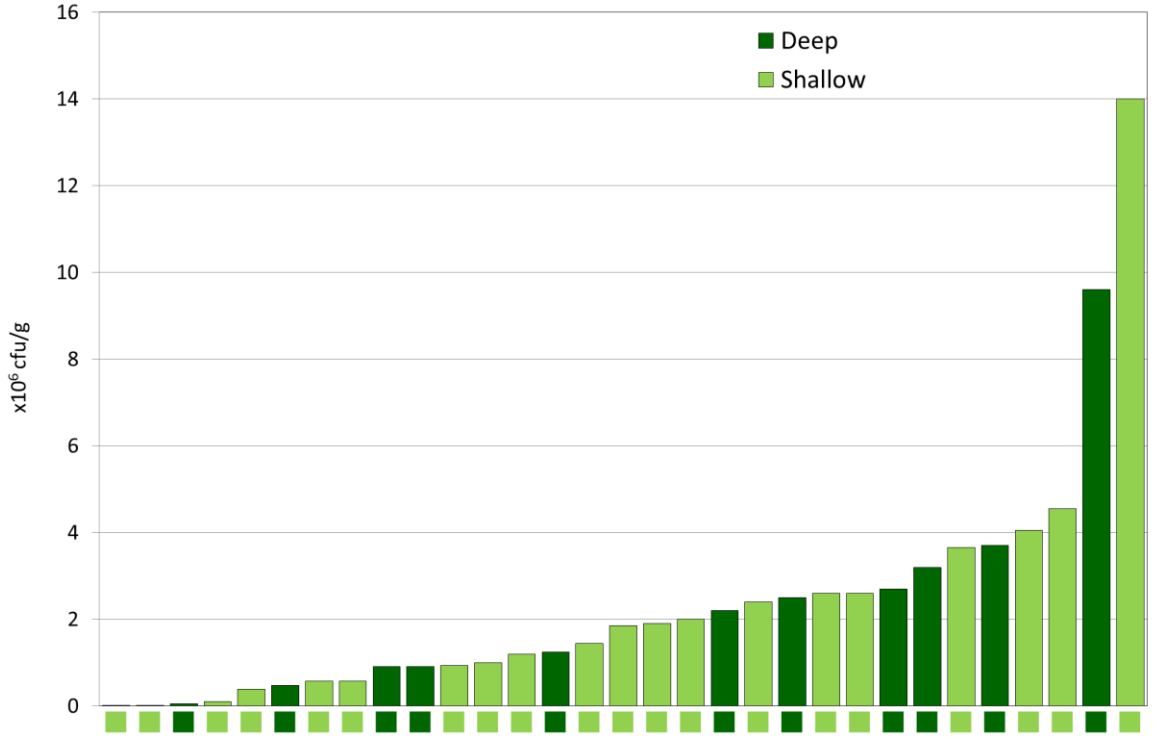


Figure 2.25: An illustration of the psychrotrophic counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.

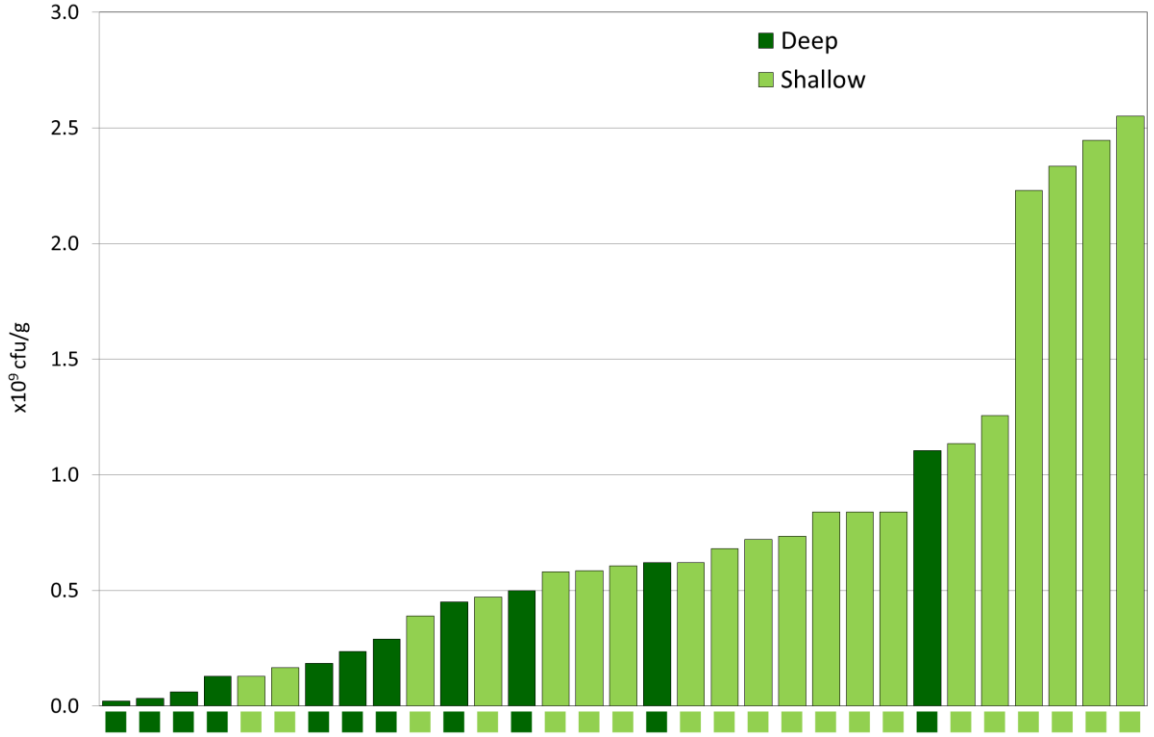
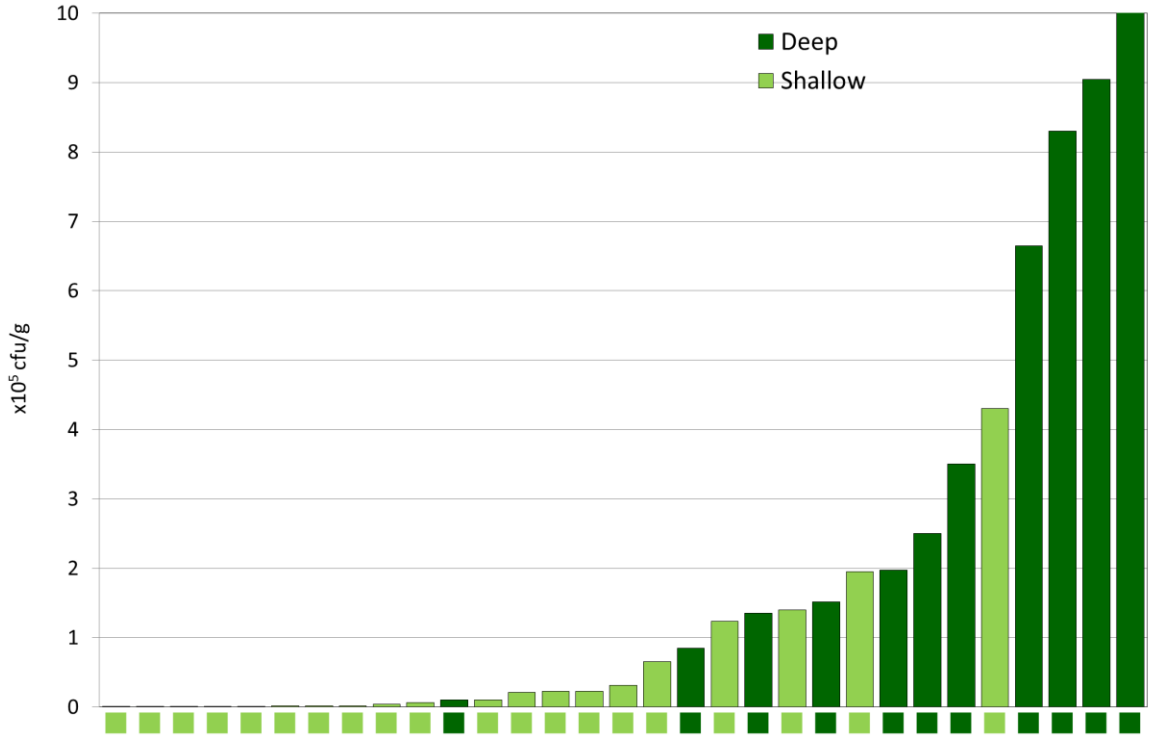


Figure 2.26: An illustration of the *Bacillus cereus* counts (cfu/g wet weight) in used bedding across farms utilising deep or shallow RMS beds.



Data was explored, and specific hypotheses tested in an attempt to understand any relationships between bed and bedding management of RMS farms and the number of bacteria in used bedding.

No significant relationship could be identified between the frequency of bedding and bacterial counts in bedding, though there was a trend for *Streptococcus* spp counts to be lower in beds to which fresh material was applied daily (1.08×10^8 *vs* 2.80×10^8 cfu/g; p=0.057).

There was no effect of whether the bedding was separated under cover on dry matter of the fresh bedding on the day of production, the dry matter of used material or bacterial counts in used bedding material.

When the impact of conditioner on bacterial counts in used bedding was investigated across all RMS farms, no relationships could be identified. Farmers using RMS in shallow beds were significantly more likely to employ a conditioner than those who were not (7/13 *vs* 1/10; p=0.003). However, when the impact of conditioner on bacterial counts in used bedding was restricted to RMS farms with shallow beds it was still not possible to identify any relationships. Given that conditioner was commonly used with sawdust, which is also an organic bedding material, the impact of conditioner on sawdust beds was also investigated; however, no significant effect on bacterial numbers in used bedding could be identified.

Table 2.19: A summary of bacterial counts (cfu/ml) somatic cell counts and milk constituents in bulk milk from farms utilising deep or shallow RMS beds.

Parameter	Bedding Type	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Total Bacterial Count	Deep RMS	11	4,056	3,950	1000	12,600	1,365	4,630
	Shallow RMS	20	8,015	4,298	495	55,000	2,209	9,888
Coliform Count	Deep RMS	11	68	13	3	530	3	24
	Shallow RMS	20	332	13	3	4050	6	36
***Streptococcus* spp Count**	Deep RMS	11	626	300	25	2,450	110	1,250
	Shallow RMS	20	1,104	450	25	9,500	216	931
***Staphylococcus* spp Count**	Deep RMS	11	159	80	10	800	50	195
	Shallow RMS	20	223	80	20	2,650	50	145
Laboratory Pasteurised Count	Deep RMS	11	213	120	0	670	50	300
	Shallow RMS	20	329.5	225	65	1,665	143.8	401.3
Thermophilic Spore Count	Deep RMS	11	122	35	7	980	20	65
	Shallow RMS	20	54	45	0	180	15	77.5
Psychrotrophic Count	Deep RMS	11	670	125	35	4,650	80	695
	Shallow RMS	19	901	240	60	7,750	125	460
***Bacillus cereus* Count**	Deep RMS	11	0.91	0	0	5	0	0
	Shallow RMS	20	0	0	0	0	0	0
Fat (%)	Deep RMS	11	4.02	3.98	3.86	4.42	3.97	4.05
	Shallow RMS	20	3.99	3.99	3.31	4.60	3.85	4.16
Protein (%)	Deep RMS	11	3.32	3.34	3.18	3.49	3.24	3.39
	Shallow RMS	20	3.36	3.36	3.07	3.53	3.28	3.44
Lactose (%)	Deep RMS	11	4.79	4.79	4.69	4.88	4.75	4.84
	Shallow RMS	20	4.83	4.83	4.74	4.94	4.79	4.87
Total Solids (%)	Deep RMS	11	12.87	12.86	12.63	13.36	12.77	12.92
	Shallow RMS	20	12.94	12.97	12.17	13.55	12.66	13.16
SCC (x10^3 cells/ml)	Deep RMS	11	226	171	65	629	132	243
	Shallow RMS	20	157	145	42	320	112	185

Figure 2.27: An illustration of the total bacterial counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

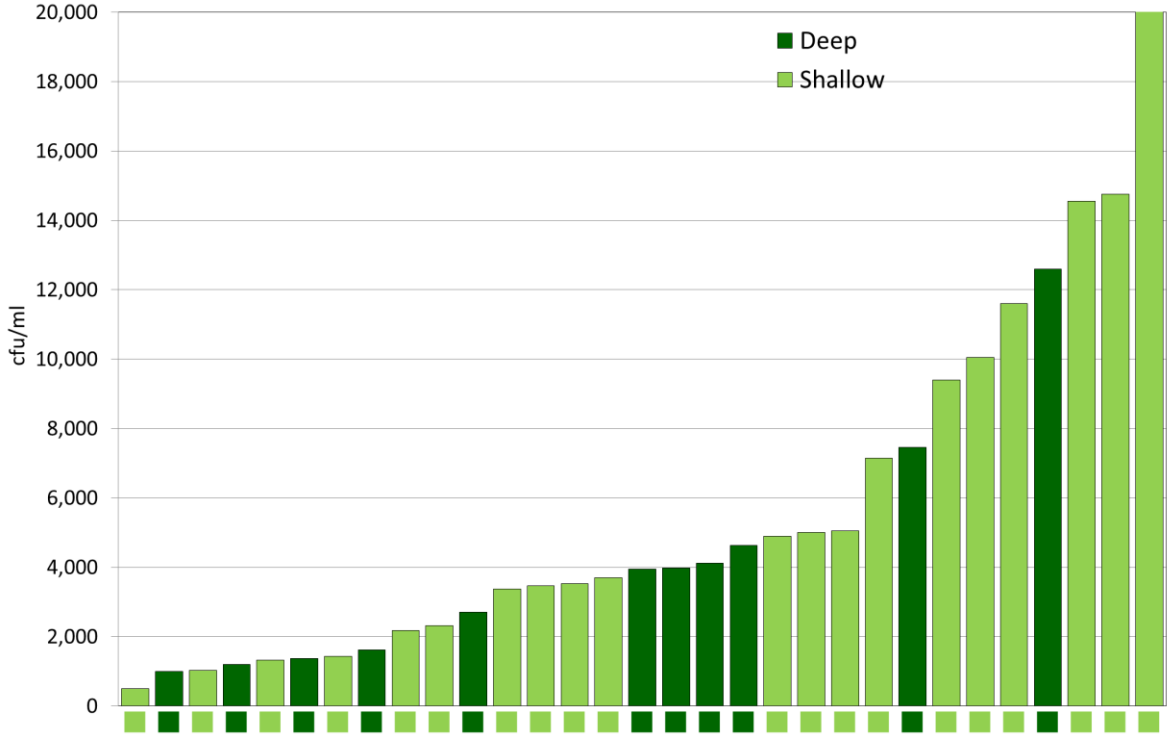


Figure 2.28: An illustration of the coliform counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

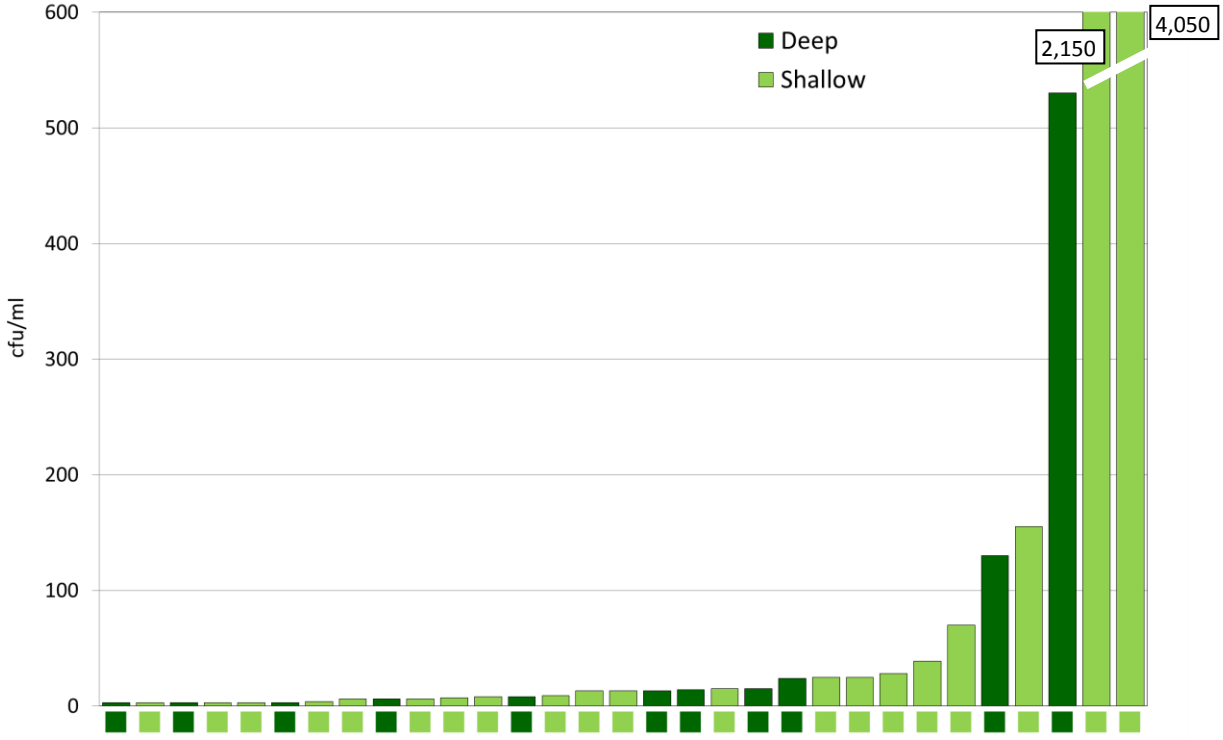


Figure 2.29: An illustration of the *Streptococcus* spp counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

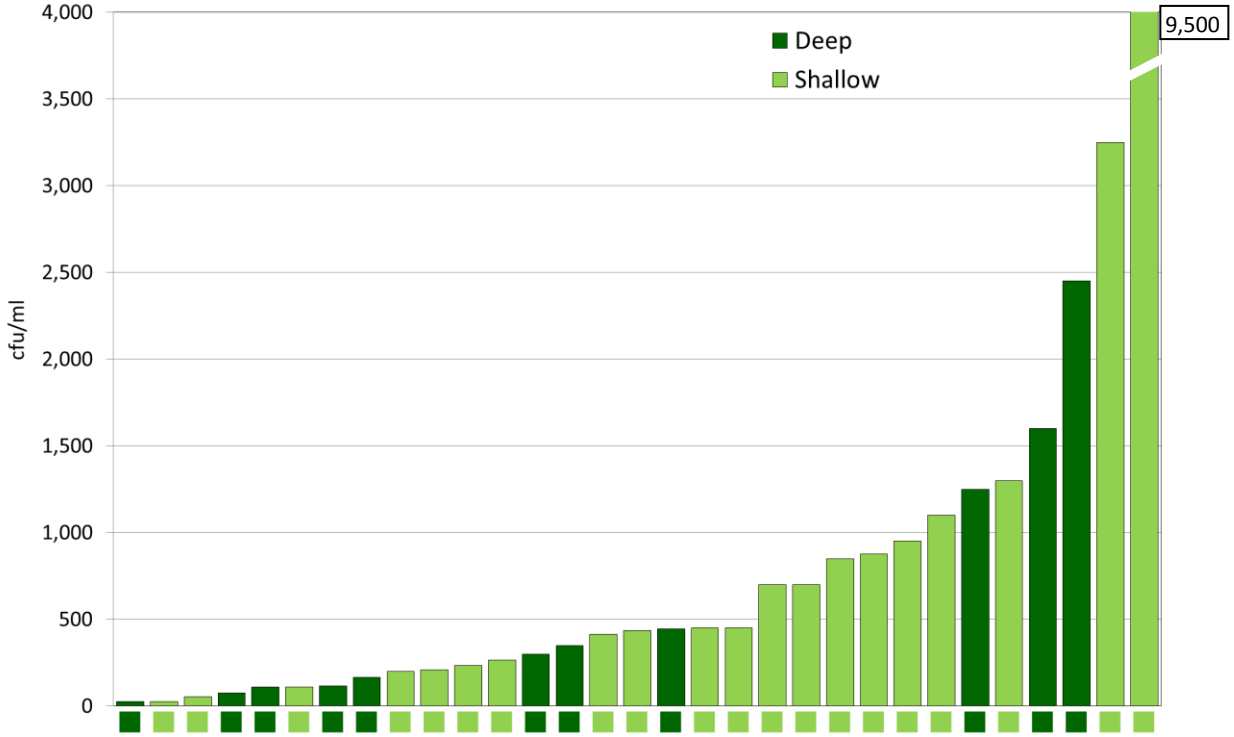


Figure 2.30: An illustration of the *Staphylococcus* spp counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

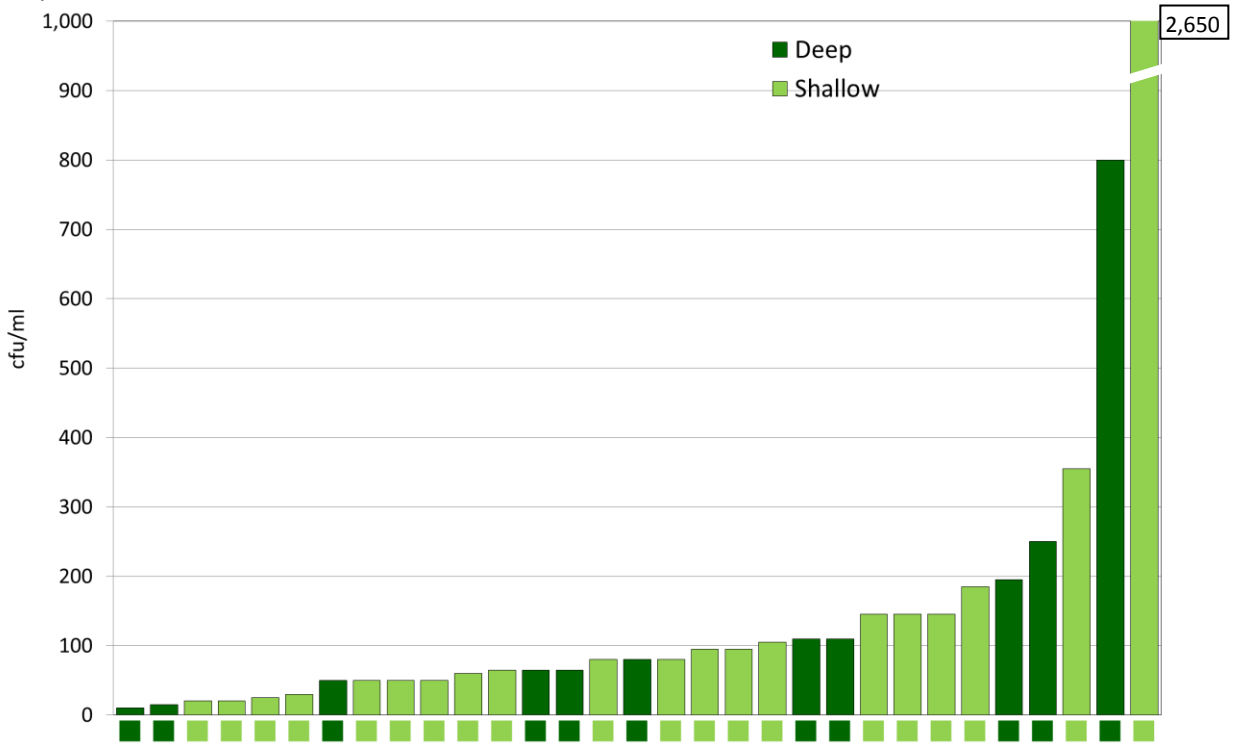


Figure 2.31: An illustration of the laboratory pasteurised counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

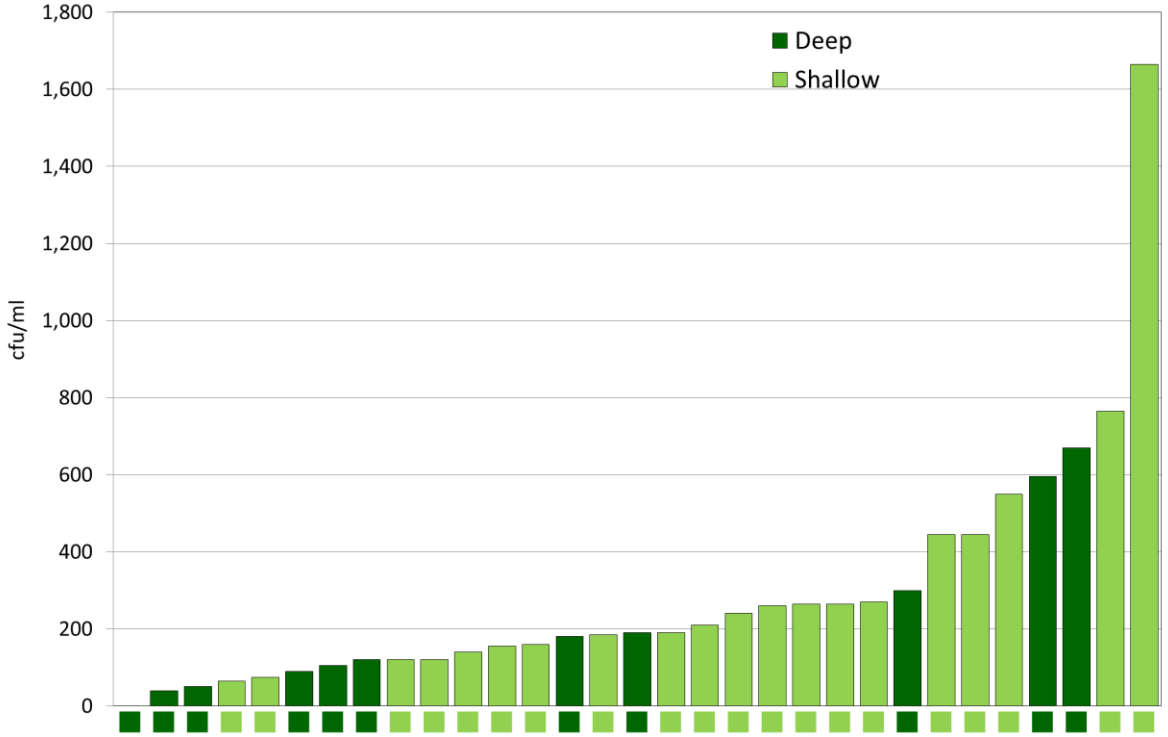


Figure 2.32: An illustration of the thermophilic spore counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

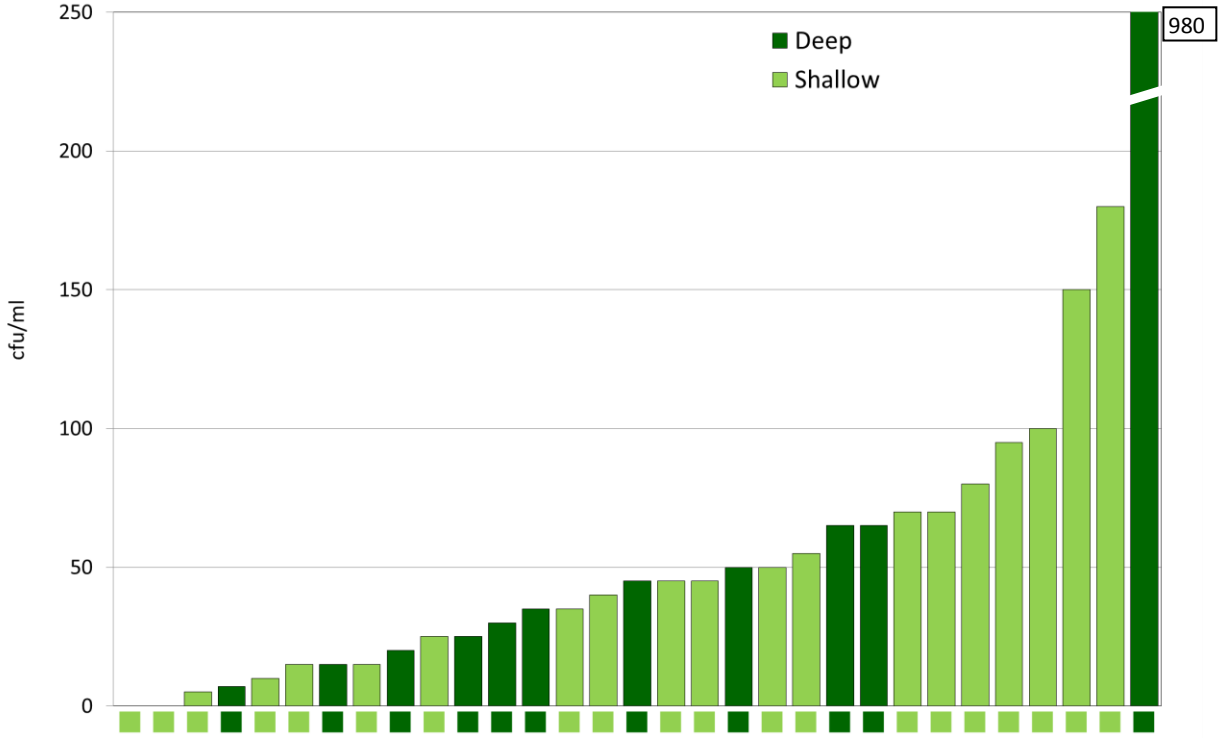


Figure 2.33: An illustration of the psychrotrophic counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

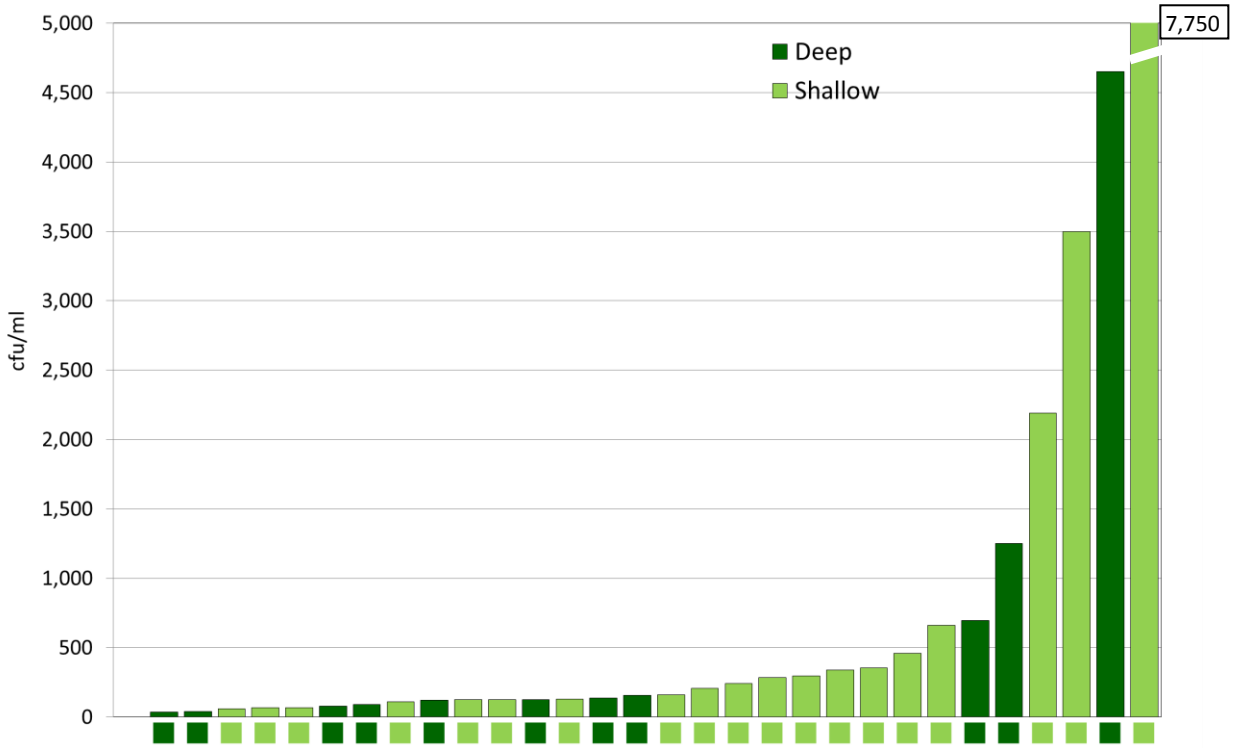


Figure 2.34: An illustration of the *Bacillus cereus* counts (cfu/ml) in bulk milk from farms utilising deep or shallow RMS beds.

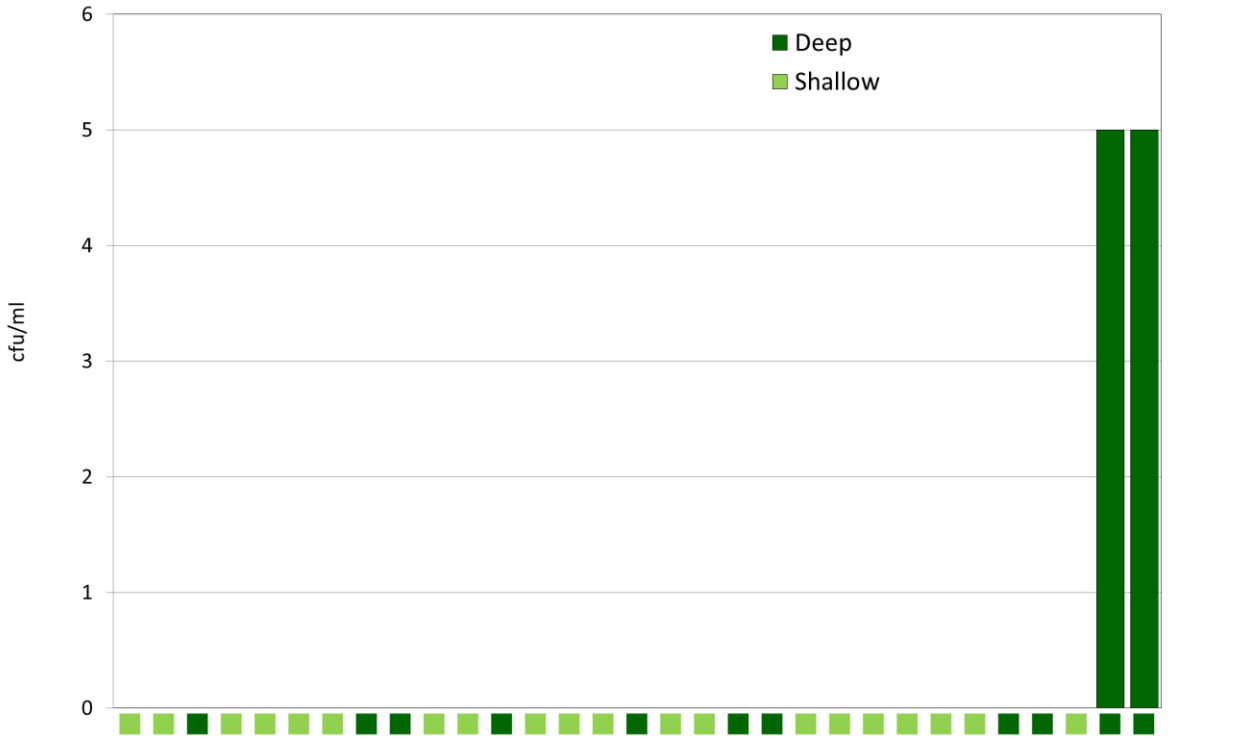
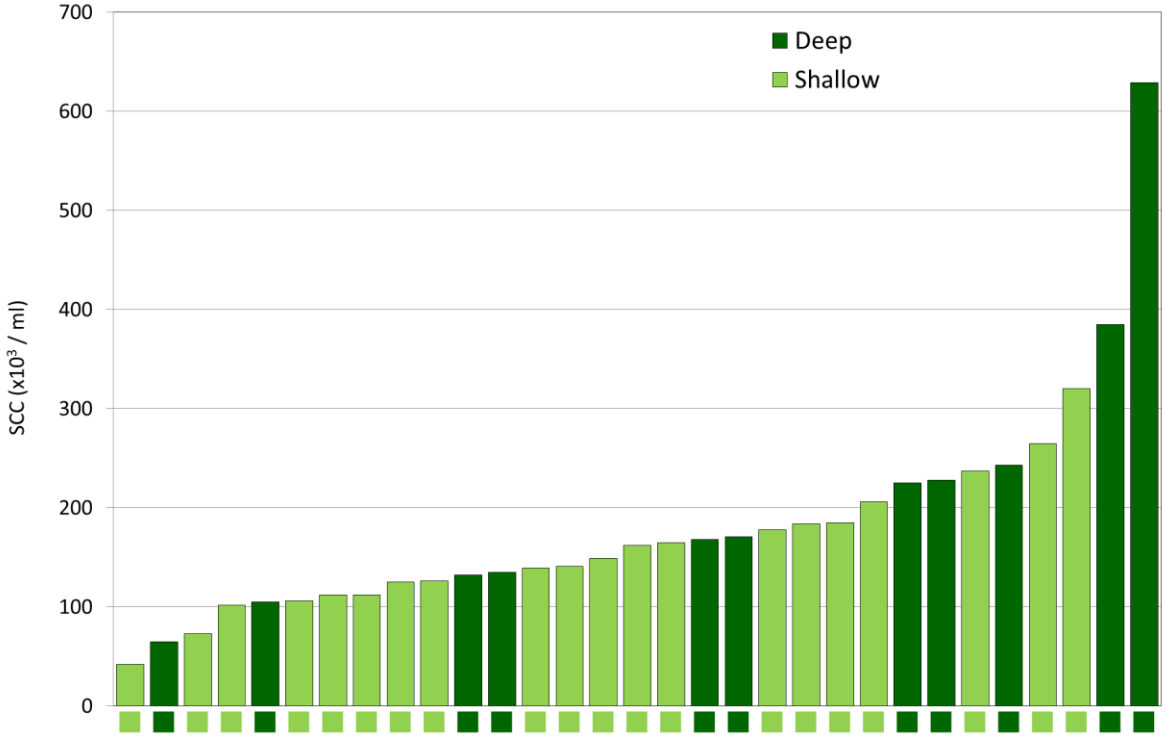
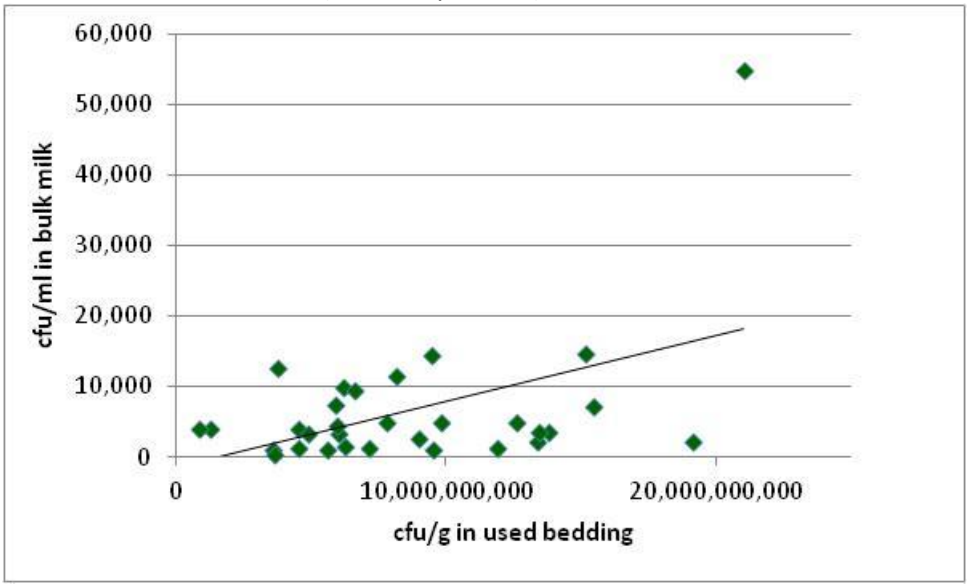


Figure 2.35: An illustration of somatic cell counts in bulk milk across the survey farms.



Data was explored in an attempt to identify any correlations between the number of bacteria in bedding and in bulk milk. A positive relationship was identified between the total bacterial count in bedding and in bulk milk (r=0.465; p=0.008) in RMS bedded farms as illustrated in Figure 2.36. No other relationships were identified between bacterial numbers in bedding and in milk.

Figure 2.36: An illustration of the relationship between total bacterial number in bedding and in bulk milk in RMS bedded farms (r=0.465; p=0.008).

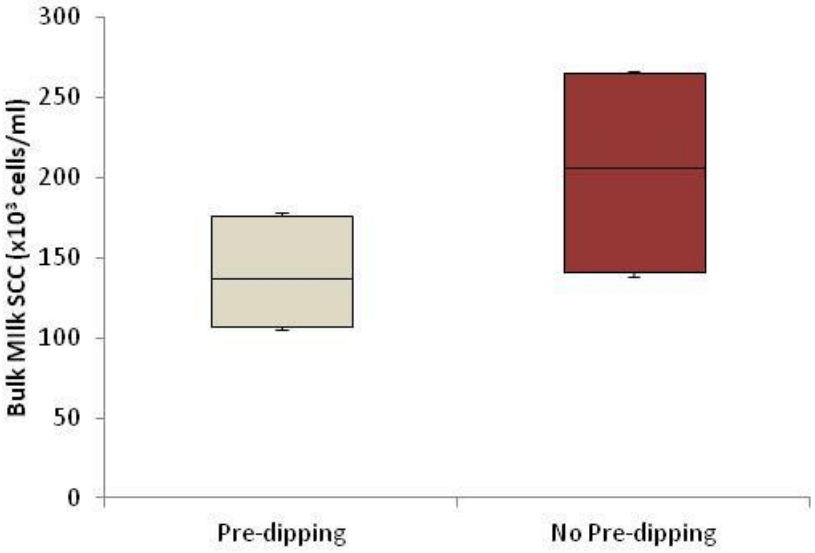


In addition, the impact of milking practices on bacterial counts in bulk milk was investigated.

Fore-milking was not associated with a reduction in any bacterial counts in bulk milk in herds bedded on RMS.

Pre-milking teat preparation that involved a pre-dip followed by wiping dry was associated with a lower psychrotrophic count in bulk milk (130 *vs* 660 cfu/ml; p=0.023), but not with any other bacterial species/grouping. Somatic cell counts in bulk milk were also significantly lower in RMS herds that employed pre-dipping (137 *vs* 206 x10^3 cells/ml; p=0.037) as illustrated in Figure 2.37.

Figure 2.37: An illustration of the relationship between bulk milk somatic cell counts and pre-dipping on RMS farms.



Cluster disinfection was not found to be associated with lower bacterial counts in milk or bulk milk SCC on RMS bedded farms. There was no association of a hot wash after every milking with any bacterial counts on RMS bedded farms.

2.3.5 Udder Health

One hundred and eighteen herds provided electronic management data for analysis. Four herds could not supply recent (2015) milk recording data and were discarded from the analysis. Of the 114 remaining herds, 39 recorded with the Cattle Information Service (CIS), 67 with National Milk Records (NMR) and 8 with Quality Milk Management Services Ltd (QMMS). Thirty-seven herds submitted data from on-farm software; 19 using Interherd (PAN Livestock Services, University of Reading), 11 using Uniform Agri (UNIFORM-Agri UK, Taunton), four using Total Dairy (SUM-IT Computer Systems Ltd, Thame) and three using Dairy Plan C21 (GEA United Kingdom, Warrington). Of all the herds that submitted data, either in the form of a milk recording organisation Common Data Layer (CDL) file or via on-farm software, 64 (56%) reported clinical mastitis records of varying quality in electronic format, including six herds which supplied clinical mastitis data in spreadsheet format. The exact number of herds available for analysis of clinical mastitis data varied according to the period of analysis, but typically data robust enough for analysis was only available from approximately one third of farms.

The udder health performance in the month, quarter and year ending 31st March 2015, of herds utilising each of the bedding materials is summarised in Tables 2.20, 2.21 and 2.22 respectively. Performance as measured by the rate of new infection in lactation, the proportion of cows chronically infected and the rate of clinical mastitis of apparent lactating period origin in the previous quarter is illustrated in Figures 2.38, 2.39 and 2.40 respectively.

Analysis confirmed that the herds did not differ with respect to yield, age of the herd, calving index or days in milk. Some of the mean ages reported in some datasets were higher than was plausible (suggesting in one case a mean age of >10 years in the herd); for this reason and to ensure a robust analysis, nonparametric statistics were used.

No significant differences were identified between farms utilising the different bedding materials, in any of the measures of udder heath, based on either SCC or clinical mastitis cases, in any of the periods of analysis.

None of the analyses of change in rates of udder health parameters over time revealed any significant effect of the change to RMS from a different bedding material when herds changing were compared to herds not changing. Figure 2.41 illustrates one such analysis on the rate of new infection in lactation between 2014 and 2015 for herds which had been on RMS for less than one year, but longer than 3 months.

The relatively recent introduction of RMS meant that insufficient farms had a long enough history of use to allow a robust analysis of the impact of length of time of use, of RMS, on udder health parameters.

Data was also analysed to determine if there was any difference in lactation based udder health parameters in herds bedding on deep or shallow RMS beds. No significant differences were identified. Similarly, a brief analysis of performance failed to identify any consistent significant correlations between the bacterial numbers in bedding at the time of the farm visit and subsequent udder health - this area was not explored extensively as given the variation in bacterial counts seen in bedding it was not considered biologically plausible to attempt to explain udder health over an extended period of time, or at a time remote from the time of analysis of bacterial numbers in bedding.

Table 2.20: A summary of udder health performance in March 2015, by bedding type, in survey herds providing data for analysis.

Parameter	Bedding Type	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Lactation new infection rate (%)	RMS	35	5.8	4.7	0.8	17.0	2.6	7.0
(SCC)	Sand	35	5.8	5.0	0.0	13.4	4.0	7.6
	Sawdust	37	6.7	5.7	2.1	15.5	4.4	8.3
Dry Period new infection rate (%)	RMS	35	14.5	13.6	0.0	55.6	0.0	22.2
(SCC)	Sand	35	14.2	15.0	0.0	50.0	0.0	21.1
	Sawdust	37	11.2	11.1	0.0	50.0	0.0	17.9
Dry period cure rate (%)	RMS	35	66.9	75.0	0.0	100.0	55.6	85.7
(SCC)	Sand	35	74.9	80.0	0.0	100.0	66.7	100.0
	Sawdust	37	72.9	85.7	0.0	100.0	57.8	100.0
Fresh calver infection rate (%)	RMS	35	17.1	15.8	0.0	50.0	6.3	23.2
(SCC)	Sand	35	16.1	16.0	0.0	50.0	6.0	23.5
	Sawdust	37	13.6	12.5	0.0	42.9	2.4	24.3
Chronic infection rate (%)	RMS	35	10.8	10.5	2.2	35.8	6.9	12.9
(SCC)	Sand	35	9.5	9.0	3.5	21.4	6.6	11.9
	Sawdust	37	10.3	10.3	3.7	19.4	6.8	13.9
% Cows with SCC > 200K	RMS	35	16.8	16.0	3.3	45.2	11.4	20.8
(SCC)	Sand	35	15.8	15.2	8.2	34.4	11.4	19.4
	Sawdust	37	17.2	15.3	8.6	30.6	13.1	21.2
Clinical mastitis rate	RMS	15	46.0	42.0	13.0	101.0	34.0	53.0
(cow cases/100cows/year)	Sand	19	34.2	31.0	11.0	89.0	16.0	42.0
	Sawdust	17	35.7	23.0	10.0	114.0	14.5	47.0
Apparent dry period origin	RMS	12	1.07	0.92	0.39	1.77	0.78	1.49
clinical mastitis rate	Sand	15	1.10	0.88	0.26	2.83	0.54	1.36
(cows in 12 affected)	Sawdust	10	1.12	0.94	0.27	3.39	0.61	1.18
Apparent lactating period origin	RMS	15	2.66	2.39	0.55	7.54	1.39	4.07
clinical mastitis rate	Sand	19	1.78	1.68	0.48	4.43	1.11	2.34
(cows in 12 affected)	Sawdust	15	2.02	1.84	0.70	4.06	0.98	3.07
305 day yield	RMS	35	9,617	9,616	7,227	11,911	8,858	10,647
(litres)	Sand	35	9,916	9,849	5,906	12,591	9,017	11,165
	Sawdust	37	9,611	9,659	6,238	11,636	8,862	10,273
Herd size	RMS	35	352	283	126	973	218	432
(number of lactating cows)	Sand	35	354	311	126	886	231	468
	Sawdust	37	317	255	94	759	186	416
Calving index (mean)	RMS	35	409	406	369	517	392	421
(days)	Sand	35	405	403	372	517	385	415
	Sawdust	37	408	408	377	440	397	420
Days in milk (mean)	RMS	35	185	182	120	260	172	198
(days)	Sand	35	183	179	153	260	168	194
	Sawdust	37	186	188	154	222	170	200
Age of the milking herd	RMS	35	1,708	1,654	1,322	2,665	1,578	1,729
(days)	Sand	35	1,786	1,688	1,373	2,705	1,526	1,916
	Sawdust	37	1,863	1,722	1,505	3,716	1,604	1,917
% milking herd exiting in last year	RMS	35	26.7	28.6	8.2	47.7	22.3	31.0
	Sand	35	24.3	24.8	3.0	40.4	19.8	27.6
	Sawdust	37	27.7	28.5	17.7	41.4	21.7	32.6

Table 2.21: A summary of udder health performance in the first quarter of 2015, by bedding type, in survey herds providing data for analysis.

Parameter	Bedding Type	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Lactation new infection rate (%)	RMS	33	6.3	5.8	1.4	18.2	4.0	7.2
(SCC)	Sand	35	6.0	5.3	1.9	16.2	4.1	7.5
	Sawdust	37	6.6	6.0	2.9	11.4	5.0	8.0
Dry Period new infection rate (%)	RMS	33	15.2	14.5	0.0	37.5	7.8	20.3
(SCC)	Sand	35	15.4	13.0	0.0	50.0	8.5	20.9
	Sawdust	37	12.7	11.1	0.0	35.3	7.8	16.4
Dry period cure rate (%)	RMS	33	78.2	80.0	45.1	100.0	67.8	86.6
(SCC)	Sand	35	74.7	78.4	0.0	100.0	64.3	86.2
	Sawdust	37	76.4	80.0	0.0	100.0	68.4	86.4
Fresh calver infection rate (%)	RMS	33	17.2	16.3	0.0	39.0	11.5	23.9
(SCC)	Sand	35	17.6	15.0	0.0	50.0	11.5	22.7
	Sawdust	37	14.4	14.0	0.0	36.8	9.7	18.9
Chronic infection rate (%)	RMS	33	10.6	10.9	2.5	31.0	6.8	13.1
(SCC)	Sand	35	9.8	9.4	3.0	20.9	6.7	11.6
	Sawdust	37	10.2	10.5	3.8	21.6	7.1	12.8
% Cows with SCC > 200K	RMS	33	16.9	15.9	4.2	44.9	11.9	22.7
(SCC)	Sand	35	15.8	15.3	6.5	36.0	10.8	20.0
	Sawdust	37	16.7	16.2	6.5	31.1	12.9	19.9
Clinical mastitis rate	RMS	16	40.5	34.0	16.0	118.0	24.0	49.3
(cow cases/100cows/year)	Sand	23	35.9	35.0	14.0	88.0	25.0	42.0
	Sawdust	18	39.5	25.0	16.0	107.0	20.0	50.3
Apparent dry period origin	RMS	15	0.64	0.65	0.13	1.20	0.38	0.82
clinical mastitis rate	Sand	21	1.28	0.91	0.26	6.08	0.66	1.58
(cows in 12 affected)	Sawdust	16	1.01	0.82	0.22	3.24	0.46	1.41
Apparent lactating period origin	RMS	16	2.20	2.01	0.87	4.00	1.37	3.28
clinical mastitis rate	Sand	23	1.98	1.93	0.72	3.47	1.50	2.52
(cows in 12 affected)	Sawdust	18	1.94	1.75	0.82	3.55	1.33	2.57
305 day yield	RMS	33	9,599	9,569	7,272	11,945	8,685	10,618
(litres)	Sand	35	9,910	9,825	6,037	12,564	9,024	11,151
	Sawdust	37	9,594	9,618	6,279	11,507	8,835	10,285
Calving index (mean)	RMS	33	410	407	363	518	390	426
(days)	Sand	35	406	401	372	518	386	418
	Sawdust	37	409	408	378	440	397	421
Days in milk (mean)	RMS	33	181	180	123	261	163	196
(days)	Sand	35	180	178	130	261	166	185
	Sawdust	37	182	187	136	215	167	193

Table 2.22: A summary of udder health performance in the year to March 2015, by bedding type, in survey herds providing data for analysis.

Parameter	Bedding Type	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Lactation new infection rate (%)	RMS	20	7.5	7.3	3.5	12.7	5.4	9.0
(SCC)	Sand	34	6.8	6.6	3.1	16.4	4.6	8.2
	Sawdust	36	7.1	7.1	3.7	11.7	5.8	8.2
Dry period new infection rate (%)	RMS	20	14.7	14.9	2.9	30.0	10.7	17.5
(SCC)	Sand	34	14.9	14.6	2.6	41.5	9.6	18.1
	Sawdust	36	13.5	13.8	5.2	26.4	9.3	16.4
Dry period cure rate (%)	RMS	20	76.4	77.7	55.9	95.2	68.4	84.0
(SCC)	Sand	34	78.5	77.8	61.3	97.4	73.7	84.2
	Sawdust	36	78.4	78.4	67.4	90.8	72.1	83.6
Fresh calver infection rate (%)	RMS	20	17.2	16.8	3.9	34.6	12.1	20.4
(SCC)	Sand	34	16.5	17.0	4.8	32.8	11.1	20.7
	Sawdust	36	15.6	15.4	5.8	27.9	12.6	18.2
Chronic infection rate (%)	RMS	20	11.5	11.3	2.6	23.6	6.9	15.5
(SCC)	Sand	34	10.5	9.7	2.9	21.8	7.2	13.0
	Sawdust	36	10.8	10.8	5.4	21.8	8.5	13.4
% cows with SCC > 200K	RMS	20	18.8	18.6	7.1	34.3	14.1	23.9
(SCC)	Sand	34	17.0	16.3	7.6	35.6	12.5	19.4
	Sawdust	36	17.7	17.7	9.4	31.7	14.6	21.0
Clinical mastitis rate	RMS	7	46.0	42.0	24.0	77.0	30.0	72.0
(cow cases/100cows/year)	Sand	23	39.2	38.0	12.0	66.0	29.0	49.0
	Sawdust	19	39.6	36.0	11.0	110.0	22.0	47.0
Apparent dry period origin	RMS	7	0.69	0.75	0.12	1.12	0.43	0.87
clinical mastitis rate	Sand	23	0.95	0.96	0.17	2.25	0.56	1.23
(cows in 12 affected)	Sawdust	19	0.80	0.69	0.06	2.56	0.36	1.06
Apparent lactating period origin	RMS	7	2.60	2.33	1.74	3.71	2.03	3.43
clinical mastitis rate	Sand	23	2.16	2.12	1.13	4.32	1.77	2.45
(cows in 12 affected)	Sawdust	19	2.11	2.26	0.62	3.91	1.38	2.70
305 day yield	RMS	20	9,430	9,148	7,052	11,728	8,454	10,686
(litres)	Sand	34	9,657	9,536	5,801	12,359	8,877	10,853
	Sawdust	36	9,450	9,508	6,135	11,098	8,647	10,292
Calving index (mean)	RMS	20	9430	9148	7052	11728	8454	10686
(days)	Sand	34	9107	9468	419	12359	8762	10853
	Sawdust	36	9450	9508	6135	11098	8647	10292
Days in milk (mean)	RMS	20	197	192	117	313	176	208
(days)	Sand	34	176	179	11	289	167	196
	Sawdust	36	186	185	149	255	175	196

Figure 2.38: An illustration of the rate of new infection during lactation (as measured by SCC changes) in the first quarter of 2015, across the survey farms.

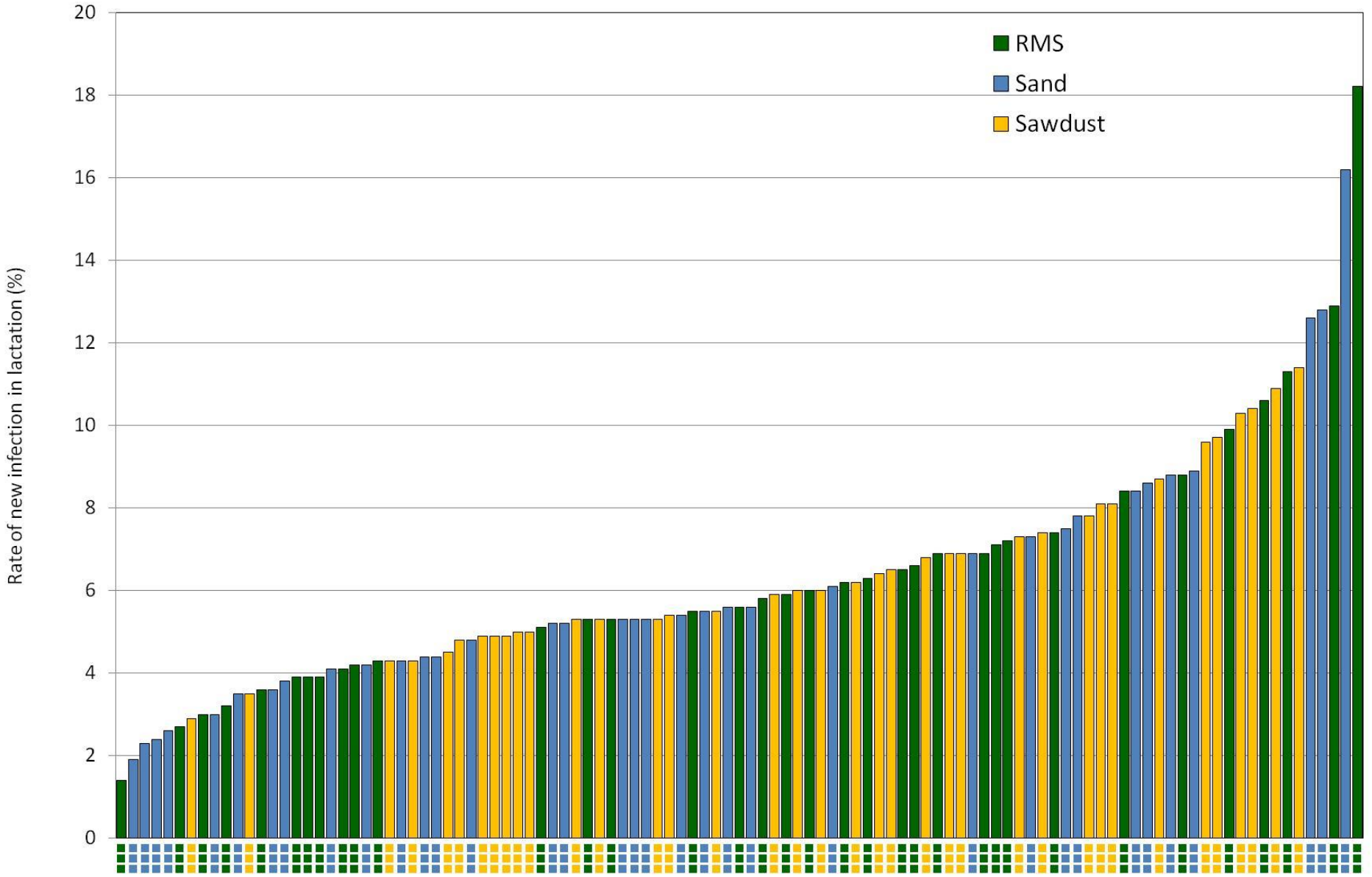


Figure 2.39: An illustration of the proportion of cows chronically infected (as measured by SCC changes) in the first quarter of 2015, across the survey farms.

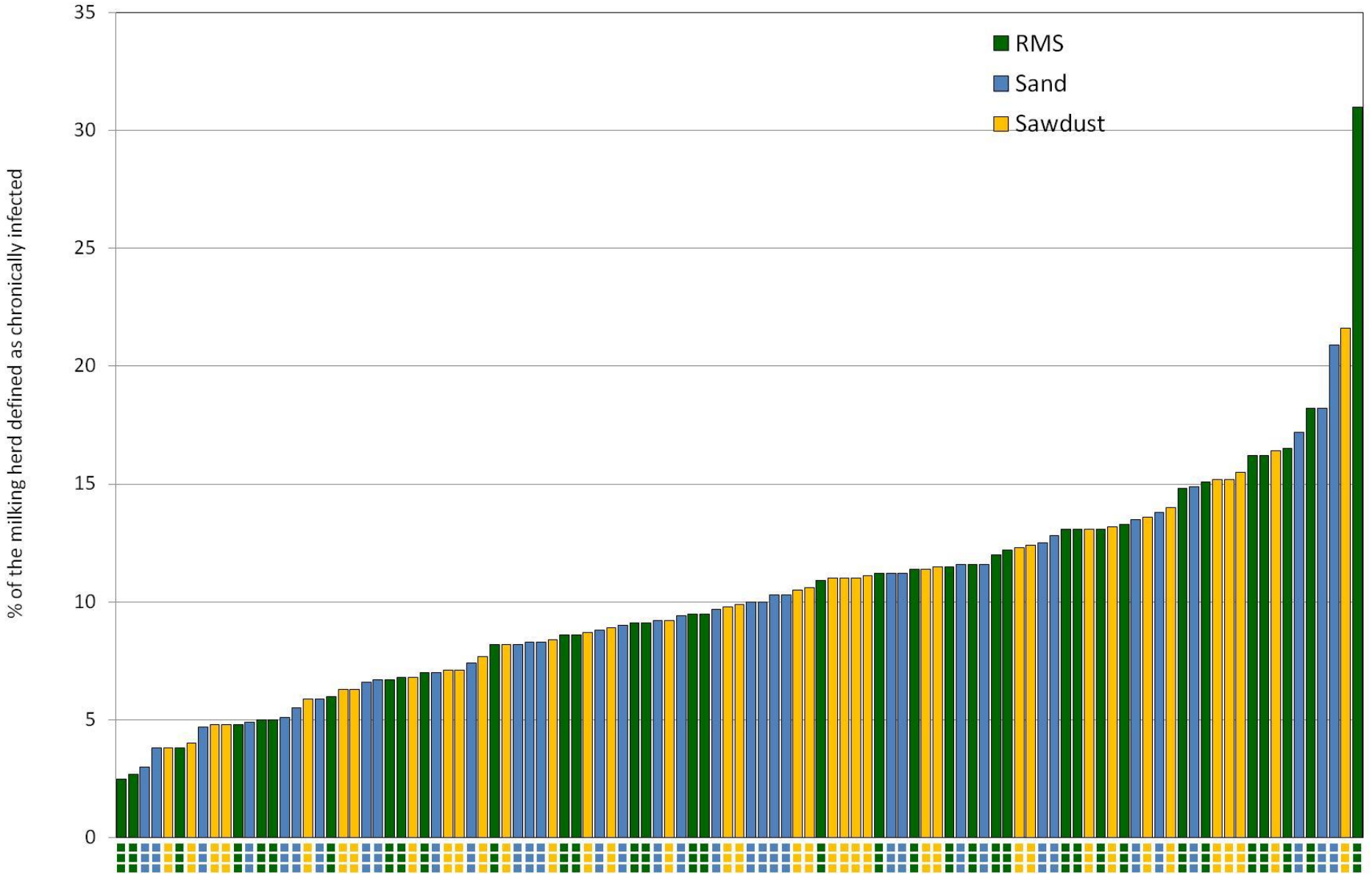


Figure 2.40: An illustration of the rate of clinical mastitis of apparent lactating period origin, in the first quarter of 2015, across the survey farms.

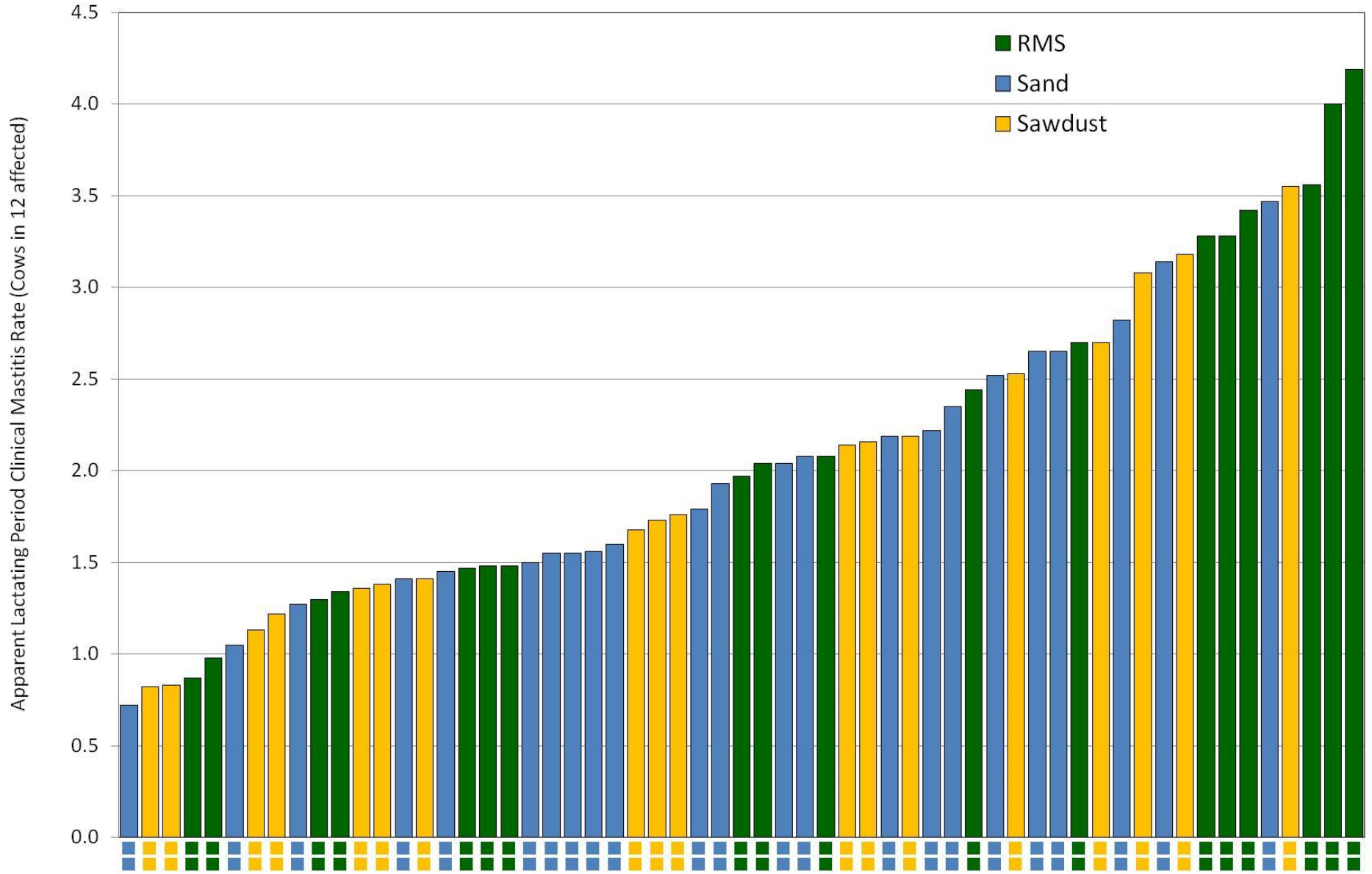
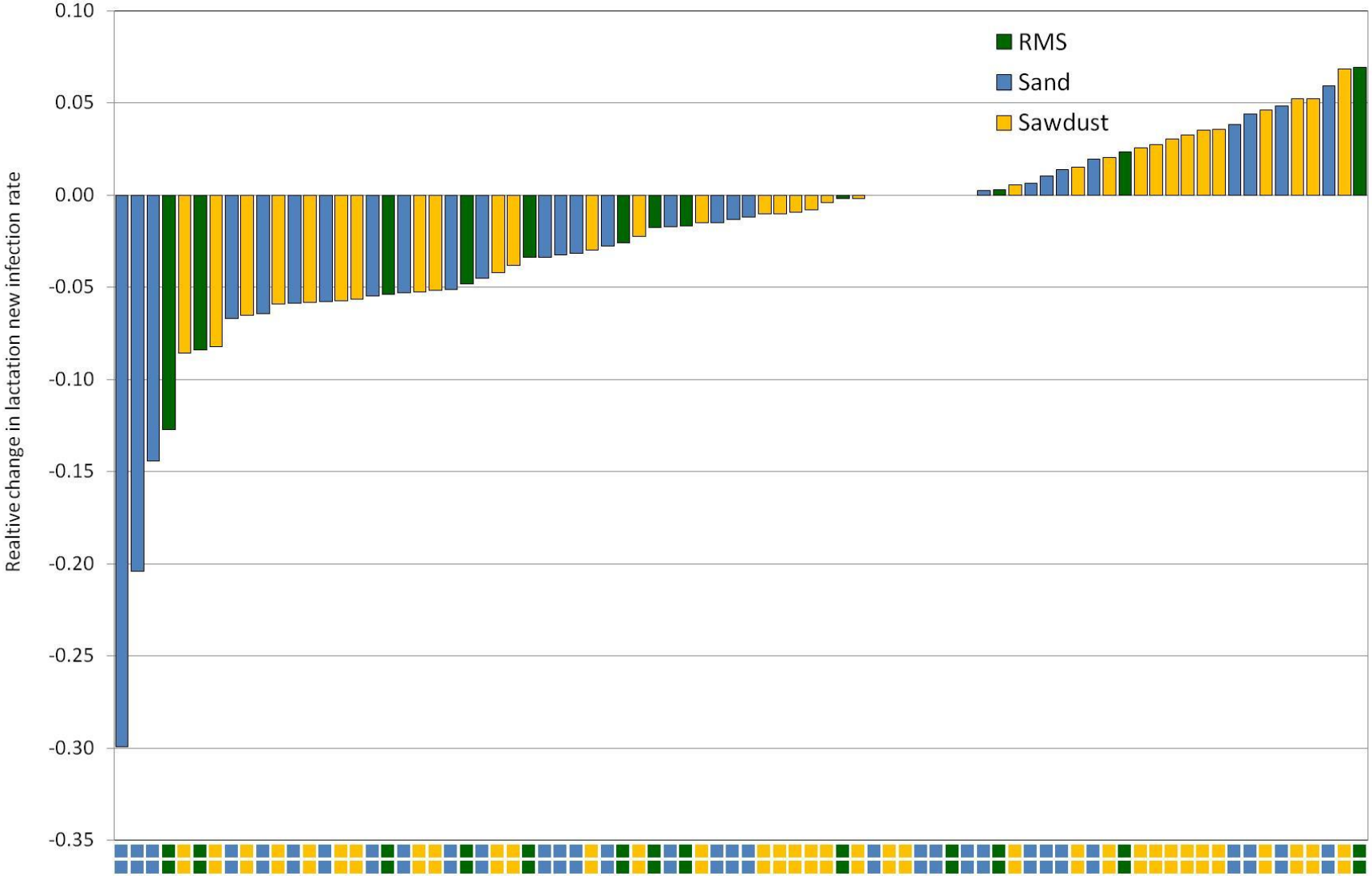


Figure 2.41: An illustration of the relative change in new infection during lactation (as measured by SCC changes) between the first quarter of 2014 and 2015, across the survey farms, including RMS farms that adopted this bedding more than 3 months ago and less than 1 year ago.



2.3.1 Cow Comfort and Welfare Indicators

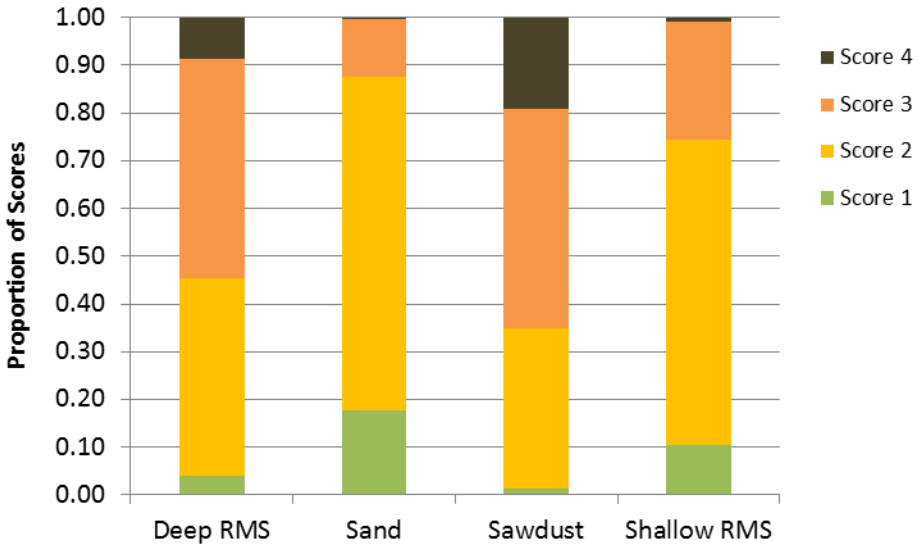
A subset of 109 farms was used to assess cow comfort and welfare indicators; farms with mixed (*ie* deep and shallow) or atypical bed designs were excluded. Eleven farms using deep RMS, 18 using RMS on mats ("shallow RMS"), 37 using deep sand and 42 using sawdust on mats were available for analysis. Scores were available from a total of 3258 cows for cleanliness and from 3252 cows for hocks.

The proportion of cows within each bedding group given each score for cleanliness and hock attributes are summarised in Table 2.23. Individual attribute scores are illustrated in Figures 2.43 to 2.47.

2.3.5.1 Cleanliness Scores

Scores 3 and 4 were combined for analysis. Lower leg cleanliness in cows in RMS bedded herds was intermediate between sand (cleanest) and sawdust (dirtiest) (see Figure 2.43). All groups differed significantly ($p<0.001$) in the proportion of lower legs scoring 1 (Sand > Shallow RMS > Deep RMS > Sawdust) and in the proportion of lower legs scoring 3 or 4 (Sand < Shallow RMS < Deep RMS < Sawdust).

Figure 2.43: An illustration of lower leg cleanliness scores by bedding group.



Upper leg and flank scores differed significantly between all treatments ($p<0.001$), with the exception of score 1 on sand and shallow RMS. Upper leg and flank were cleanest on deep RMS, and dirtiest on sawdust (Figure 2.44).

Table 2.23: A summary of cleanliness and hock attributes by bedding group.

Parameter			
Lower leg cleanliness (% of cows scored, scoring)			
Score	**1**	**2**	**3 or 4**
Deep RMS	3.9[a]	41.4	54.7[a]
Sand	17.6[b]	70.0	12.4[b]
Sawdust	1.2[c]	33.8	65.0[c]
Shallow RMS (on mat)	10.4[d]	64.1	25.5[d]
Upper leg and flank cleanliness (% of cows scored, scoring)			
Score	**1**	**2**	**3 or 4**
Deep RMS	46.4[a]	51.2	2.4[a]
Sand	25.4[b]	62.8	11.8[b]
Sawdust	10.0[d]	43.0	47.0[c]
Shallow RMS (on mat)	21.8[b]	56.2	22.0[d]
Udder cleanliness (% of cows scored, scoring)			
Score	**1**	**2**	**3 or 4**
Deep RMS	49.7[a]	39.7	10.6[a]
Sand	43.1[b]	48.7	8.2[a]
Sawdust	17.1[c]	48.1	34.8[b]
Shallow RMS (on mat)	36.4[d]	45.7	17.9[c]
Hock swelling (% of cows scored, scoring)			
Score	**0 or 1**	**2**	**3**
Deep RMS	70.6[a]	27.3	2.1[a]
Sand	81.8[b]	16.0	2.2[a]
Sawdust	49.4[c]	37.4	13.2[b]
Shallow RMS (on mat)	66.6[a]	27.3	6.2[c]
Hock hair loss and lesions			
	No hairloss or lesion	**Hairloss (with or without lesion)**	**Lesion**
Deep RMS	62.1	37.9[a]	1.8[a]
Sand	81.7	18.3[b]	2.3[a]
Sawdust	38.2	61.8[c]	8.5[b]
Shallow RMS (on mat)	45.3	54.7[d]	5.9[b]

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).
For each parameter the lowest score is optimal

Udders were cleanest on deep RMS and sand, followed by shallow RMS and sawdust (Figure 2.45). All groups differed significantly in the proportion of udders scored 1, and 3 or 4 ($p<0.05$), with the exception of sand and deep RMS which did not differ in the proportion of udders scoring 3 or 4.

Figure 2.44: An illustration of upper leg and flank cleanliness scores by bedding group.

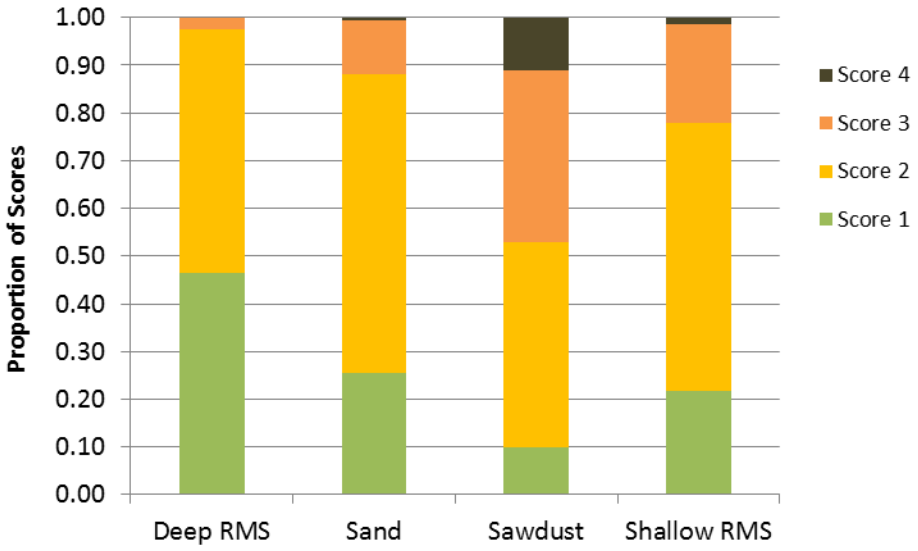
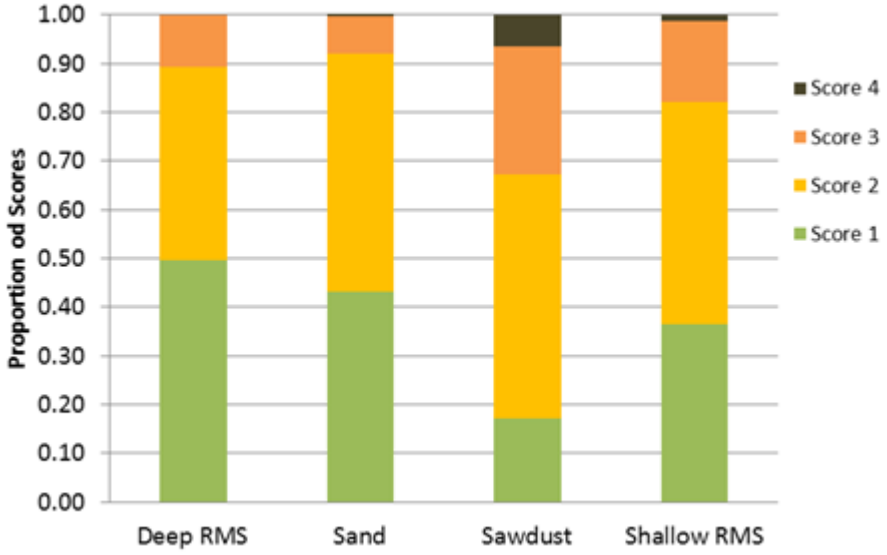


Figure 2.45: An illustration of udder cleanliness scores by bedding group**.**



2.3.5.2 Hock Swelling

The proportion of cows with no or mild hock swelling (score 0 or 1) on both designs of RMS beds was significantly lower than on sand, but higher than on sawdust (Figure 2.46). This proportion did not differ significantly between deep and shallow RMS. Score 3 swelling was relatively rare, being equally low on deep RMS and sand, and higher on sawdust than on all other treatments.

A summary of the separate analysis of the subset of farms where hock swelling scores 0 and 1 were distinguished is summarised in Table 2.24. Hocks with no swelling (Score 0) were rare other than on deep sand beds. Hocks with no swelling (score 0) was recorded less often on deep RMS beds than on sand beds (9% *vs* 23%; $p<0.0001$). There was no significant difference in proportion of hocks with no swelling recorded on mats, whether bedded with RMS (2%) or sawdust (3%), though both scores were significantly lower than on either of the deep beds.

Figure 2.46: An illustration of hock swelling scores by bedding group.

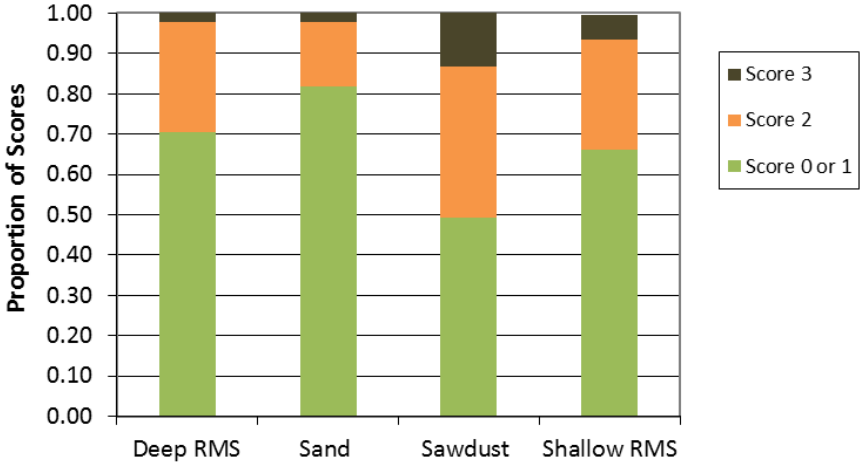


Table 2.24: A summary of the percentage of hocks exhibiting no swelling (score 0) or swelling (score >0) on the four different bed types.

	No Swelling Score 0	Swelling Score >0
Deep RMS	9.3[a]	90.7
Sand	22.7[c]	77.3
Shallow RMS	3.4[bd]	96.6
Sawdust	2.0[d]	98.0

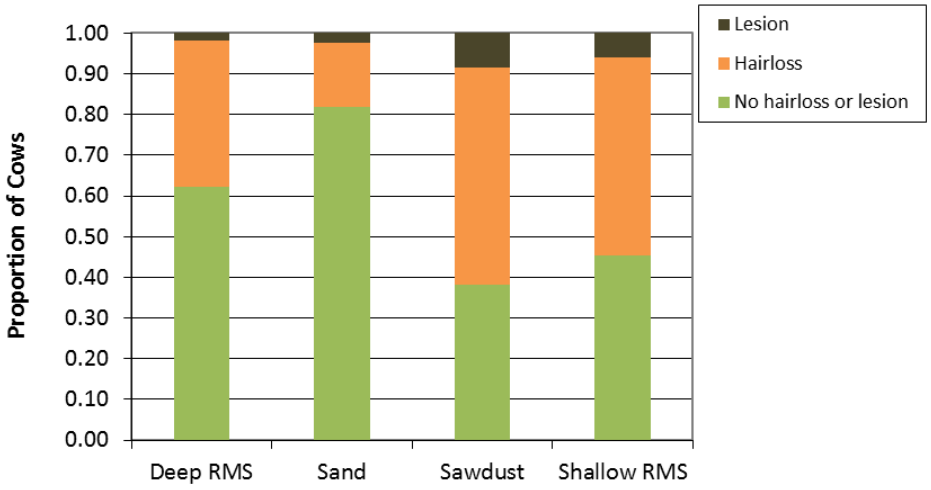
[a,b] Values with different superscripts within a column differ ($p < 0.0001$)

2.3.5.3 Hock Hair Loss and Lesions

The proportion of hocks with hair loss was lowest on sand and highest on sawdust (18.3% *vs* 61.8% respectively); deep RMS and shallow RMS were intermediate (37.9% and 54.5% respectively) (see Figure 2.47). All differences between treatments were significant ($p<0.01$).

The proportion of hocks with a lesion varied significantly between deep beds and mats, but not within bedding materials on each of these bed types. Lesion prevalence was lowest on deep sand beds (1.8%) and highest on mats bedded with sawdust (8.5%).

Figure 2.47: An illustration of the proportion of hocks demonstrating hair loss or a lesion, by bedding group.



2.4.1 Feedback from Farmers Discontinuing the Use of RMS

Seven farmers were identified who had begun use of RMS as bedding but had subsequently discontinued. Four agreed to be interviewed and their feedback is summarised in this section.

The farmers had tried use of RMS bedding for between six weeks and six months. Three farms began their trial period in the autumn and one, which housed cows continuously, in the spring (May). Previous bedding materials included sand, sawdust and a paper based product. One farmer was already separating slurry for ease of handling, but, the machine was not of the specification intended for creating bedding. Reasons for beginning were related to the desire for a "sustainable alternative" to the current bedding material, particularly in view of bedding purchase costs and unreliable availability of good quality sawdust.

The separators in question were of four different types. Two were sited under cover and two in the open. In addition to the slurry from the milking herd and parlour washings, the input material on one farm contained the output from a floodwash system. None of the farms allowed waste whole milk to enter the reception tank.

Two farmers used the bedding for dry cows as well as milkers. Three farmers considered the buildings where the bedding was used to be well ventilated; on one farm there were two buildings, one of which had poorer ventilation. There were examples of use both on mattresses, with amounts varying from "a top-dressing as previously with sawdust", to a "3 inch" layer, and in deep beds. On two farms, "deep beds" were engineered by adding a wooden board 10-13 cm high at the back of the cubicles. Bedding was applied within 12 hours of beginning separation, with the exception of a short experiment with delayed application on one farm. Two different management techniques designed to attempt to increase dry matter content of the material were described; spreading the material out to dry before use and using a mechanical rake to disturb the material on the beds. However, it was found that both these practices caused heating and were discontinued.

All farmers cited problems with udder health among their reasons for giving up use of RMS bedding. On a farm changing from sand, there were also problems with cows injured through slipping, and an

opinion that overall welfare and comfort was poorer than on sand. Three farmers mentioned that the bedding produced was “not dry enough”. In one case, the machine was found to be an inappropriate model for producing bedding. In another case, the machine needed adjustment to produce suitably dry material. Following this the farmer is considering resuming use. One farmer perceived inconsistency in the slurry entering the machine as limiting the ability to produce sufficiently dry material.

The udder health problems cited manifested as both increases in bulk milk somatic cell count and clinical mastitis prevalence. Initial bulk tank somatic cell counts of 120 - 150,000 cells/ml were reported to rise to above 200,000 cells/ml, and in one case above 400,000 cells/ml. Two farms reported similar increases in mastitis rates, from 40 cases/100 cows/year before use, to the equivalent of 90 cases/100 cows/year during use. One farm reported a change from diagnoses in which *E. coli* predominated to *S. uberis*, while another reported that the majority of cases during use were “*E. coli*” (many manifesting as sick cows). For all farmers the opinion that SCC and mastitis had reached unacceptable levels contributed to the decision to abandon RMS bedding. Particular risk factors for mastitis that individual farmers perceived were a change of bedding coinciding with the major calving season, and cows returning to the bedding with open teats.

The farmers interviewed offered some pointers for others considering a move to RMS as bedding – these are summarised in Table 2.25. It should be stressed that these are individual opinions and not necessarily supported by any more than one farmer’s experience.

Table 2.25: A summary of suggestions for farmers considering the use of RMS as bedding from farmers who experienced problems with RMS use.

DO	DON'T
DO go and see plenty of other farms using RMS in various conditions and set-ups before starting (and possibly talk to some farmers who have given it up).	**DON'T** introduce at a time when cows are under extra stress *eg* calving time for block calving herd, when building work is going on/just finished.
DO be sure that you have the right specification of separator machine and ensure it is properly set up and adjusted.	**DON'T** use in poorly ventilated buildings.
DO be aware that non-grooved floors can be slippery with RMS - one farmer had lost some cows through injury after converting from sand.	**DON'T** use in buildings with very open ridges, where rain may fall onto beds.
DO make sure that cows are lying in the correct position in cubicles, so that the udder is not in contact with wet areas of the bed.	**DON'T** try spreading RMS out to dry in a building and using it later as it is impossible to get a thin enough layer for it to dry out sufficiently.
DO pay careful attention to teat preparation in the parlour - pre-dipping routing needs to be very good. One farmer considered an automatic brush with peracetic acid alone to be insufficient, citing better udder health once manual pre-dipping was added to the routine.	**DON'T** use the material on beds if it is not sufficiently dry.

2.4 Discussion

This comprehensive survey has facilitated the collation of a large dataset from which important and relevant conclusions can be drawn. The data collected has provided new insights into the interaction of bedding with udder heath, has dispelled some previously held beliefs, and suggests, in as far as the data currently permits, that bedding on recycled manure need not pose a significant threat to human or animal health, though any longer term implications still need to be assessed.

Bacterial counts were consistently higher in RMS beds, but not always significantly so. However, a significant factor that needs to be considered is the difference in bedding management styles for different materials. Sawdust beds were typically bedded much more frequently, and may not have performed as well if bedded as infrequently as RMS beds. Sand beds were bedded significantly less frequently and yet from a bacteriological perspective were at least as good as sawdust beds. This difference in management styles and consequent labour costs needs to be considered by any farmer considering a switch to RMS bedding.

Comparison of deep and shallow RMS beds often, and perhaps counter-intuitively, revealed higher bacterial counts in shallow rather than deep beds; this may have been influenced by the relative

proportion of 'fresh' faecal matter present on the beds at the time of sampling. However, organisms that favoured 'composting' type conditions were typically high in the deep beds.

One interesting and perhaps surprising finding was the higher prevalence of *Listeria* spp in sand beds compared to either RMS or sawdust. This may have been due to the presence of this 'soil borne' organism in the fresh bedding (given sand is often sourced from inland pits rather than the coast), but this would need to be tested to be confirmed. This finding however does challenge the widely held assumption that sand is inert and therefore must be the 'cleanest' of bedding materials, though this must be tempered by the disparity in bedding frequency seen with different bedding materials.

Salmonella spp were isolated from either bedding or milk from all three farm bedding groups. It is an interesting observation that it was not recovered until the latter stages of the survey (in March) when ambient temperatures had started to rise. The finding of *Salmonella* spp in RMS farms was followed up by testing of fresh recycled RMS, on those farms, over a period of weeks, which confirmed the continued presence of the organism in both cases. In both instances the *Salmonella* spp was 'exotic' and the continued presence could have resulted from continued input from an outside source (*eg* wildlife or feed), but may have been as a result of the recycling of the manure solids. Further studies would need to be conducted to confirm or refute these hypotheses.

An important and key finding in this study was the apparent overall lack of influence of bacterial numbers in bedding on milk quality. Across all the beddings, no correlations between bedding bacterial counts and bacterial counts in milk were made. This must have been in no small part down to the efforts of the farmers to prevent such 'transmission'. Milking hygiene will be crucial in preventing transmission of organisms form the environment to milk and this is substantiated by the fact that fore-milking, pre-milking teat disinfection and cluster flushing all had an impact on reducing the presence of at least some bacterial species and groups in milk. Another possible reason for a lack of correlation could be the potential for other sources of bacteria reaching milk to confound any effects, though it seems unlikely that this would always have been the case.

However, one needs to be mindful that the lack of correlation across all bedding between bacterial numbers in bedding and bacterial numbers in milk may have been confounded by the ease of cleaning of teats coated with each of the beddings. This conclusion may be supported by the correlation between total bacterial count in RMS beds and bulk milk from RMS bedded farms - this further reinforces the importance of good milking hygiene.

Another interesting finding was the apparent impact of pre-milking on the bulk milk SCC of herds bedded on RMS. Pre-milking has not been reported as impacting SCCs and has always been associated with decreasing the risk of clinical mastitis. However, given the reports of *Klebsiella* spp being more prevalent as a cause of intramammary infection and clinical mastitis in RMS bedded herds and the proclivity of this organism to cause persistent intramammary infection (compared to other coliforms), this could in part explain this finding. The whole area of clinical mastitis incidence and aetiology in RMS bedded herds warrants further investigation.

Factors potentially important in the management of RMS beds were explored, but generally failed to demonstrate any significant effects. The frequency of bedding had a small effect on *Streptococcus* spp numbers, but no onward impact on milk quality. Separation under cover conveyed no apparent benefits, though this would have been confounded by the fact that farmers did not separate solids

outdoors in adverse weather conditions - that said the flexibility offered by a covered separation area should not be overlooked. Whilst bedding conditioners were used on many farms (most commonly on sawdust farms) no impact of their use on bacterial numbers in used bedding was identified - this may have been because of a lack of power in the study, or could be because only herds which had experienced a problem commenced the use of a conditioner. Alternatively it could be that conditioners have no sustained impact on bacterial numbers in bedding. This is an area in need of further research and well controlled clinical trials. Although not directly measured in the survey, it is worthy of note that with the commencement of the controlled trial conducted at Sewborwens Farm the use of a conditioner was discontinued in RMS beds without apparent ill effects.

Although there is anecdotal evidence that RMS is beneficial for cow cleanliness, these results suggest that this evaluation varies with the body area in question, the bed design, and the comparative bedding material. For udders, the advantage of RMS is apparent in comparison with sawdust on mats rather than with sand in deep beds. In contrast to this is the cleaner upper leg and flank in cows on deep RMS beds compared with sand. A possible explanation is that the RMS absorbs more moisture from the alleyways, so that tails are cleaner and cows less likely to flick dirt onto their flanks. Also, automatic scrapers which will scrape the passages frequently are more likely to be used with RMS than with sand. Shallow RMS was associated with cleaner lower legs than deep RMS or sawdust, but dirtier lower legs than sand. Again, there may be an interaction with the passageway scraping method.

The majority of cows had some hock swelling whatever the bedding type or bed design. Farmers often report “better hocks” on conversion to RMS, but these results suggest that the effect seen will depend on the previous material and bed design and the length of time cows have been on the new system. Deeper bedding has previously been reported as protective against hock swelling (Brenninkmeyer *et al*, 2013). In view of this, the highest prevalence of hocks with no or mild swelling being on sand and the lowest on sawdust is as expected. However it is interesting that the intermediate prevalence of scores 0 and 1 on RMS did not differ significantly with the bed design, though this may change with time spent on the bedding. This may be because farmers are willing to apply a deeper layer of RMS to mats. Hock hair loss and lesions were clearly related to bed design, being more prevalent on mats than deep beds. Within a bed design, hair loss is also influenced by bedding material. Failure to demonstrate a similar relationship for hock lesions may have been related to the relatively low prevalence of such lesions in this survey, or because hair loss will recover sooner after a bedding change to deep beds than will chronic hock swelling.

2.5 Conclusions

The overall, and probably the single most important conclusion of this survey is that there is a larger variation in all aspects of performance within herds and cows bedded on a given bedding material than between different bedding materials.

The use of RMS as a bedding material is still in its infancy in the UK and Europe and whilst early indications are that there need not be an adverse effect on udder and animal health this will need to be monitored, as and if more herds adopt this technology, particularly with respect to clinical mastitis

(where data collation and recording is often poor). Again performance varies more within bedding groups than between them.

The survey highlights the importance of good milking hygiene and demonstrates clearly that it is possible to mitigate the impact of high bacterial loads in bedding to prevent their transmission to bulk milk and the human food chain.

With respect to cow comfort and welfare (excluding udder health), RMS beds would appear to offer advantages with respect to cow comfort and cleanliness; deep RMS beds typically performed as well as sand beds and when used on mats RMS demonstrated clear advantages over sawdust.

3 Controlled Trial - An Investigation of the Impact of Bedding Type on Pathogen Load in Bedding, Udder Health and Milk Quality

3.1 Introduction

As outlined earlier in this report, there is a general lack of understanding of the impact of different bedding materials, and their management, on milk quality and udder health. The aim of this study was to make a quantitative, four-way comparison of the impact of bedding type on pathogen load in bedding, milk quality, udder health and cow comfort using RMS on mats, sawdust on mats, RMS as a deep bed and sand as a deep bed.

This study comparing different cubicle bedding materials and bed design was carried out near Penrith, Cumbria, (NY 492303) where the climatic conditions are not dissimilar to those experienced in many parts of Wales (evidenced by available data (http://www.metoffice.gov.uk/public/weather/climate/)) ensuring that results of this study are transferable and relevant to the Welsh environment.

3.2 Methods

3.2.1 Experimental Site and Design including Cow Allocation

The study was conducted at Sewborwens Farm, Newton Rigg, Penrith, Cumbria, (NY 492303).

A modified crossover design was employed using four groups of 40 cubicles within a single shed. Each group of cubicles had a different bed type: deep sand, deep RMS, RMS on mats and sawdust on mats. Four cow groups, each of 40 cows, were rotated around the four bedding areas; spending two weeks on each bedding type, and cycling twice around the four treatments (16 weeks in total).

Cows were initially grouped, in blocks, by parity and days in milk, prior to being randomly allocated, within blocks, to one of four groups. With four exceptions, cows only left the group on the fortnightly "changeover days" – when the group moved to the next bedding type. Cows removed for drying off were typically replaced with freshly calved cows entering the next parity. Cows leaving for other reasons (n = 4) were replaced with cows of equal parity and as similar days in milk as possible.

3.2.2 Housing and General Management of Cows and Beds

The building was approximately 6 m high (at the eaves), with doors and space boarding at both gable ends. The sides of the building were solid to approximately 2 m, above which were automatically operated curtains. An impression of the shed design can be gained from Figures 3.1 and 3.2.

Cows were fed a total mixed ration once a day and milked twice a day in a 30:30 semi-rapid-exit herringbone parlour in an adjacent building. The order of milking of the groups was randomised and altered each day according to a pre-arranged schedule that ensured that each possible order of milking was evenly represented, both over the whole period, and on days when group milk samples were taken.

3.2.2.1 Bed Design

The four treatments were beds of deep sand (approx 8 cm depth over a hardcore base), deep RMS (approx 8 cm depth over a hardcore base), shallow RMS on mats (Luxury Mattress; Quattro, Penrith) and sawdust on mats (Luxury Mattress; Quattro, Penrith).

Figure 3.1: An illustration of main shed accommodating cows in the controlled trial of bedding materials.

Figure 3.2: An illustration of the deep RMS beds as designed and used in the controlled trial.

Quarry sand was obtained locally and was stored outdoors. Fine powdered kiln dried sawdust was obtained in sealed bags which were stored under cover.

The deep RMS beds were already in existence at the start of the study. The deep sand beds were created by replacing the existing RMS with sand two days before the start of the study. The mat based beds were created from existing deep beds, which were filled with sub-base and concrete to recreate conventional concrete cubicles to which the mats were fitted during the week prior to the trial commencing.

3.2.2.2 Recycled Manure Solids Preparation

RMS for use during the study was produced from the slurry scraped from the study area, excluding the sand cubicles, and an adjacent shed housing approximately 30 milking cows which were not included in the study. This slurry, along with water from washing down the parlour, and washing the milking plant (but no whole milk) was collected in a reception pit (volume 100 cubic metres). Twice a week the contents of a footbath containing formalin and copper sulphate also entered the reception pit. To make the bedding material, following agitation, slurry was pumped from this pit through a FAN Press Screw Separator F10113782 (PSS 3.3-780) with standard 1mm screens. The screens of the separator were cleaned every two weeks.

Bedding was prepared on two days per week. Dry matter content of the material was monitored subjectively, by feel, aiming for a dry matter content of at least 35%, based on previous experience of the operator when making regular measurements using a small portable oven. If the material was considered too wet, it was not used for bedding. The material was collected under cover, beneath the separator, and was applied to the beds within four hours of separation.

3.2.2.3 Bed and Passageway Management

Fresh bedding was applied to sand cubicles every two weeks, to RMS beds twice a week and sawdust beds twice daily. The sawdust was put out with a barrow and shovel twice a day, the RMS was distributed with a mechanical dispenser twice weekly, and the sand was dropped in with a telehandler once weekly and levelled by hand. At each application sand beds typically received an addition of approximately 4 cm depth, deep RMS 8 cm, and mats 5 cm of RMS or 0.5 cm of sawdust. Slightly more RMS was delivered to the front of the cubicles to allow for movement 'back' over the following days. Twice a day, when cows were absent for milking, all beds were inspected and any dung removed, and bedding raked from front to back of cubicles. A tractor mounted rake was used to level the deep RMS beds twice a week. All passageways were scraped twice daily, by a tractor mounted scraper.

3.2.3 Sample Collection – bedding and milk

3.2.3.1 Bedding Sampling

At two week intervals, 5 days after groups had changed, both fresh and used bedding was sampled. This was scheduled to coincide with the day prior to proportional inline milk sampling (see below) and to fit with farm management and laboratory testing schedules. Samples were taken of unused bedding material from stock held or from freshly separated RMS. Used bedding was collected, immediately

before fresh bedding was applied, from the top 2.5 cm of the bed, from the rear of the cubicles, in the same manner as that used in the survey (in Chapter 2). For each treatment, samples were taken from ten cubicles and combined to give a sample of at least 500 ml in volume. Samples were packed in an insulated box with icepacks and transported in order to reach the laboratory within 24 hours of collection.

3.2.3.2 In-line Milk Sampling

Samples of the milk produced by each treatment group were collected weekly, at the same morning milking, using a proportional in-line sampling device previously developed by QMMS Ltd. The device was designed to collect approximately 0.5 ml of milk per litre passing down the main pipeline (the collection rate could be regulated by restriction of the 'outflow' from the sampler). The sampler was placed between the plate cooler and the bulk tank. Sampled milk was collected into a previously sterilised bottle and stored on ice. Prior to sampling the first group and between groups, the device was 'purged' by allowing milk to flow to waste for a few seconds in order to minimise carry-over between groups.

After collection, samples were packed in an insulated box with icepacks and transported in order to reach the laboratory within 24 hours of collection.

Following each milking when it was used, the apparatus was removed, cleaned and sterilised prior to the next scheduled sampling date.

Figure 3.3: Illustration of the in-line sampling device used for collection of a proportion sample from each treatment group.

3.2.3.3 Individual Quarter Somatic Cell Counts

On entry to the study, individual quarter samples were taken from each cow for somatic cell count measurement. Prior to milking, and following teat preparation, 20-30 ml of foremilk was expressed

from each quarter. Thereafter approximately 30 ml of milk was collected from each quarter into a pot containing 8mg Myacide Pharma BP (2-bromo-2-nitropropane-1,3-diol) and Natamycin 0.30g (Broadspectrum MicroTabs® II; Advanced Instruments Inc, Norwood, MA). Sampling was repeated at the final milking of each treatment period. Samples were also taken on entry or exit from any cows that were added to or removed from the study unexpectedly at other times.

3.2.4 Yield and Additional Cow Data

Ten day average yields were collated for individual cows from the study the day prior to the end of each two week period. Yields were measured using Fullwood meters and collated into Fullwood Crystal Software on farm.

Additional data relating to cow treatments and events and significant herd events were also recorded.

3.2.5 Clinical Mastitis Sampling

Farm personnel monitored cows for the presence of clinical mastitis throughout the study period and collected a pre-treatment aseptic quarter milk sample when cases occurred. These samples were frozen on farm and stored until the next scheduled shipment of samples to the laboratory. Farm personnel were trained in detection, grading and aseptic sampling of clinical mastitis following standard operating procedures. Clinical mastitis cases were scored for clinical severity (Grade 1 = milk changes only; Grade 2 = milk and/or udder changes; Grade 3 = a cow exhibiting signs of systemic disease (*eg* loss of appetite, change in demeanour, elevated rectal temperature (>39.2^{o}C)) with or without milk or udder changes; Grade 4 = a cow showing signs of severe depression and toxaemia/toxic shock).

3.2.6 Cow Observations

Observations of cow behaviour were made at 10 am on four days each week for 11 weeks. This was approximately four hours after feeding and three hours after all cows had returned from milking. For each group, the number of cows a) lying in cubicle, b) standing in cubicle (all four feet), c) perching in cubicle (two feet), d) standing feeding (head through feed barrier), or e) standing not feeding was recorded.

Cows were assessed for cleanliness on the last day of the first treatment period. Scores were allocated according to the method of Cook (2012) as described earlier. This allowed an assessment of changes in cow cleanliness after 2 weeks on each of the bedding materials before this was confounded by cows rotating around other bedding treatments.

3.2.7 Environmental and Climatic Data Collection

Temperature and relative humidity were measured inside and outside the building using electronic data loggers (Digitron Monolog2®). Further data was obtained from the Galebreaker™ mini weather stations that control the automatic curtains. Daily rainfall data were obtained from www.penrithweatherstation.com.

3.2.8 Laboratory Methods

3.2.8.1 Bacteriological Analyses - Bedding and Bulk Milk Analysis

Thirty grams of thoroughly mixed bedding material was added to 270 ml of maximum recovery diluent (MRD) and mixed in a stomacher for 1 minute at 100rpm prior to aliquoting for preparation of serial dilutions. Serial dilutions of milk and the bedding aliquots were then made in MRD to encompass the 2 or 3 dilutions anticipated to reflect likely counts. When necessary and where appropriate, further dilutions were undertaken to allow an accurate enumeration of colony forming units (cfu) to be determined.

Growth was evaluated and enumerated on selective media 'pour plates', with the aim of allowing counts of a number of 'putative' bacterial populations to be made - the media used and the bacterial species enumerated are outlined below. Positive and negative controls were also utilised to demonstrated profuse growth and 'no growth' respectively.

Total Viable Count (TVC): Samples incubated in milk agar for 66-72 hours at 30^{o}C (±2^{o}C).

Coliform Count (CC): Samples incubated in VRB(MUG) agar for 66-72 hours at 37^{o}C (±2^{o}C).

Laboratory Pasteurised Count (LPC): Samples heated to 63.5^{o}C (±0.5^{o}C) for 35 minutes prior to being incubated in milk agar for 66-72 hours at 30^{o}C (±2^{o}C).

***Streptococcus* spp Count (StrC):** Samples incubated in Edwards agar for 66-72 hours at 37^{o}C (±2^{o}C).

***Staphylococcus* spp Count (StaC):** Samples incubated in Baird Parker agar for 48 hours at 37^{o}C (±2^{o}C). Colonies demonstrating morphology typical of *S. aureus* were then enumerated.

Thermophilic Spore Count (TSC): Samples heated to 80^{o}C (±1^{o}C) for 10 minutes prior to being incubated in milk agar for 24-48 hours at 55^{o}C (±2^{o}C).

Psychrotrophic Count (PsyC): Samples incubated in milk agar for 6 days at 5^{o}C (±2^{o}C).

***Bacillus cereus* Count (BCerC):** Samples heated to 80^{o}C (±1^{o}C) for 10 minutes prior to being incubated in *Bacillus cereus* agar for 18-24 hours at 35^{o}C (±2^{o}C). Plates were re-examined after a further 18-24 hours at room temperature.

In addition, specific enrichment and plating techniques to facilitate detection of additional pathogens of interest were undertaken as outlined below:

***Salmonella* spp:** 25 g of bedding or 25 ml of milk was inoculated into 225 ml of Buffered Peptone Water (BPW) and incubated at 37^{o}C (±2^{o}C) for 18-24 hours. Following incubation, 100 ul of the BPW was inoculated into 10 ml of Rappaport-Vassiliadis (RV) enrichment broth and incubated at 42^{o}C (±2^{o}C) for 24-48 hours. Following this second incubation 10 ul of the RV broth was inoculated in duplicate onto Brilliant Green Agar and XLD Agar plates and incubated at 35^{o}C (±2^{o}C) for 18-24 hours. Suspicious colonies were identified by MALDI-TOF MS (matrix assisted laser desorption/ionization time-of-flight mass spectrometry) (MALDI Biotyper, Bruker Daltonics) and submitted for typing to the APHA.

***Listeria* spp:** 25 g of bedding or 25 ml of milk was inoculated into 225 ml of Listeria Enrichment Broth (LEB) and incubated at 30^{o}C (±2^{o}C) for 7 days. LEBs were then sub-cultured at 1, 2 and 7 days onto

Listeria Selective Agar (LSA) and incubated at 35°C (±2°C) for up to 48 hours. Suspicious colonies were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

***Yersinia enterocolitica*:** 100 ul of the 10^{-1} dilution of milk or bedding was inoculated on *Yersinia* selective agar and incubated for 18-24 hours at 32°C (±2°C). Suspicious colonies were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

A direct plating onto sheep blood agar, Edwards agar and MacConkey agar was also undertaken to assist the identification and recovery of key pathogens. Where necessary the identity of micro-organisms was confirmed by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics).

3.2.8.2 Bacteriological Analyses - Clinical Mastitis Analysis

Microbiological investigation was carried out using the standard milk sample examination techniques, which exceeded the standard recommended by the International Dairy Federation (Bulletin No 132, 1981), International Standard 13366-1:1997 (E) and 13366-2:1997 (G). More specifically, ten μL of secretion was inoculated onto sheep blood agar and Edward's agar; 100 μL of secretion was inoculated onto MacConkey agar to enhance the detection of *Enterobacteriaceae*. Plates were incubated at 37°C and read at 24, 48, and 72 h. Organisms were identified and quantified using standard laboratory techniques (NMC, 1999; Quinn *et al*, 1994) and MALDI-TOF (MALDI Biotyper, Bruker Daltonics).

3.2.8.3 Somatic Cell Count Determination

SCCs were determined using the Fossomatic method (Delta CombiScope - Model FTIR 400, Drachten, The Netherlands), according to the FIL . International Dairy Federation 141 C: 2000(Infrared).

3.2.8.4 Milk Compositional Analysis

Milk constituents were determined by near infrared analysis (Delta CombiScope - Model FTIR 400, Drachten, The Netherlands), according to the FIL . International Dairy Federation 148 A: 95 norm.

3.2.8.5 Dry Matter Determination

Dry matter content and bulk density of fresh and used bedding were determined. Two subsamples of 50 g sand, 20 g sawdust or 20 g RMS were taken for determination of dry matter content, by drying to constant weight in an oven. Bulk density was determined by determining the weight of material in a 150 ml container filled in a standard manner (NRAES, 1992).

3.2.9 Udder Health Evaluation

Udder health was explored and evaluated in a number of ways as outlined below:

New intramammary infections at the quarter level (QIMI): Quarters with an SCC <101,000 cells/ml were defined as 'uninfected' and were therefore considered eligible for a new intramammary infection (IMI) in the subsequent 2 week block. A new QIMI was defined by such quarters having an SCC >100,000 cells/ml at the end of the two week block under analysis.

Cure of an intramammary infection at the quarter level (QCURE): Quarters with an SCC >100,000 cells/ml were defined as 'infected' and were therefore considered eligible for a cure in the subsequent 2

week block. A QCURE was defined by such quarters having an SCC <101,000 cells/ml at the end of the two week block under analysis.

3.2.10 Data Collation and Statistical Analysis

Data were collated and initially analyzed using Excel and Access 2003 (Microsoft Corp) and Minitab 15.1 (Minitab Inc). Descriptive and graphical analyses were carried out to explore the data. Where appropriate, groups were compared using ANOVA or the Kruskal-Wallis Test if data were not normally distributed. Pairwise comparisons were made using either the Two Sample T-test or Mann-Whitney U test as appropriate. Univariable analysis of treatment efficacy was performed using the Chi-Square test to investigate differences in proportions between groups; a layered Bonferroni correction was used to allow for multiple comparisons where appropriate (Darlington, 1990).

For the purposes of analysis of udder health, all eligible quarters from all two week blocks on each of the bedding materials were collated and the proportion of quarters developing a new QIMI or QCURE compared. In addition the rate of new QIMI and QCURE was calculated for each two week block and rates compared between the different bedding treatments.

The impact of the previous bedding treatment for a group of cows was evaluated by examining the impact of the bedding used in one two week block on new QIMI and QCURE rates in the subsequent two week block.

3.3 Results

3.3.1 Bacteriology of Bedding and Milk

Bacterial numbers, dry matters and milk constituents (where appropriate) in fresh and used bedding and in milk are summarised in Tables 3.1, 3.2 and 3.3 respectively.

As might be expected there was significant variation in the bacterial load of the unused bedding, with counts being significantly and consistently higher in the recycled manure solids than the other bedding materials. Numerically, the lowest counts were typically in the kiln dried sawdust, with the exception of the psychrotrophic count which was significantly higher in the unused, kiln dried, sawdust than the unused sand (2.6×10^4 *vs* 1.45×10^5; $p<0.05$).

More variation was evident in the used bedding. Again counts were typically higher in the deep and shallow RMS beds, and most frequently highest in the shallow RMS beds. In summary, TBCs varied significantly between the four bedding materials; being highest in shallow RMS and lowest in sawdust. Coliform counts were lowest in sawdust beds, and this was significant when compared to deep or shallow RMS, but not significantly different from sand. *Streptococcus* spp counts were highest in shallow beds, with sawdust and shallow RMS showing no significant difference. *Staphylocccus* spp counts were lowest in sand and sawdust and significantly higher in shallow RMS than in other used bedding materials. The LPCs were highest in deep RMS and lowest in sand, whilst thermophilic spore counts were high in both deep and shallow RMS beds. Psychrotrophic counts were significantly lower in sawdust beds than in other bedding materials. *Bacillus cereus* counts were significantly higher in deep RMS beds being 3 logs higher than in sand or shallow RMS beds; very little *Bacillus cereus* was identified in sawdust beds.

The huge variation in bacterial numbers evident in used bedding materials did not occur to the same extent in milk, and treatment effects in milk were less evident. With the exception of *Streptococcus* spp and *Staphylococcus* spp counts no significant differences were detected in the quality of the milk produced by animals bedded on the different bedding materials. *Streptococcus* spp counts were significantly lower ($p<0.05$) in milk from cows on deep beds whilst variation was less predictable in *Staphylocccus* spp counts.

Salmonella spp, and *Yersinia enterocolitica* were not identified in any of the used bedding samples. However, *Yersinia enterocolitica* was identified on one occasion in a sample of unused sand. Similarly neither *Salmonella* spp nor *Yersinia enterocolitica* were identified on any occasion in any of the milk samples.

Listeria spp were identified in a number of samples as outlined in Table 3.4. These were typically identified as *L. monocytogenes,* but on occasion could not be definitively differentiated from *L. innocua*. Whilst *Listeria* spp were more frequently found in unused sand than other unused bedding materials there was no significant difference between the groups. However, used sand was significantly more likely to contain *Listeria* spp than either sawdust or deep RMS. With respect to milk, *Listeria* spp were more frequently isolated from cows bedded on sand than on RMS and this was a significant finding.

Table 3.1: A summary of bacterial counts and dry matter in unused bedding used for bedding cubicles during the study (all bacterial counts are cfu/g wet weight).

Parameter	Bedding	n	Mean	Median	Minimum	Maximum	25th Percentile	75th Percentile
Total Bacterial Count	RMS	8	403,250,000	412,500,000[a]	143,500,000	735,000,000	301,875,000	478,750,000
	Sand	5	610,900	415,000[b]	61,500	2,000,000	72,250	1,247,500
	Sawdust	6	20,850	23,750[c]	3,300	35,500	8,175	30,625
Coliform Count	RMS	8	150,625	135,000[a]	25,000	270,000	93,750	231,250
	Sand	5	65	20[b]	20	200	20	132.5
	Sawdust	7	9.29	0[c]	0	45	0	15
***Streptococcus* spp Count**	RMS	8	25,750,000	14,500,000[a]	7,000,000	81,000,000	10,625,000	39,375,000
	Sand	6	221	15[b]	-	1,100	-	421
	Sawdust	7	183.6	90[b]	-	600	20	375
***Stpahylococcus* spp Count**	RMS	6	14,417	12,500[a]	1,500	25,000	7,875	25,000
	Sand	5	1.0	0[b]	0	5.0	0	2.5
	Sawdust	8	17.5	2.5[b]	0	100	0	18.8
Laboratory Pasteurised Count	RMS	8	678,750	615,000[a]	405,000	1,010,000	490,000	921,250
	Sand	7	13,307	15,100[b]	1,500	25,000	1,950	24,500
	Sawdust	8	1,431	975[c]	250	4,500	325	2,263
Thermophilic Spore Count	RMS	8	453,500	472,500[a]	135,000	765,000	307,000	616,250
	Sand	7	2,636	1,400[b]	-	5,850	200	5,050
	Sawdust	8	763	550[b]	250	2,450	275	925
Psychrotrophic Count	RMS	8	112,743,750	95,750,000[a]	9,450,000	265,000,000	24,625,000	193,375,000
	Sand	7	25,786	26,000[b]	6,000	55,500	7,500	41,500
	Sawdust	8	188,750	145,000[c]	70,000	420,000	91,250	326,250
***Bacillus cereus* Count**	RMS	8	2,011	1,258[a]	340	5,050	545	3,875
	Sand	7	52.1	20[b]	0	195	0	110
	Sawdust	8	3.13	2.5[b]	0	10	0	5
Dry Matter (%)	RMS	8	33.5	33.3[a]	28.5	41.1	29.4	36.8
	Sand	7	91.8	91.3[b]	89.4	94.9	89.7	94.0
	Sawdust	8	92.2	92.6[b]	89.5	92.7	92.5	92.6

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

Table 3.2: A summary of bacterial counts and dry matter in used bedding from cubicles during the study (all bacterial counts are cfu/g wet weight).

Parameter	Bedding	n	Mean	Median	Minimum	Maximum	25th Percentile	75th Percentile
Total Bacterial Count	Deep RMS	8	8,375,000,000	8,300,000,000[a]	5,350,000,000	12,050,000,000	6,087,500,000	10,488,000,000
	Sand	8	3,005,000,000	3,442,500,000[b]	345,000,000	5,650,000,000	801,250,000	4,568,750,000
	Sawdust	8	552,062,500	385,000,000[c]	113,000,000	2,000,000,000	204,625,000	542,500,000
	Shallow RMS	8	13,713,000,000	13,175,000,000[d]	8,500,000,000	19,450,000,000	11,613,000,000	16,888,000,000
Coliform Count	Deep RMS	8	11,750,000	10,500,000[a]	2,050,000	23,500,000	4,737,500	20,875,000
	Sand	8	5,621,250	237,500[a,b]	15,000	38,500,000	105,000	3,687,500
	Sawdust	8	1,932,756	34,750[b]	3,550	14,950,000	9,750	265,000
	Shallow RMS	8	7,850,000	1,450,000[a]	860,000	42,500,000	1,360,000	8,050,000
***Streptococcus* spp Count**	Deep RMS	8	35,312,500	39,500,000[a]	6,000,000	64,000,000	14,875,000	52,500,000
	Sand	8	56,375,000	51,500,000[a,b]	7,500,000	110,000,000	23,000,000	87,625,000
	Sawdust	8	181,687,500	122,500,000[b,c]	6,000,000	660,000,000	68,125,000	196,250,000
	Shallow RMS	8	554,375,000	557,500,000[c]	75,000,000	1,070,000,000	227,500,000	921,250,000
***Staphylococcus* spp Count**	Deep RMS	8	680,000	525,000[a]	50,000	1,700,000	217,500	1,162,500
	Sand	7	62,214	35,000[b]	500	150,000	25,000	150,000
	Sawdust	8	295,000	280,000[a,b]	25,000	700,000	93,750	395,000
	Shallow RMS	8	2,562,500	1,200,000[c]	400,000	10,000,000	775,000	3,275,000
Laboratory Pasteurised Count	Deep RMS	8	8,475,000	6,100,000[a]	2,900,000	19,050,000	4,962,500	12,975,000
	Sand	8	821,875	742,500[b]	275,000	1,255,000	483,750	1,243,750
	Sawdust	8	2,095,625	772,500[b,c]	165,000	9,400,000	293,750	3,177,500
	Shallow RMS	8	3,661,875	1,717,500[a,c]	840,000	11,050,000	1,455,000	5,562,500
Thermophilic Spore Count	Deep RMS	8	1,614,375	1,372,500[a]	360,000	3,950,000	800,000	2,212,500
	Sand	8	292,625	265,500[b]	175,000	565,000	181,250	363,750
	Sawdust	8	1,248,000	305,000[a,b]	19,000	8,000,000	197,500	496,250
	Shallow RMS	8	2,280,000	1,547,500[a]	835,000	5,150,000	952,500	3,612,500
Psychrotrophic Count	Deep RMS	8	212,437,500	204,500,000[a]	18,000,000	590,000,000	51,625,000	280,000,000
	Sand	8	88,468,750	67,250,000[a,b]	4,250,000	240,000,000	29,000,000	158,500,000
	Sawdust	8	6,586,456	2,650,000[c]	1,650	23,600,000	1,130,000	13,212,500
	Shallow RMS	8	846,250,000	662,500,000[d]	320,000,000	1,765,000,000	423,750,000	1,361,250,000
***Bacillus cereus* Count**	Deep RMS	8	990,938	1,305,000[a]	32,500	1,740,000	216,250	1,527,500
	Sand	8	12,008	3,975[b]	1,040	45,000	1,656	19,700
	Sawdust	8	354	185[c]	45	1,025	69	739
	Shallow RMS	8	3,017	1,625[b,d]	585	8,050	750	5,538
Dry Matter (%)	Deep RMS	8	50.4	50.6[a]	39.9	56.0	48.2	54.9
	Sand	8	94.4	94.9[b]	90.9	97.8	92.1	96.1
	Sawdust	8	78.9	80.4[c]	69.8	84.0	75.3	83.4
	Shallow RMS	8	55.7	56.1[a]	45.5	68.1	47.4	63.2

[a,b] Values with different superscripts within columns, within parameters, differ (p<= 0.05).

Table 3.3: A summary of bacterial counts and milk constituents in milk from cows bedded on different materials during the study.

Parameter	Bedding	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Total Bacterial Count	Deep RMS	16	1,608	955	505	9,400	766	1,424
	Sand	16	1,547	955	280	6,800	499	2,068
	Sawdust	16	22,933	768	265	347,500	685	1,565
	Shallow RMS	16	3,460	1,458	735	22,500	920	4,225
Coliform Count	Deep RMS	16	84.8	12.5	1	1,000	4	29.8
	Sand	16	221	8	2	2,800	3	73
	Sawdust	16	66.3	8	1	545	3	46
	Shallow RMS	16	200	10	1	2,750	3	65
***Streptococcus* spp Count**	Deep RMS	16	84.4	40[a]	10	410	20	93.8
	Sand	16	94.1	55[a]	5	635	15	118.8
	Sawdust	16	766	180[b]	70	7,500	108	354
	Shallow RMS	16	749	263[b]	85	5,000	148	408
***Stpahylococcus* spp Count**	Deep RMS	16	18.44	20[a,b]	0	40	10	25
	Sand	16	16.25	15[a]	5	40	5	28.75
	Sawdust	16	17.5	17.5[a,b]	0	45	6.25	23.75
	Shallow RMS	16	41.6	32.5[b]	10	185	20	48.8
Laboratory Pasteurised Count	Deep RMS	16	35.94	27.5	10	95	15	52.5
	Sand	15	37.7	20	0	140	15	35
	Sawdust	16	47.8	30	5	210	21.3	48.8
	Shallow RMS	16	65.6	65	10	235	26.3	75
Thermophilic Spore Count	Deep RMS	16	27.81	25	0	70	6.25	45
	Sand	16	39.69	35	0	105	7.5	75
	Sawdust	16	60.3	42.5	0	190	16.3	76.3
	Shallow RMS	16	49.7	35	0	130	6.3	95
Psychrotrophic Count	Deep RMS	16	319	173	0	2025	75	351
	Sand	16	671	153	25	7500	81	366
	Sawdust	16	19,276	123	0	306,000	68	240
	Shallow RMS	16	282.8	177.5	20	1,355	102.5	392.5
***Bacillus cereus* Count**	Deep RMS	16	0.63	0	0	10	0	0
	Sand	16	0	0	0	0	0	0
	Sawdust	16	0	0	0	0	0	0
	Shallow RMS	16	0.31	0	0	5	0	0
Butterfat	Deep RMS	16	4.53	4.52	3.91	5.55	4.19	4.73
	Sand	16	4.44	4.38	4.00	4.86	4.29	4.63
	Sawdust	16	4.44	4.45	3.43	5.38	4.13	4.76
	Shallow RMS	16	4.30	4.38	3.01	4.74	4.26	4.58
Protein	Deep RMS	16	3.41	3.43	3.19	3.56	3.34	3.48
	Sand	16	3.44	3.43	3.36	3.56	3.37	3.51
	Sawdust	16	3.43	3.45	3.31	3.52	3.38	3.48
	Shallow RMS	16	3.44	3.42	3.34	3.58	3.37	3.52
Lactose	Deep RMS	16	4.74	4.76	4.22	4.86	4.75	4.80
	Sand	16	4.77	4.77	4.69	4.89	4.75	4.79
	Sawdust	16	4.78	4.79	4.62	4.88	4.75	4.84
	Shallow RMS	16	4.79	4.81	4.68	4.84	4.77	4.84
Total Solids	Deep RMS	16	13.44	13.43	12.84	14.14	13.15	13.69
	Sand	16	13.40	13.37	12.90	13.93	13.17	13.61
	Sawdust	16	13.40	13.41	12.11	14.42	13.10	13.69
	Shallow RMS	16	14.12	13.42	11.90	26.78	13.13	13.61
SCC	Deep RMS	16	83	83	31	165	64	101
	Sand	16	87	77	49	189	61	108
	Sawdust	16	82	70	46	170	62	97
	Shallow RMS	16	76	72	56	117	62	83

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

Table 3.4: A summary of presence/absence of *Listeria* spp from different fresh and used bedding materials and milk.

	Number of samples Positive	Number of samples Negative	Proportion samples Positive	Proportion of samples Negative
Unused Bedding				
RMS	1	7	0.125	0.875
Sand	3	5	0.375	0.625
Sawdust	1	7	0.125	0.875
Used Bedding				
Deep RMS	1	7	0.125[a]	0.875
Sand	7	1	0.875[b]	0.125
Sawdust	1	7	0.125[a]	0.875
Shallow RMS	3	5	0.375[a,b]	0.625
Milk (Cows bedded on…)				
Deep RMS	0	16	0[a]	1.000
Sand	5	11	0.313[b]	0.688
Sawdust	1	16	0.059[a,b]	0.941
Shallow RMS	0	16	0[a]	1.000

[a,b] Values with different superscripts within columns, within parameters, differ (p<= 0.05).

3.3.2 Production and Udder Health

The four groups established at the start of the study and their composition as the study progressed were assessed to ensure that groups were 'balanced' with respect to potentially confounding factors. Analysis demonstrated no significant differences between the groups with respect to yield, days in milk or parity at the start of the study or any of the subsequent bedding periods. In addition, the impact of the bedding in the previous bedding treatment was investigated as a potentially confounding factor on the rate of new infection in the period of analysis; there was no significant difference (p>0.5) in the rate of new infection (or apparent cure) in the period of analysis when analysed on the basis of bedding in the previous period, as illustrated in Figure 3.4.

The overall proportion of eligible quarters, across all two week blocks of bedding treatments, experiencing an apparent new intramammary infection (QIMI) or an apparent cure (QCURE) are outlined in Table 3.5 and Figure 3.5. There was significant variation between the bedding types (p=0.015). New QIMIs were significantly less likely to occur in cows on sawdust beds than on deep RMS (47/961 *vs* 84/965; p=0.012) or sand beds (47/961 *vs* 78/965; p=0.04). No impact of bedding material on the likelihood of a quarter curing could be identified.

Figure 3.4: An illustration of the overall rate of apparent new intramammary infection by bedding in the previous bedding period.

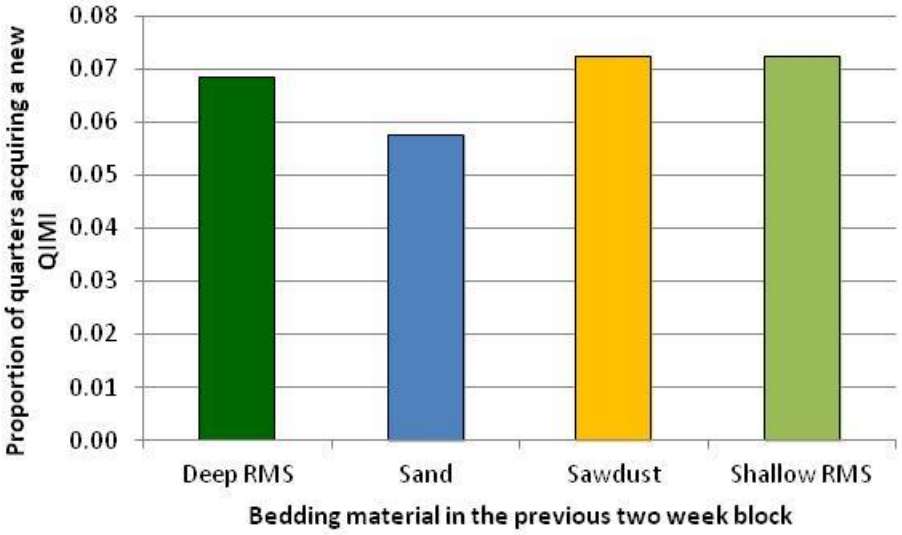


Table 3.5: A summary of the proportion of quarters experiencing an apparent new intramammary infection (QIMI) or apparent cure (QCURE) by bedding type.

Bedding Type	Number of quarters eligible for a new QIMI	Number of quarters acquiring a new QIMI	Proportion of quarters acquiring a new QIMI
Deep RMS	1049	84	0.0801[a]
Sand	1043	78	0.0748[a]
Sawdust	1008	47	0.0466[b]
Shallow RMS	1014	69	0.0680[a,b]
Bedding Type	**Number of quarters eligible to QCURE**	**Number of quarters experiencing a QCURE**	**Proportion of quarters experiencing a QCURE**
Deep RMS	271	48	0.1771
Sand	264	54	0.2045
Sawdust	284	34	0.1197
Shallow RMS	276	55	0.1993

[a,b] Superscripts within columns, within parameters are significantly different (p<= 0.05)

In addition the rate of new infection across each of the 2 week treatment blocks was calculated and is outlined in Table 3.6 and illustrated in Figure 3.6. There was significant variation in the rate of QIMI between the different bedding groups (p=0.032), However, whilst there was a strong trend for quarters in cows bedded on sawdust to be less likely to acquire a QIMI than in cows bedded on deep RMS, the effect was not significant (p=0.0516).

Data was explored in an attempt to identify any correlations and associations between bedding bacterial counts and udder health. However, no consistent, biologically plausible, repeatable correlations were found across the different bedding groups.

Figure 3.5: An illustration of the overall rate of apparent new intramammary infection by bedding group (columns with different superscripts differ p<0.05).

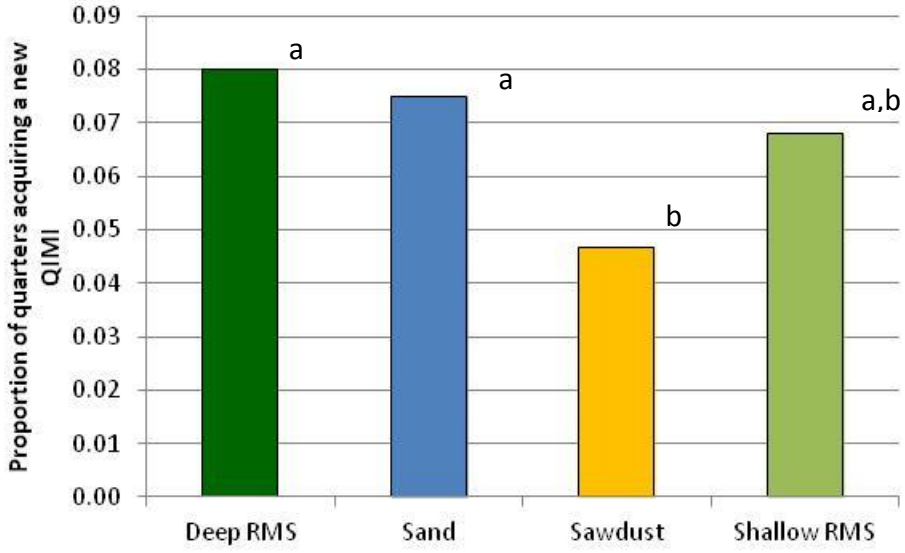
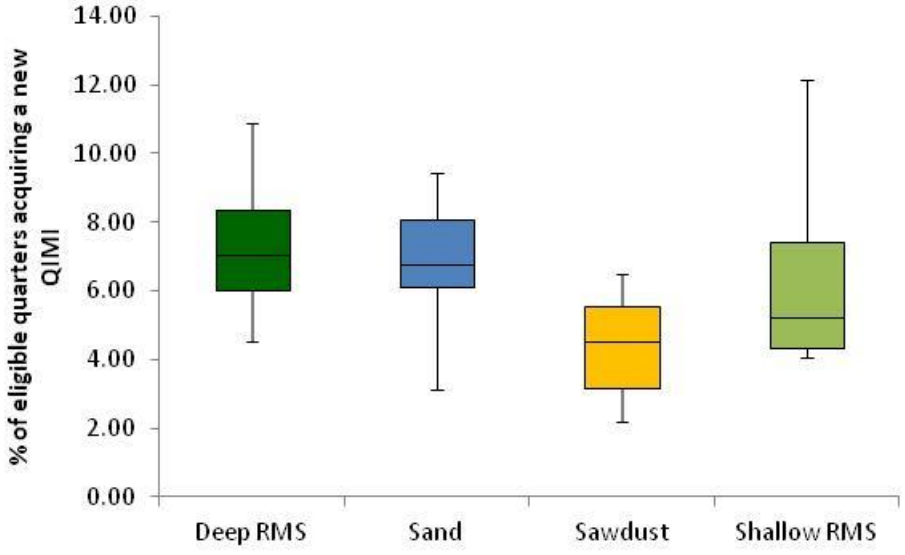


Table 3.6: A summary of the rates of new quarter intramammary infections by bedding group and two week block.

Date of start of two week block	Bedding Material			
	Deep RMS	**Sand**	**Sawdust**	**Shallow RMS**
28/01/2015	6.52	9.45	3.33	5.56
11/02/2015	6.21	3.15	2.68	4.07
25/02/2015	7.59	7.69	4.55	7.69
11/03/2015	10.14	6.06	4.48	4.38
25/03/2015	7.75	6.80	2.17	4.92
08/04/2015	4.55	6.16	6.47	4.20
22/04/2015	5.48	6.76	6.11	7.35
06/05/2015	10.88	9.27	5.37	12.16
Mean	**7.39**	**6.92**	**4.39**	**6.29**
Median	**7.05**	**6.78**	**4.51**	**5.24**
Minimum	**4.55**	**3.15**	**2.17**	**4.07**
Maximum	**10.88**	**9.45**	**6.47**	**12.16**
25th percentile	**6.03**	**6.14**	**3.17**	**4.33**
75th percentile	**8.35**	**8.09**	**5.55**	**7.44**

Figure 3.6: An illustration of the rate of apparent new intramammary infection (QIMI), per two week 'treatment' block, by bedding group.



A total of 10 cows developed clinical mastitis during the 16 weeks of the study; these cases are summarised in Table 3.7. *Klebsiella* spp were the most frequently isolated causal organism. Whilst there was no significant difference in the proportion of cows developing clinical mastitis across the four treatment groups, there was a trend for cows bedded on RMS to be at higher risk of developing clinical mastitis than cows not bedded on RMS (7/73 *vs* 2/78; p=0.086).

Table 3.7: A summary of the cases of clinical mastitis occurring in cows during the study period

Date	Cow	Bedding at time of clinical case	Days case occurred after introduction to bedding	Previous bedding material	Diagnosis
07/02/2015	505	Shallow RMS	10	Deep RMS	*Enterococcus sccharolyticus*
19/02/2015	584	Sawdust	8	Sand	*Klebsiella oxytoca*
26/02/2015	1064	Shallow RMS	1	Sawdust	No Sample
16/03/2015	971	Shallow RMS	5	Sawdust	*E. coli*
18/03/2015	869	Shallow RMS	7	Sawdust	*Klebsiella pneumoniae*
21/03/2015	1104	Sand	10	Deep RMS	*E. coli*
29/03/2015	433	Deep RMS	4	Sand	*Klebsiella pneumoniae*
14/04/2015	1026	Deep RMS	6	Shallow RMS	*Klebsiella pneumoniae*
26/04/2015	971	Deep RMS	4	Deep RMS	*E. coli*
26/04/2015	869	Deep RMS	4	Shallow RMS	*Klebsiella pneumoniae*

Ten day average yields, collated for each group at the end of each 2 week treatment block, did not vary significantly between the treatment groups (p=0.714) being 34.0, 34.4, 34.4 and 33.5 litres for cows bedded on deep RMS, sand, sawdust and shallow RMS respectively.

3.3.3 Cow Comfort and Welfare

The number of cows, from a total of 40 per group, observed performing selected behaviours over 44 separate daily observations is summarised in Table 3.8. Throughout the whole study duration, no cows were observed lying in the passageways. The median number of cows lying down ranged from 22 on sawdust to 25 on deep RMS. There was a significant effect of group on the number of cows lying (p<0.001). Regardless of bedding type, the number of cows lying on deep beds was higher than on

shallow beds (24 *vs* 22; p<0.05). Significantly more cows were recorded as lying on deep RMS than on sawdust (p<0.05); although showing a numerical difference, the advantage of sand over sawdust did not remain significant once the correction for multiple comparisons had been made. Very few cows were observed "perching" with two feet in the cubicles and the number did not differ between treatments. There was no effect of treatment on the number of cows standing not feeding.

Table 3.8: A summary of the number of cows out of 40 performing selected behaviours in the four treatment groups - summary of 44 daily observations

Behaviour	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Lying								
	Deep RMS	44	24.2	25.0[a,c]	14	30	22.0	26.8
	Sand	44	23.7	24.0[a,b]	15	32	22.0	26.8
	Sawdust	44	21.9	22.0[c]	10	32	19.3	23.8
	Shallow RMS	44	23.0	23.0[a,c]	15	32	20.3	26.0
Perching (2 feet in cubicle)								
	Deep RMS	44	2.0	2.0	0	7	1.0	3.0
	Sand	44	1.7	1.5	0	5	0.0	3.0
	Sawdust	44	2.0	2.0	0	5	1.0	3.0
	Shallow RMS	44	1.7	1.0	0	5	1.0	2.0
Standing not feeding								
	Deep RMS	44	4.7	4.0	1	10	2.3	6.0
	Sand	44	4.6	4.5	0	10	3.0	6.8
	Sawdust	44	5.5	5.0	1	15	3.0	7.0
	Shallow RMS	44	5.0	4.5	0	13	4.0	6.0

[a,b] Values with different superscripts within columns, within parameters, differ (p<= 0.05).

The results of assessment of cow cleanliness are summarised in Table 3.9. Differences between groups were apparent after two weeks on different beds. A change to mats resulted in a deterioration in cleanliness within two weeks, regardless of the bedding material (RMS or sawdust), and a change to sand resulted in dirtier lower legs. The following differences were significant at p<0.05. Lower leg, udder and upper leg + flank were all dirtier on mats, regardless of bedding material. Udders were significantly dirtier on sawdust that on deep RMS or sand (p<0.05). Within deep beds, lower legs were cleaner on RMS than sand. Comparing the two types of RMS beds, both lower and upper leg were cleaner on the deep RMS, but there was no difference in udder cleanliness.

Table 3.9: A summary of cow cleanliness after the first two week period on each of the bedding treatments

	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Udder								
	Deep RMS	40	1.18	1[a]	1	3	1	1
	Sand	40	1.38	1[a]	1	3	1	2
	Sawdust	39	1.67	2[b]	1	3	1	2
	Shallow RMS	40	1.25	1[a,b]	1	3	1	1
Upper leg and flank								
	Deep RMS	40	1.43	1[a]	1	3	1	2
	Sand	39	1.38	1[a]	1	3	1	2
	Sawdust	39	1.85	2[b]	1	3	1	2
	Shallow RMS	40	1.75	2[b]	1	4	1	2
Lower leg								
	Deep RMS	40	1.63	2[a]	1	2	1	2
	Sand	39	1.85	2[b]	1	3	2	2
	Sawdust	40	2.38	2[c]	1	4	2	3
	Shallow RMS	40	2.20	2[c]	2	4	2	2

[a,b] Values with different superscripts within columns, within parameters, differ ($p <= 0.05$).

3.3.4 Impact of Environmental Conditions on Attributes of Bedding (DM and Bacteriology)

Data was explored in an attempt to identify any correlations and associations between bedding bacterial counts and other attributes (*eg* dry matter) and environmental conditions both within and outside the shed. However, no consistent, repeatable correlations were found, though it was apparent that different bedding may respond in ways to different combinations of temperature, humidity and airflow, with inorganic bedding and deep beds being relatively less influenced by these factors.

3.4 Discussion

This study represents one of the most comprehensive investigations of the impact of different bedding materials and bedding regimes on udder health and milk quality conducted to date, being one of the few carefully controlled studies to investigate this particular topic.

The findings of this study are not inconsistent with those of the current survey and results from other published studies looking at the influence of bedding on udder health and cow comfort. However, the study is obviously constrained by virtue of the fact that it was conducted over only four months, on only one site, and encompassing only one management technique for each of the bedding materials/bed designs.

Analysis of bacteria numbers in bedding has confirmed previously held beliefs, though the lower '*Streptococcus* spp' counts in deep beds suggests that the environment in these beds is less conducive to their growth. This finding is of interest as these are potential udder pathogens, but also because these counts also encompassed the enterococci and these may be important in any perpetuation of antimicrobial resistance. Interestingly the higher '*Streptococcus* spp' counts in bedding were the only

ones to be reflected in higher counts in milk. *Listeria* spp were more prevalent in bulk milk samples from cows bedded on sand which also reflected the higher prevalence of *Listeria* spp in used sand bedding.

From the perspective of udder health and overall milk quality, the conclusion might be drawn that sawdust on mats is the most suitable bedding material for cows; however, in this study, sawdust was applied twice daily compared to RMS twice weekly and sand once a fortnight, so it could be that different management of the bedding materials could influence these results. Looking at the data, it could be concluded that counts in used sawdust are already very high, despite fresh bedding being applied twice daily and bedding being removed by cow habitation - in fact in many instances bacterial numbers in sand are lower after two weeks than in sawdust after 12 hours; this observation is supported by the findings of the farm survey conducted as part of this research.

Unlike the assessment of udder health using SCCs, the analysis of clinical mastitis suggests that RMS as a bedding material may increase the risk of clinical mastitis. This is an area that warrants further research; it is difficult to draw definitive conclusions with respect to clinical mastitis as one cannot necessarily assume that the intramammary infection was acquired immediately prior to presentation with clinical signs. In fact, this is highlighted in this study by cow 869 which suffered a recurrent episode of clinical mastitis due to *Klebsiella pneumoniae*. *Klebsiella* spp were by far the most common diagnoses supporting the anecdotal reports of the importance of this pathogen in RMS bedded systems and further confounding the analysis of clinical mastitis given the proclivity of this organism to cause persistent infection.

An interesting observation from this study is the lack (with perhaps the exception of *Listeria* spp and *Streptococcus* spp) of correlation between bacterial numbers in bedding and in milk. Analysis suggests that the proportional sampling device worked well and there was minimal 'carry over' as evidenced by the lack of *Listeria* spp found in samples collected after cows were milked that had been housed on sand. This lack of correlation can probably best be explained by the thorough pre-milking routine adopted on the farm which would have minimised transfer to milk via contaminated teats.

In this study *Listeria* spp were found in the unused sand. This is perhaps not unsurprising given that this organism is commonly found in soil and the sand was sourced from a local quarry rather than from the coast. The identification of *Listeria* spp in both the unused sand samples in the first month of the study prompted the authors to seek an alternative source of sand. This separate source also proved to be intermittently contaminated with *Listeria* spp. The presence of *Listeria* spp in sand bedding was also associated with the presence of this organism in milk from cows housed on this material – this may in part have been explained by the anecdotal reports of sand being more difficult to remove from cows' teats prior to milking.

With respect to cow comfort there would appear to be distinct advantages of deep beds, and possibly of deep RMS over sand. Based on the very simplistic assessment presented here, deep beds, and within these, RMS, appear to be associated with more cows lying at a standard time. However, such a "snapshot" of lying is a crude measure of cow comfort. Analysis of data including number and length of lying bouts, collected using the 'IceQube®' pedometers employed alongside this study will allow more detailed analysis and may afford a better insight into any long term benefits. It was surprising that differences in cow cleanliness, between treatment groups, became apparent within 2 weeks. Making

the assumption that all groups began the trial at an equal level of cleanliness, acquired on deep RMS, a change to mats resulted in a deterioration in cleanliness, regardless of the bedding material, and a change to sand resulted in dirtier lower legs. Although staff milking the cows remarked on the fact that both sand and sawdust were more difficult to remove from the teats, the cleanliness scoring (performed by the same staff) did not indicate that udders were visually significantly more dirty on sand than RMS. The crossover design of the study did not allow a longer term analysis of the impact on cow cleanliness which over time may have a cumulative effect on udder hygiene, health and milk quality.

3.5 Conclusions

There were significant differences in the bacterial challenge to teats in different types of bedding and between bed designs when considering RMS.

With the exception of *Streptococcus* spp and *Listeria* spp there was no clear relationship between bacterial numbers in bedding and in bulk milk, although this may, in part, reflect the hygiene practices during milking and the challenges incumbent in preparing teats on cows arriving from beds constructed with three different bedding materials.

In this study, sawdust, applied to mats twice daily, appeared to offered the best protection against new intramammary infection (as measured by SCC).

Deep beds offered the highest level of cow comfort, although RMS was relatively protective when used on shallow beds.

4 Bed Building Study

4.1 Introduction

Deep beds of recycled manure solids can provide comfortable beds for cows. However, due to the nature of the material, farmers have reported that the bedding may not dry out optimally if a large amount of bedding is initially put into the bed during the process of bed establishment. In addition, heating may occur, probably as a result of composting and microbial activity. Such microbial growth could have an effect on udder health. As a consequence, it has been suggested that building up the beds gradually may be preferable to an initial application of a large amount of bedding. In addition, it is not known whether the presence of cows during the bed building phase affects the dry matter (DM) and temperature of the beds. This experiment was designed to test whether building beds gradually was associated with an increase in the DM content of the bedding and less heating. In addition the impact of the presence of cows during the bed building phase was assessed.

Hypotheses:

1) Creating a deep bed by adding layers gradually will minimise heating of the bed.
2) Creating a deep bed by adding layers gradually will result in a dryer bed.
3) The absence of cows during the bed building process will result in a more predictable process resulting in less heating and a dryer bed.

4.2 Methods

4.2.1 Experimental Site

The trial was carried out at Sewborwens Farm, Newton Rigg, Cumbria, in a cubicle shed housing early lactation cows (eaves height 6-8 m). One side of the shed was fitted with curtains which could be automatically operated to adjust ventilation. Up to the 12th April, these remained closed; thereafter, they were set to close if the ambient temperature fell below 5^{o}C.

4.2.2 Experimental Design

In total, sixteen cubicles were used for the trial, with 4 cubicles allocated to each of four treatments:
Treatment 1: Beds filled to capacity with RMS on day 1 with cows having access (Rapid Fill Cows -RC)
Treatment 2: Beds filled to capacity with RMS on day 1 with no cows having access. (Rapid Fill no Cows -RNC)
Treatment 3: Beds filled with shallow layers of RMS daily, aiming to reach full capacity on Day 7 with cows having access. (Slow Fill Cows -SC)
Treatment 4: Beds filled with shallow layers of RMS daily, aiming to reach full capacity on Day 7 with no cows having access. (Slow Fill no cows -SNC)
In addition to the 16 trial cubicles there were two “buffer cubicles” located between treatments 1 and 2, and 3 and 4 respectively, as illustrated in Figure 4.1. Measurements were not made in these cubicles.
The trial was replicated twice in two different sets of cubicles in the same shed. The first replicate commenced on 16th March 2015 and the second on 13th April 2015.

4.2.3 Bed Preparation

On day one existing RMS bedding was removed from the cubicles, until the solid base of hard-core and compacted organic material from the previous established beds was reached, taking care not to disturb the aggregated surface. The design of the beds and previous use meant that the depth was somewhat variable, but at the rear of the cubicles, the hard-core base surface was 10-15 cm below the concrete "heelstone".

Freshly separated manure solids were placed in the cubicles, to a depth of approximately 15-25 cm for treatments 1 and 2, and 4-5 cm for treatments 3 and 4.

Thirty-four cows were given access to the trial area which included another 18 deep bedded RMS cubicles not used in the study. Cows were excluded from cubicles in treatments 2 and 3 by means of a rope tied across the back of these cubicles.

On days two to seven, additional freshly prepared bedding was added to treatments 3 and 4, in layers of approximately 3 cm depth. No further bedding was added to treatments 1 and 2 during this time.

4.2.4 General Management

In accordance with the main herd management practices, any surface dung was removed from cubicles twice daily, when cows were absent for milking. After day eight, the beds in the occupied treatments were topped up twice a week with approximately 7.5 cm depth of freshly separated manure solids.

4.2.5 Measurements and Sampling

All measurements and sampling were carried out in duplicate (on the left and right) at the rear of each cubicle. Samples were taken and measurements made within a frame measuring 30 cm x 21 cm, the edge of which was placed 15 cm from the heelstone of the cubicle to coincide with the area in contact with cows' udders as illustrated in Figure 4.2.

On days one to seven, the depth of the bedding and the temperature at 2.5 cm depth were recorded daily in each cubicle. Where possible these measurements were repeated at 5 cm depth. After the first day of each trial, these measurements were made immediately prior to the addition of fresh bedding to treatments 3 and 4. On days one to seven, a sample (approximately 100g) of bedding was collected from the top 2.5 cm of the bed for determination of dry matter.

Figure 4.1: An illustration of the layout of the trial cubicles.

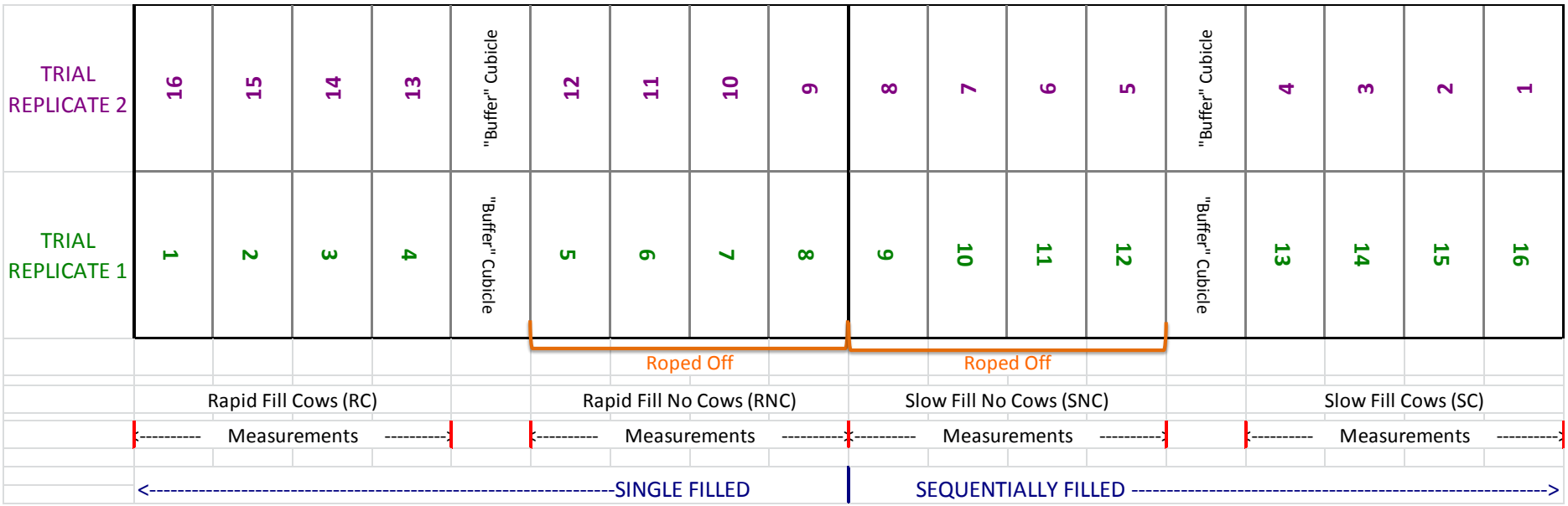
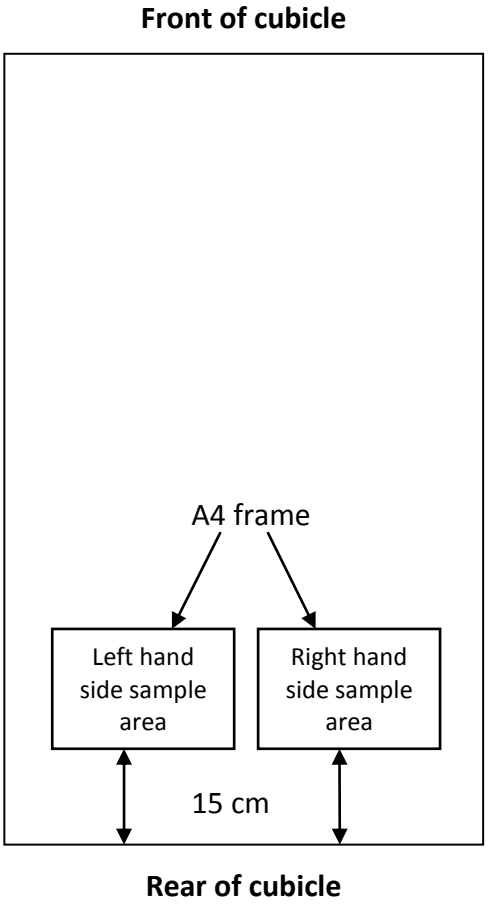


Figure 4.2: Position of measurements and sample collection



Footnote: Samples were taken from within each frame. Temperatures were taken and the depth of bedding measured at the centre of each frame.

On days eight, 15 and 22 approximately 100 g of bedding material was collected from the top 2.5 cm layer of each cubicle. Samples from within each treatment group were then comingled and thoroughly mixed prior to a subsample being taken. These samples were packed in insulated boxes with icepacks and immediately shipped to the laboratory for bacteriological analysis.

On day 29, the total depth of bedding was recorded. Temperatures were measured at 2.5 cm depth intervals and samples were collected in 2.5 cm layer intervals for DM analysis.

4.2.6 Bacteriological Analysis

Analysis of total bacterial count and coliforms was carried out as described for the survey in Chapter 2.

4.2.7 Environmental Conditions

Temperature and wind speed were collated from sensors recording these variables every 10 minutes which controlled the automatically operated curtains of the shed. The curtains remained closed throughout the first trial period. Relative humidity was monitored inside and outside an adjacent, similarly designed, shed using Digitron Monolog2® data loggers recording every hour.

4.2.8 Statistical Analysis

The two trial replicates were analysed separately as initial review of the data made it clear that the findings varied considerably between the two replicates.

Within each replicate, data for individual days of Week 1 were pooled for the purposes of statistical analysis, allowing an assessment of the overall conditions of the beds during the initial establishment week.

Data were tested for normality. Due to large variation and/or skewed distributions, non parametric tests were used to test hypotheses. The Kruskal-Wallis test was used to test the Null Hypothesis of no difference between the four treatments in temperature and DM at various depths and points in time. If the Null Hypothesis was disproved ($p<0.05$), Mann-Whitney tests were used to make pairwise comparisons, between the four individual treatments, and also testing the effect of rapid v slow fill and presence and absence of cows, with layered Bonferroni adjustment for multiple comparisons. Comparisons were only made if there were at least six values per treatment (the number of values for some measures was affected by variation in the depth of beds).

4.3 Results

4.3.1 General Observations

Assessment of the bedding at the rear of the occupied cubicles was complicated by the presence of cows as consistent bedding levels could not be maintained.

4.3.2 Replicate One

4.3.2.1 Environmental Conditions

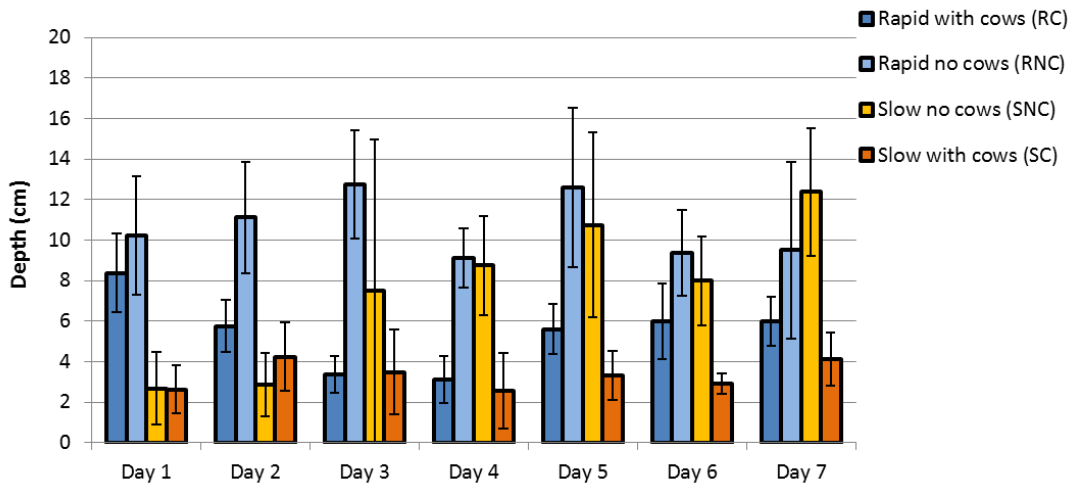
Mean daily average ambient temperature in the experimental shed was 10^{o}C during week one (range 6.2 to 13.8^{o}C). Daily mean relative humidity (RH) was 66% in the adjacent shed (range 45% to 82%) and 61% in the external environment (range 49% to 85%).

There was a tendency for bedding DM to increase with ambient temperature in rapid filled cubicles, and decrease with ambient temperature in slow filled. Relationships between bedding DM content and relative humidity at the time of sampling, were inconsistent.

4.3.2.2 Bed Depth - Week One

The mean depth of beds on each day in the first week of the trial is illustrated in Figure 4.3. As would be expected, beds were deeper in the absence of cows, as bedding was not compacted or removed by cows during use. In the absence of cows the slow fill beds gradually increased in depth during the first week, whereas bed depth did not increase in the presence of cows.

Figure 4.3: All illustration of the depth of beds in each treatment during week one of Replicate 1 (mean and SD).



4.3.2.3 Bed Temperatures - Week One

Temperatures at 2.5 cm and 5 cm depth are illustrated in Figures 4.4 and 4.5. Temperatures in all treatments increased up to day three, and RC and SNC increased further on day four. In general all treatments followed the pattern of ambient temperature. For the first two days, all bedding materials were above ambient temperature. From day three onwards, SNC was very close to ambient temperature, whilst the other treatment groups remained above the ambient temperature. The rapidly filled cubicles in which cows were present typically exhibited the highest temperatures.

Figure 4.4: An illustration of median bed temperatures at 2.5 cm depth over the first week of Replicate 1 (Ambient temperatures are illustrated for comparison).

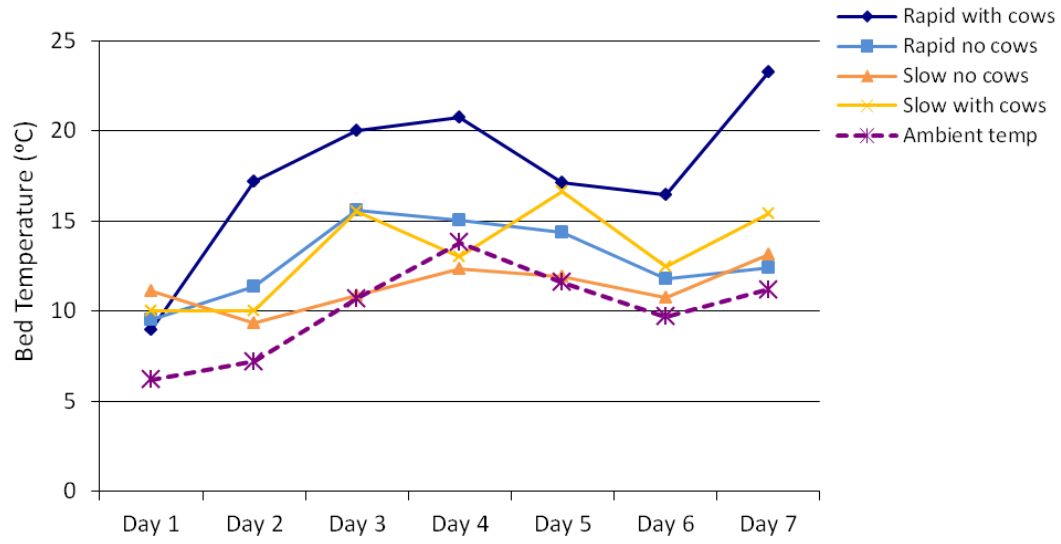
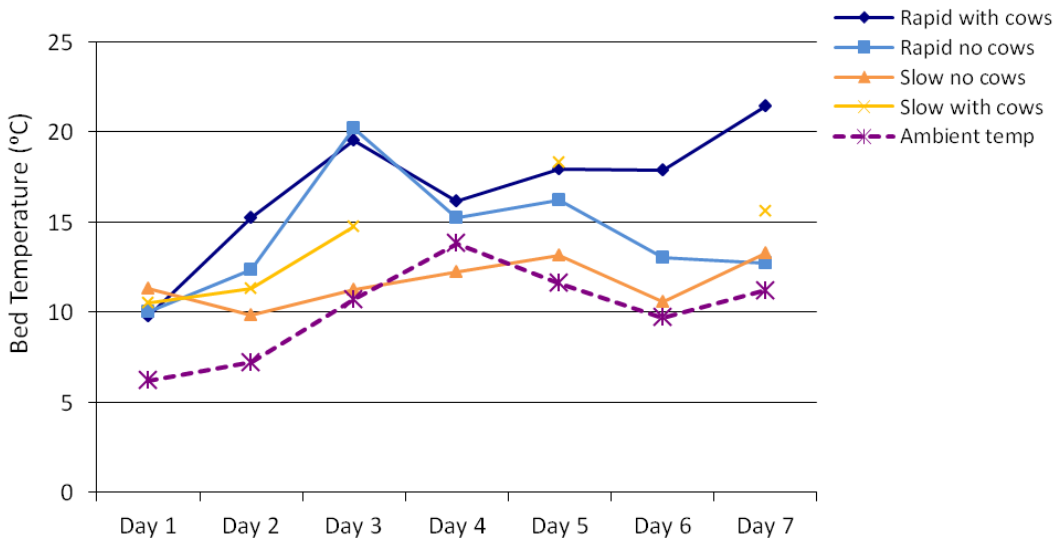


Figure 4.5: An illustration of median bed temperatures at 5 cm depth over the first week of Replicate 1 (Ambient temperatures are illustrated for comparison).



*NB Data points excluded where insufficient (<6) measurements are available.

Summary statistics for pooled data across all seven days for each treatment are presented in Table 4.1. There was a wide range of temperatures within each treatment during the week. Median temperature at 2.5 cm varied significantly between treatment groups ($p<0.0001$), being highest in RC (17.0°C) and lowest in SNC (11.4°C). Temperature was higher with rapid filling (14.3°C v 11.6°C, $p<0.001$) and with cows (14.5°C v 12.0°C, $p<0.001$). Pairwise comparisons showed that the median temperature at 2.5 cm was significantly higher in RC than in all other treatments ($p<0.001$). RNC was significantly higher than SNC (12.4 vs 11.4°C ($p<0.001$)), but RNC did not differ from SC or SC from SNC.

Median temperature at 5 cm depth showed a similar range within treatments. Treatments were ranked in the same order as for temperature at 2.5 cm and showed the same individual significant differences.

Table 4.1: Summary statistics for pooled data on depth, temperature and surface DM of bedding from all days of week one - Replicate 1.

Parameter	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Depth (cm)	RC	56	5.5	5	2	11	4	7
	RNC	56	10.5	10	0	20	9	12
	SC	56	3.3	3	1	8	2	4
	SNC	56	7.6	7	1	25	3.3	10
Temp at 2.5 cm (^{o}C)	RC	56	18.3	17.0^{a}	8.3	31.6	12.5	24.6
	RNC	56	13.3	12.4^{b}	9	28.9	11.3	14.6
	SC	56	14	12.3^{bc}	9	28.5	10.5	15.9
	SNC	56	11.5	11.4^{b}	8.7	19.9	10.4	12.4
Temp at 5 cm (^{o}C)	RC	50	17.5	17.2^{a}	9.4	28	13.4	23
	RNC	56	14.4	13.2^{b}	9.7	36.1	12.2	15.6
	SC	35	14.2	11.8^{bc}	9.5	24.9	10.6	18
	SNC	56	11.8	11.7^{b}	9	16.8	10.6	12.8
Dry Matter (%)	RC	56	39.3	38.3^{a}	33.5	47.2	37.1	41.2
	RNC	55	38.6	38.7^{a}	33.2	44.5	36.9	39.7
	SC	56	38	38.4^{a}	28.3	47.2	35.8	39.5
	SNC	56	37.2	36.5^{b}	31.9	52.5	34.8	38.4

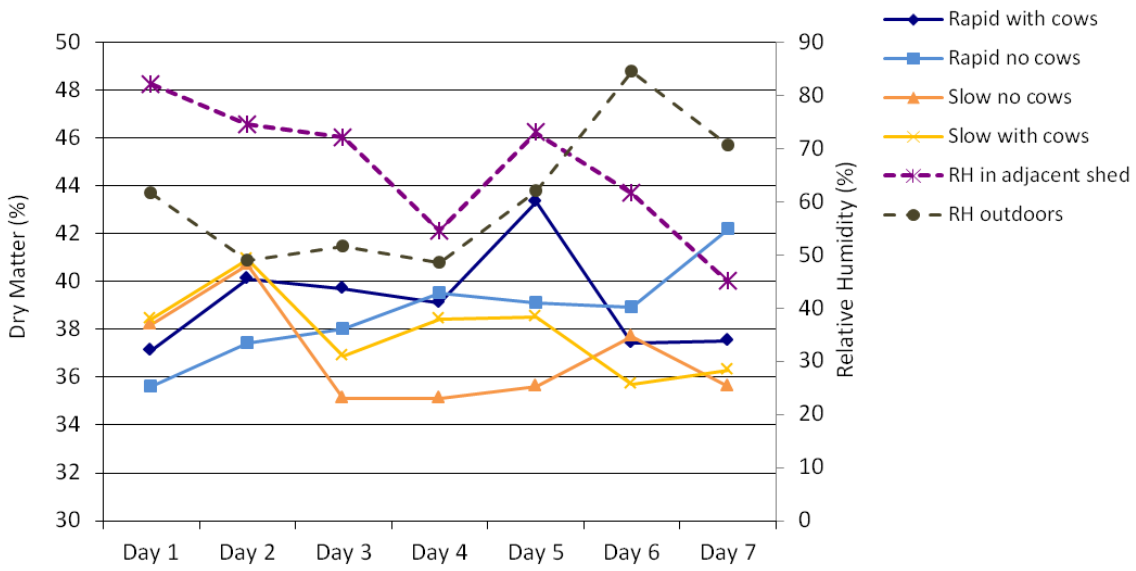
^{a,b} Values with different superscripts within a parameter differ ($p<0.05$)

4.3.2.4 Dry Matter - Week One

Median dry matter of samples from the surface 2.5 cm are illustrated in Figure 4.6. The slow fill treatments showed a greater range of DM content over the first week than the rapid fill treatments (see Table 4.1). There was a significant effect of treatment on DM. Rapid fill resulted in higher DM than slow filling (38.6% v 37.5%; $p<0.001$) and beds with cows were drier than those without (38.4% v 37.9%, $p<0.05$). Comparing all treatments, DM was highest for RNC (38.7%) and lowest for SNC (36.5%). Both RC ($p<0.0001$), and RNC ($p<0.001$) were drier than SNC.

The DM content did not show any clear relationship with the ambient temperature or relative humidity measured outdoors or in an adjacent shed within any of the treatments.

Figure 4.6: An illustration of median bedding DM content, at 2.5 cm depth, over the first week of Replicate 1. (Relative humidity is illustrated for comparison).



4.3.2.5 Results after Four Weeks - Replicate 1

Descriptive statistics for depth, and temperature and DM content at 2.5 and 5 cm depth, for all treatments in Replicate 1 after four weeks are summarised in Table 4.2.

Mean depths of beds on Day 29 are shown in Figure 4.7. After four weeks, both treatments with cows had stabilised at a depth of approximately 6 cm prior to the addition of fresh material.

Figure 4.7: An illustration of the depth of beds in each treatment after 4 weeks in Replicate 1 (mean and SD).

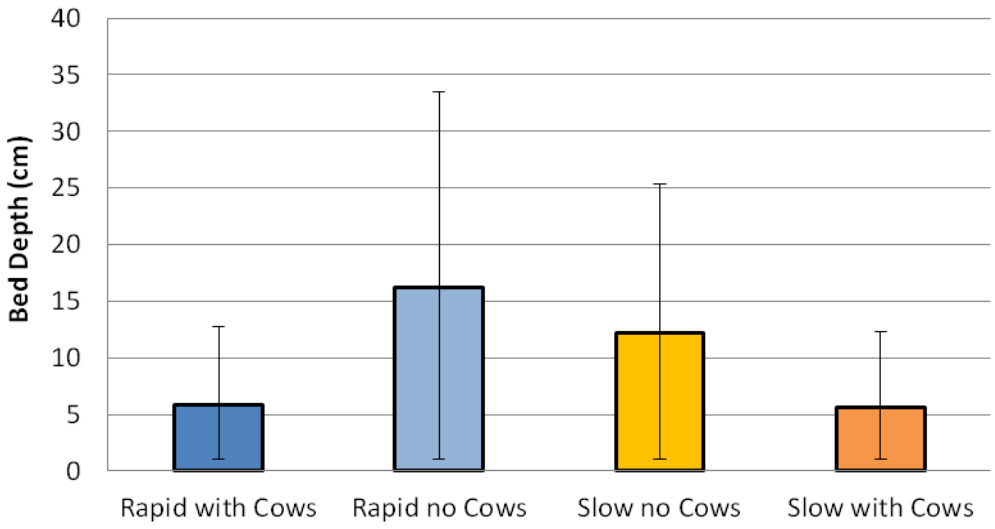


Table 4.2: Summary statistics for pooled data on depth, temperature and surface DM of bedding after four weeks in Replicate 1.

Parameter	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Depth (cm)	RC	8	5.8	5	5	7.5	5	7
	RNC	8	16.3	15	10	25	12.5	20
	SC	8	6.3	6.3	5	7.5	5	7.5
	SNC	8	11.3	10	7.5	17.5	10	12.5
Temp at 2.5 cm (^{o}C)	RC	8	19.6	20.7	12	25	14.5	23.6
	RNC	8	14.3	13.7	11	20.2	12.5	15.5
	SC	8	12.7	11.9	10.2	17.9	11	14
	SNC	8	12.4	12.2	11.7	13.5	11.9	13.2
Temp at 5 cm (^{o}C)	RC	8	20.5	21.1^{a}	15	24.5	17.5	23.4
	RNC	8	18.4	17.1^{ac}	13.2	28.7	14.1	21.5
	SC	8	12	12.7^{bc}	1.2	20.5	11.3	13.3
	SNC	8	13.6	13.4^{b}	12.4	15.2	13.1	14
DM at 2.5 cm (%)	RC	8	47.4	46.5	36.1	60.2	39.8	54.1
	RNC	8	54.7	52.7	45.2	65.2	49.6	62.9
	SC	8	59.4	61.1	45.1	70.1	55.9	63.2
	SNC	8	62.6	63.7	46.2	80.2	48.9	76
DM at 5 cm (%)	RC	8	39.5	37.4^{ac}	33.5	48.8	35.2	45.3
	RNC	8	41.4	40.8^{ac}	36.2	49.5	38.5	43.3
	SC	8	56.6	58.1^{bc}	41.9	66.4	52.5	61.8
	SNC	8	51	48.7^{ad}	38.3	76.2	39.9	59.8

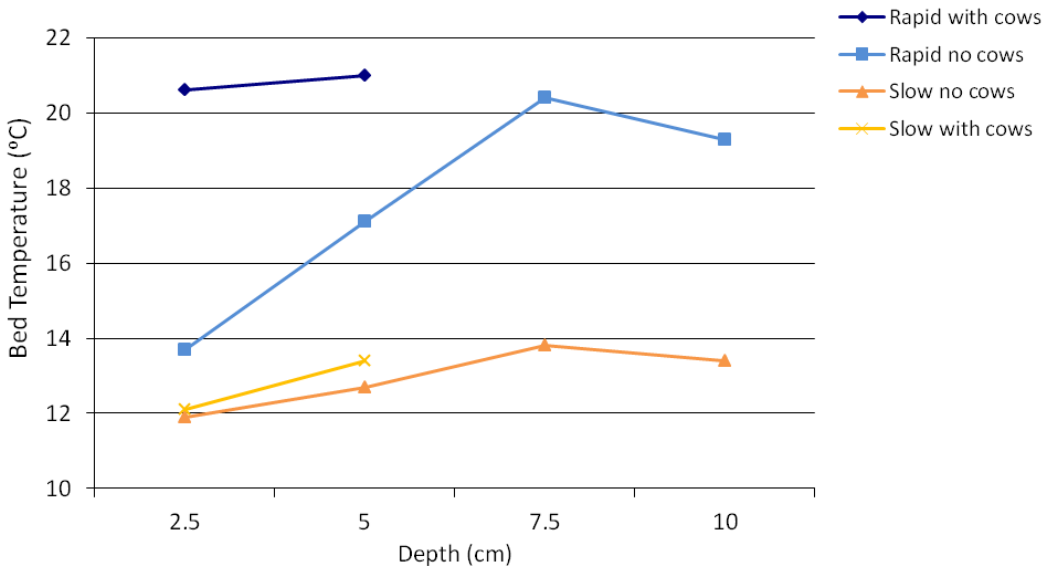
^{a,b} Values with different superscripts within a parameter differ ($p<0.05$)

Temperatures, at different depths, after four weeks are shown in Figure 4.8. Temperature increased with depth, most markedly in the RNC treatment. Due to variation in the depth of the beds, statistical comparison could only be made between all four treatments at 2.5 and 5 cm depths.

At 2.5 cm, median temperature ranged from 20.6^{o}C for RC to 11.9 for SNC. Although the Kruskal-Wallis test indicated a significant effect of treatment on temperature at 2.5 cm depth ($p<0.05$), and that rapid filled beds were warmer than slow filled beds (15.3^{o}C v 12.1^{o}C; $p<0.001$), when individual pair-wise comparisons were made, significant differences could not be identified.

Temperature at 5 cm depth varied significantly by treatment ($p<0.0001$). Values were higher for rapid fill than slow fill beds (20.1^{o}C v 13^{o}C; $p<0.001$) but the presence of cows did not have a significant effect (15.1^{o}C with cows vs 13.6^{o}C without; $p=0.152$). The temperature of rapid filled beds in the presence of cows (RC) (21.1^{o}C) was higher than both SC (12.7^{o}C; $p<0.01$)) and SNC (13.4^{o}C; $p<0.05$), RNC (17.1^{o}C) was also higher than SNC ($p<0.05$).

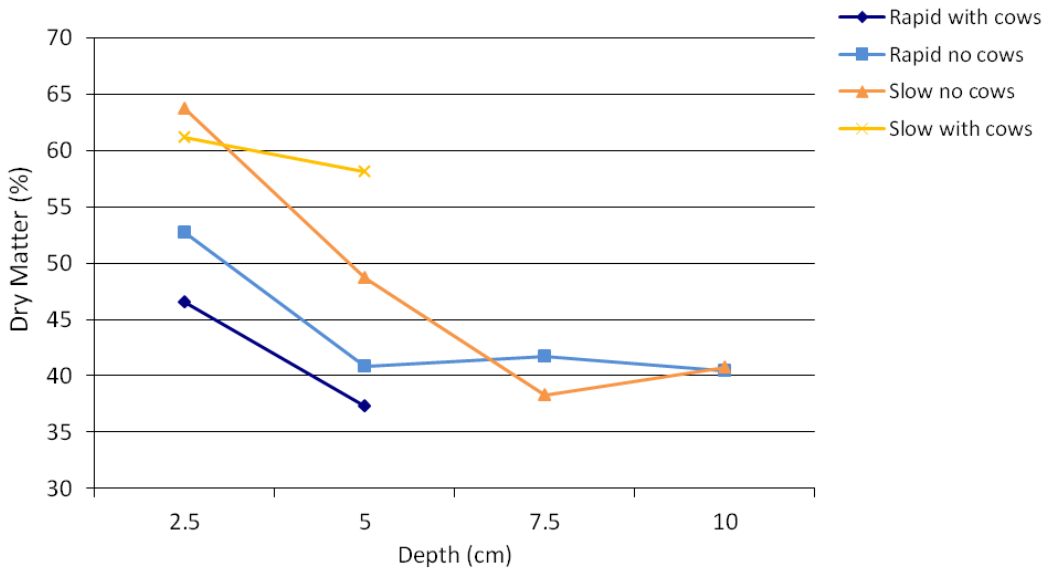
Figure 4.8: An illustration of median temperatures on Day 29 of Replicate 1, at depths where at least 6 data points were available.



Footnote: Bedding depth was reduced in occupied beds due to cows lying and compacting the bedding material.

Dry matter content of bedding various depths is illustrated in Figure 4.9. The Kruskal-Wallis test indicated a significant effect of treatment on the DM content of surface material on Day 29 ($p<0.05$). In this instance, the slow fill treatments reached higher DM than the rapid fill treatments (62.5% v 50.8%; $p<0.05$). The presence of cows overall did not have a significant effect (median 55.6% with cows and 55.5% without). Despite a clear visual trend in the plotted medians, no individual pairwise comparisons between the four treatments remained significant after a layered Bonferroni adjustment.

Figure 4.9: An illustration of median dry matter content of bedding material on Day 29 of Replicate 1, at depths where at least 6 data points were available.



Footnote: Bedding depth was reduced in occupied beds due to cows lying and compacting the bedding material.

At 5cm depth, the treatment effect was significant at $p<0.01$. Slow filling resulted in higher DM (53% v 40%; $p<0.001$) but the effect of presence of cows was not significant (51% with cows vs 42% without; $p = 0.763$). Comparing all four treatments, SC (58.1%) was significantly drier than both RC (37.4%) and RNC (40.8%) ($p<0.05$).

In summary, Replicate 1 demonstrated that bedding in rapid filled cubicles reached higher temperatures during the week of establishment. Although rapid filling overall resulted in higher DM content, the only significant difference in week one DM content between the four separate treatments, was a lower value in SC (36% v 38-39%). After four weeks the influence of speed of filling on temperature was still apparent (rapid building hotter), while the effect of speed of filling on DM content was reversed (slow filled now drier). The only significant individual treatment differences were higher temperatures at 5 cm depth in RC than other treatments, and higher DM in SC compared with the two rapid filled treatments. The effect of occupation by cows was no longer significant. From a practical point of view, the slow built beds were significantly cooler at 5 cm depth and tended to have a higher DM content at Day 29 than rapidly filled beds.

4.3.3 Replicate Two

4.3.3.1 Environmental Conditions

Mean ambient temperature during week one in replicate 2 was 11.9°C, (range 9.4°C to 15°C). This mean was 1.9°C higher than for week one of Replicate 1. Relative humidity in the adjacent shed was 52% (range 40 to 66%), and in the external environment was 63% (range 5 to 74%) (lower than for Replicate 1). There were weak positive relationships between bedding DM content and ambient temperature at time of sampling for all treatments, and weak negative relationships between bedding DM content and relative humidity at the time of sampling.

4.3.3.2 Bed Depth - Week One

Bed depths in week one of Replicate 2 are illustrated in Figure 4.10. Rapid fill beds were filled to a greater initial depth in Replicate 2 - (mean initial depth 24 cm for RNC). By day seven the mean depth of RC was 2.8 cm (compared with 3.3 cm in Replicate 1).

Summary statistics for depth, temperature and DM content of beds in week one of Replicate 2 are shown in Table 4.3.

Figure 4.10: All illustration of the depth of beds in each treatment during week one of Replicate 2 (mean and SD).

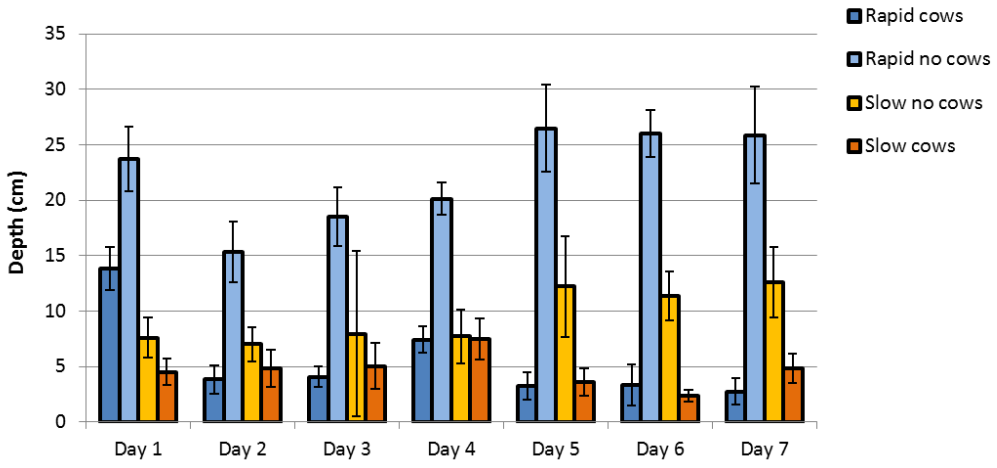


Table 4.3: Summary statistics for pooled data on depth, temperature and surface DM of bedding from all days of week one - Replicate 2.

Parameter	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Depth (cm)	RC	56	5.5	4	1	16	3	6.1
	RNC	56	22.3	22.6	10	35	17.1	26.8
	SC	56	4.7	5	1	10	3	6.2
	SNC	56	9.6	8.6	4	19	6.8	12.2
Temp at 2.5 cm (^{o}C)	RC	56	20.4	20.0^{a}	11.5	32	15.6	23.7
	RNC	56	23.5	19.0^{b}	12.1	51.6	15.6	27.6
	SC	56	14	14.0^{c}	10.5	22.1	12.4	15.4
	SNC	56	14.5	14.2^{c}	11.2	21	13.1	15.4
Temp at 5 cm (^{o}C)	RC	48	21.6	21.6^{a}	12	31.2	18.1	25.3
	RNC	48	28.7	23.8^{a}	13.8	58.8	17.7	42.8
	SC	48	14.1	13.8^{b}	10.9	21	12.8	14.8
	SNC	48	15.3	14.3^{b}	12.4	22.3	13.5	16.1
Dry Matter (%)	RC	50	39.46	38.1^{a}	32.4	62.4	35.2	42.2
	RNC	46	37.81	36.5^{a}	26.1	56.7	33.3	41.4
	SC	46	44.39	41.7^{b}	32.4	66.9	38.4	47.5
	SNC	48	41.99	39.3^{ab}	33.4	65	36.3	45.9

^{a,b} Values with different superscripts within a parameter differ ($p<0.05$)

4.3.3.3 Bed Temperatures - Week One

Bedding surface temperatures followed the general pattern of ambient temperature as illustrated in Figures 4.11 and 4.12. Slow fill treatments were very close to ambient temperature in absolute value, while rapid fill treatments were higher, particularly in the absence of cows (RNC). RNC showed the greatest range in temperature at both depths. RNC had the highest temperatures for days one to four but fell below RC from day five onwards. The peak daily median temperature reached (in RNC) was 43^{o}C on day 2; 19^{o}C higher than the Replicate 1 peak of 24^{o}C on day seven in the RC treatment. Peaks at 5 cm showed a similar trend.

There was a significant effect of treatment on temperature at 2.5 cm ($p<0.0001$). Median temperature with rapid fill beds was higher than with slow fill beds (20^{o}C vs 14.2^{o}C; $p<0.0001$). The effect of cows present was not significant (median 15.4^{o}C with and without cows). The highest median temperature was for RC treatment (20.0^{o}C) followed by RNC (19.0^{o}C), SNC (14.2^{o}C) and SC (14.0^{o}C). All pairs of treatments differed significantly ($p<0.0001$) apart from SC and SNC. There was also a significant effect of treatment on temperature at 5cm depth ($p<0.0001$). At 5 cm rapid fill beds were also hotter (22.2^{o}C vs 14.0^{o}C, $p<0.0001$) and whilst the effect of cows was insignificant, there was a trend for treatments with cows present to be cooler (15.8^{o}C vs 17.0^{o}C). All treatments differed at 5 cm depth ($p<0.0001$) apart from RC and RNC, and SC and SNC. The ranking of individual treatments was altered, at 5 cm compared to 2.5 cm, in that the median temperature for RNC (23.8^{o}C) exceeded that for RC (21.6^{o}C) ($p<0.0001$). This suggests that speed of filling had more influence on temperature than did the presence of cows.

Figure 4.11: An illustration of median bed temperatures at 2.5 cm depth over the first week of Replicate 2. (Ambient temperatures are illustrated for comparison).

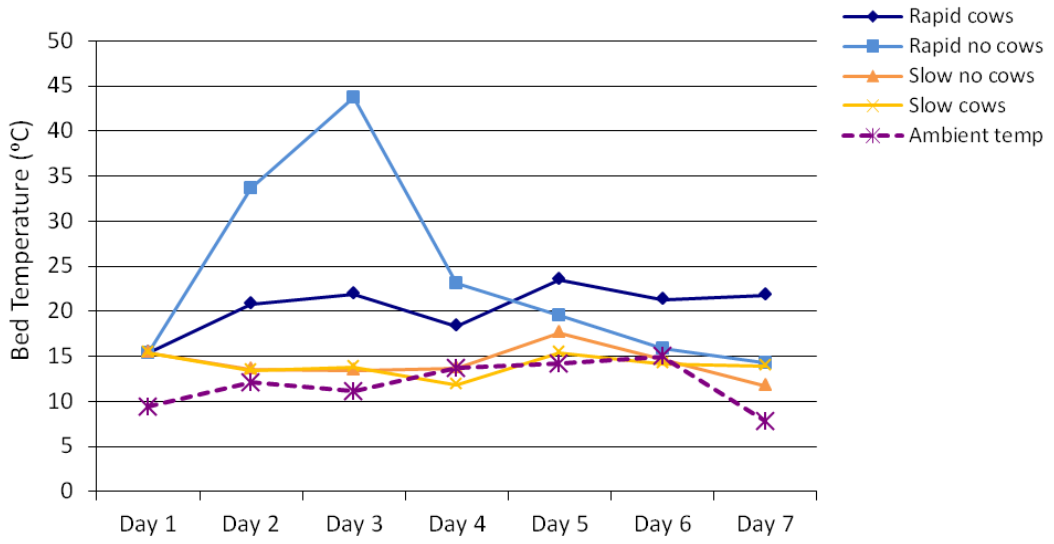
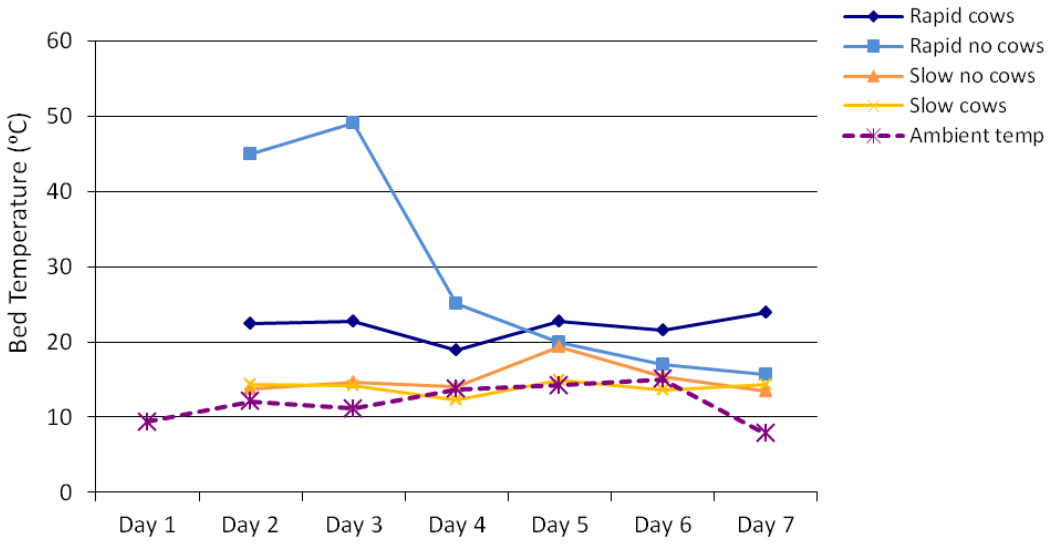


Figure 4.12: An illustration of median bed temperatures at 5 cm depth over the first week of Replicate 2 (Ambient temperatures are illustrated for comparison).

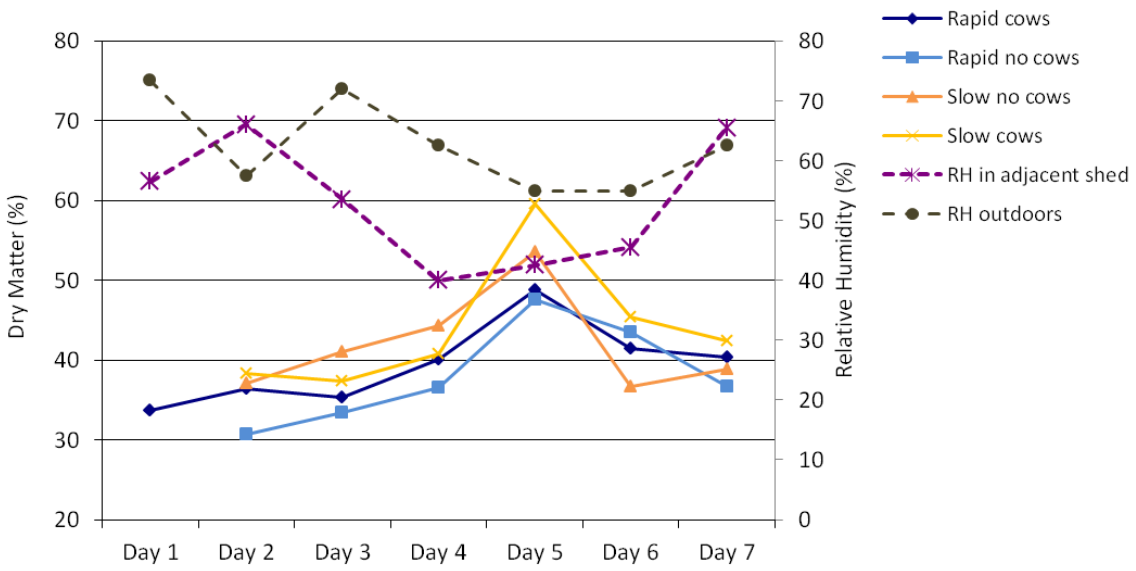


4.3.3.4 Dry Matter - Week One

The DM content of the initial material was lower for Replicate 2 (33%) than for Replicate 1 (37%). However, higher DM contents were eventually reached in week one of Replicate 2 than in Replicate 1.

In Replicate 2, all treatments increased in DM content up to a peak on day five, before falling as illustrated in Figure 4.13. The peak corresponded with a trough in external relative humidity. Median DM% across the first week varied significantly between treatments ($p<0.0001$). In this replicate, DM contents were significantly higher for the slow filled treatments (40.9% vs 37.1%; $p<0.0001$). When comparing between bed building methods, DM was significantly higher in SC compared to RC ($p<0.05$), SNC compared to RNC ($p<0.05$) and SC compared to RNC ($p<0.001$).

Figure 4.13: An illustration of median bedding DM content at 2.5 cm depth over the first week of Trial 2. (Relative humidity is illustrated for comparison)



4.3.3.5 Results after Four Weeks - Replicate 2

Depths, temperatures, and DM content after four weeks for Replicate 2 are summarised in Table 4.4.

Mean depths of beds on Day 29 during Replicate 2 are shown in Figure 4.14. As with Replicate 1 both sets of beds with cows present had stabilised at approximately 6 cm in depth.

Figure 4.14: An illustration of the depth of beds in each treatment after 4 weeks in Replicate 2 (mean and SD).

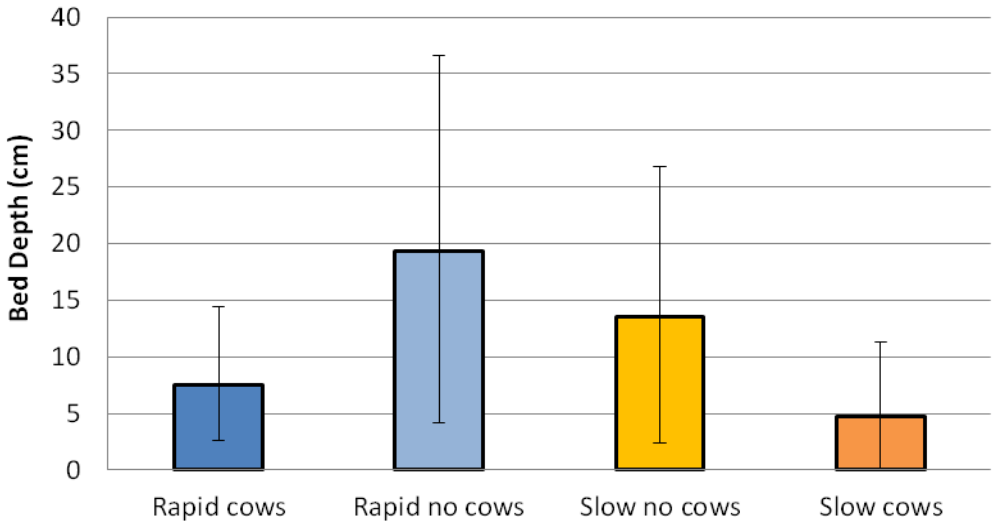


Table 4.4: Summary statistics for pooled data on depth, temperature and surface DM of bedding after four weeks in Replicate 2.

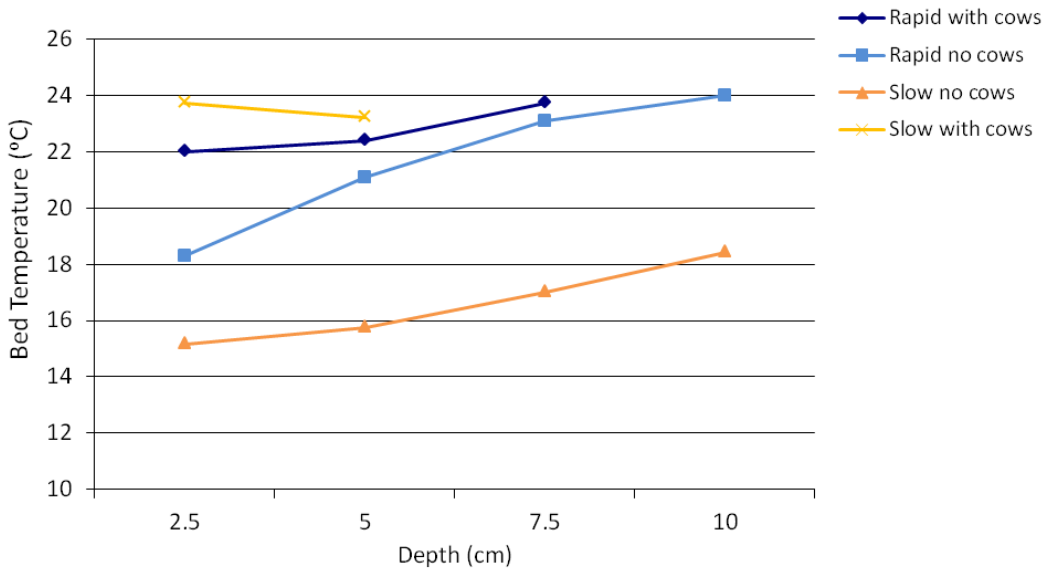
Parameter	Treatment	n	Mean	Median	Min	Max	25th Percentile	75th Percentile
Depth (cm)	RC	8	7.5	8.8	2.5	10	5	10
	RNC	8	19.4	23.8	2.5	35	10	25
	SC	8	4.7	5	2.5	10	2.5	5
	SNC	8	12.2	10	5	24	7.9	16.8
Temp at 2.5 cm (^{o}C)	RC	8	22.3	22.0[a]	19.1	26.2	19.9	24.8
	RNC	8	19.4	18.3[a]	17.3	22.7	18	21.3
	SC	8	23	23.7[a]	16.2	29.1	19	26.5
	SNC	8	15.4	15.2[b]	12.6	19.4	13.8	16.6
Temp at 5 cm (^{o}C)	RC	7	23	22.4[b]	19.7	29.1	19.8	26.2
	RNC	7	22	21.1[b]	18.5	27.2	19.5	25.6
	SC	5	23.9	23.2[b]	15.4	30	18.8	29.5
	SNC	8	15.8	15.8[a]	13.3	18.7	13.5	18
Temp at 7.5 cm (^{o}C)	RC	-	-	-	-	-	-	-
	RNC	7	24.1	23.1	19.8	30.8	21.4	28.2
	SC	-	-	-	-	-	-	-
	SNC	7	16.8	16.2	12.9	21.5	14.2	18.7
DM at 2.5 cm (%)	RC	8	47	47.3a	37.8	55.5	40.2	53.7
	RNC	8	51.3	51.9[a,c]	43.4	56.3	50.3	52.8
	SC	8	48.4	50.4[b,d]	32.1	58.5	45.7	51.5
	SNC	8	60.8	59.5[a,d]	47.9	79.2	48.1	75.4
DM at 5 cm (%)	RC	7	42.9	40.3[a]	34.9	53	36.2	49.6
	RNC	6	50.4	50.6[a,c]	45.2	55.5	46.7	54
	SC	8	50.5	48.0[b,d]	47.2	66.2	47.5	50.1
	SNC	5	41.7	40.4[a,d]	34.1	50.5	37.2	46.8
DM at 7.5 cm (%)	RC	-	-	-	-	-	-	-
	RNC	7	47.6	47.5	41.2	51.2	45.2	50.8
	SC	-	-	-	-	-	-	-
-	SNC	7	48.5	47.5	44.1	54.9	44.4	52.4

[a,b] Values with different superscripts within a parameter differ ($p<0.05$)

Median temperatures after 4 weeks are illustrated in Figure 4.15. In the absence of cows sufficient data was available for analysis to a depth of 10 cm. Comparisons could only be made between all four treatments down to 5 cm depth. With the exception of slow fill with cows present, there was a tendency for temperatures to increase with depth. At both 2.5 cm and 5 cm depth, treatment had a significant effect on bed temperature ($p<0.0001$). Rapid filled beds were hotter at 2.5 cm depth (20.0^{o}C vs 14.2^{o}C; $p<0.001$) and 5cm depth (21.3^{o}C vs 17.2^{o}C; $p<0.05$). The presence of cows was associated with a higher bed temperature at 2.5 cm (22.4^{o}C vs 17.6^{o}C; $p<0.001$) and at 5 cm depth (22.6^{o}C vs 18.5^{o}C; $p<0.01$). When comparing individual treatments, SNC was significantly cooler than all other

treatments at 2.5 cm depth ($p<0.01$) and at 5 cm depth ($p<0.05$) but no other significant differences were identified.

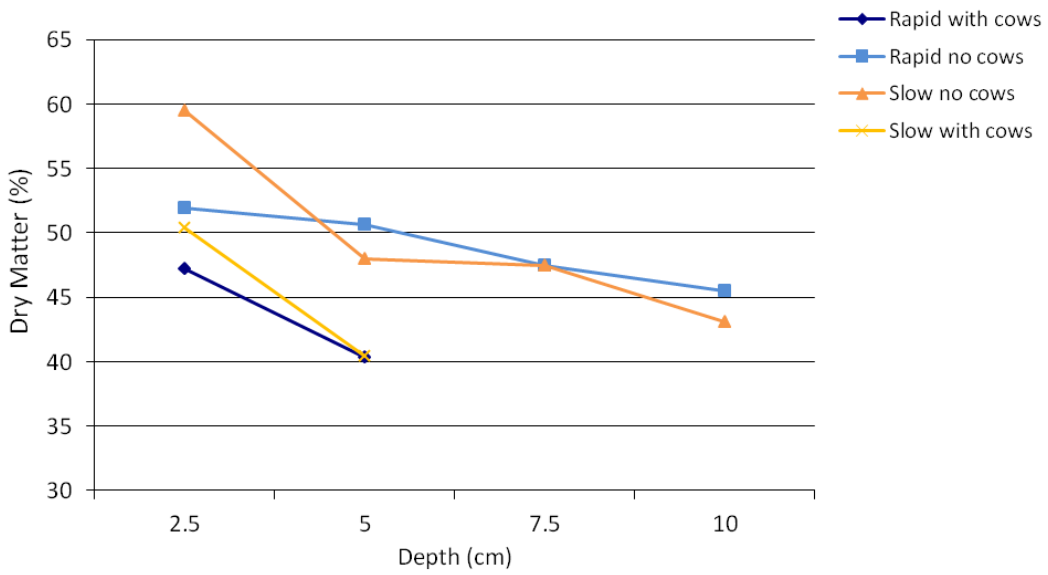
Figure 4.15: An illustration of median bed temperatures on Day 29 of Replicate 2, at depths where at least 6 data points were available.



After 4 weeks, the number of data points where dry matter could be compared across treatments was limited, due to the shallow depth of slow filled treatments. Data are illustrated in Figure 4.16. There was no significant effect of treatment on DM % at 2.5 or 5 cm depth ($p= 0.11$ at 2.5 cm; $p=0.07$ at 5 cm). Treatments without cows could be compared down to 10 cm depth, but did not differ significantly ($p= 0.848$ at 7.5 cm; $p= 0.685$ at 10 cm).

In summary, in Replicate 2, both rapid fill treatments reached higher temperatures than slow fill treatments during week one, but the presence of cows did not have a significant influence during this time. In contrast to Replicate 1, DM % was higher with slow filling during the building phase. After four weeks the temperature with cows present was higher than without, but the only significant difference between individual treatments was a lower temperature in SNC compared with other treatments.

Figure 4.16: An illustration of median dry matter content of bedding material on Day 29 of Replicate 2, at depths where at least 6 data points were available.



4.3.4 Bacterial Counts

The results of bacterial counts conducted during Replicate 1 are outlined in Table 5.6. Insufficient samples were available to facilitate a detailed statistical analysis. However, coliform counts were numerically lower in the 'slow fill' beds and in the absence of cows. Total bacterial counts appeared to be less predictable.

Table 4.6: Summary of results of bacterial counts from bedding in Replicate 1.

Time after bed creation	Rapid Fill		Slow Fill	
	with cows	without cows	with cows	without cows
Total Bacterial Count (cfu/g)				
8 days	6,100,000,000	3,030,000,000	2,450,000,000	3,190,000,000
15 days	6,150,000,000	3,100,000,000	9,150,000,000	10,000,000,000
22 days	3,700,000,000	3,715,000,000	4,250,000,000	1,930,000,000
Coliform Count (cfu/g)				
8 days	14,000,000	900,000	245,000	200,000
15 days	9,000,000	1,500,000	2,400,000	1,550,000
22 days	22,000,000	2,550,000	5,250,000	1,100,000

4.4 Discussion

Rapid building of beds elevated temperatures of bedding in the building phase by 5-8^{o}C in the presence of cows and (less consistently) by up to 8^{o}C in the absence of cows. The trial thus showed some support for the hypothesis that slow building would result in lower temperatures of the material likely to be in contact with the udder during the initial period of building beds. This in part is also supported by the observation that coliform counts at the end of the first week (likely to be raised by higher temperatures) were numerically lower in slow built beds and in the absence of cows, though this could be confounded

by the fact that cows being present may have added to the population of coliforms present in the beds. After four weeks, the overall effect of speed of building on temperature, (disregarding the presence of cows) was still apparent, although the differences between individual treatments were not necessarily still the same or significant.

The DM content of the surface layer of bedding showed a less consistent relationship with speed of bed building. In both trials the speed of fill appeared to have more influence than the presence of cows. The higher DM content with rapid building in Replicate 1 was counter intuitive and was in complete contrast to the situation in Replicate 2; this might be explained by the environmental conditions allowing more rapid drying of the freshly added material with slow building in Replicate 2.

It was unexpected that the highest surface temperature reached overall was in the slow fill beds in the presence of cows (Day 29 in Replicate 2). One possible explanation is that this was as a result of SC cubicles receiving more sunlight than others. However, as this was not measured and it is an isolated observation on a single day, it is not possible to draw definitive conclusions.

The greater range of DM values recorded for the slow fill treatments in Week one of Replicate 1 is likely to have been as a result of the addition of fresh material daily. Although the thin layers added in the slow treatment might be expected to dry more rapidly than the thicker layer created by the rapid filling, the daily addition of wetter fresh material with slow building appears to have reduced the DM content, at least in the absence of cows. The difference in environmental conditions (warmer and drier in Replicate 2) may also have had an influence. The DM content of the initial material may also have influenced the temperatures reached - higher peak and median bedding temperatures were recorded for the rapid fill treatment in the absence of cows during the building phase of Replicate 2 which began with material at 33% DM compared with 37% DM for Replicate 1.

The influence of the presence of cows is more difficult to interpret. There were fewer significant differences in physical parameters of the bedding material associated with presence and absence of cows than with speed of filling the beds, and the effect of presence of cows was inconsistent.

There are likely to be complex relationships between the DM of RMS bedding and environmental conditions, especially given the highly hygroscopic nature of the material, allowing it to readily absorb moisture. The two replicates did not show the consistent relationship that might be expected of higher DM in drier conditions. The closer relationship of atmospheric relative humidity in bedding dry matter in Replicate 2 than in Replicate 1 might be related to the higher ambient temperature in Replicate 2 and this influence this will have had on the dewpoint.

In the early stages of slow building the depth of the beds would have provided reduced comfort in comparison with the rapidly filled beds.

From a practical point of view there is some evidence that slow building of beds is more likely to result in cooler and drier beds, but environmental effects including ambient temperature, relative humidity and air flow are also likely to have a significant impact.

4.5 Conclusions

Rapid building of beds elevated temperatures of bedding in the building phase, and the effect appeared to persist for four weeks. Temperature was affected more by speed of fill than by the presence of cows.

The effect of building speed on DM content at the surface during the first week and four weeks later was inconsistent.

Dry matter content of the surface material appears to be influenced by factors in addition to the speed of bed building. These may include the presence of cows, but also environmental conditions. There appears to be a complex interaction between environmental conditions, including temperature and relative humidity, and the temperature and DM of RMS bedding.

Rapid building of beds and the consequent increases in temperature and decreases in dry matter (as seen in Replicate 1) may be associated with a higher coliform count in the bedding material in the early stages of bed establishment.

Slow building will by definition limit the depth of beds and thus the comfort provided if cows are present during the building phase.

Further research is required to understand the behaviour of this material in different environmental conditions, as this may be a key to its optimal use.

5 In silico modelling of Levels of *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) and *Salmonella* in Cattle Slurry and RMS

5.1 Introduction

Johne's Disease and Salmonellosis are severe infectious diseases of dairy cattle and Salmonellosis is known to be zoonotic. Cows infected with Johne's disease (*Mycobacterium avium* subspecies *Paratuberculosis* or MAP infection) and *Salmonella* spp are known to excrete pathogens intermittently and periodically at high levels. In a disease outbreak situation, this could result in high levels of pathogenic organisms being present in slurry. However, slurry storage comprises faeces from the whole herd and additional 'dirty water' and therefore the pathogenic load from relatively few cows will be diluted in the herd slurry store.

In this section of research, individual cow excretion patterns of MAP and *Salmonella* spp were obtained from peer reviewed literature and the potential load in herd slurry stores was modelled, using assumptions with respect to number of cows affected within a herd and factors relating to slurry storage and removal. Different scenarios of herd disease prevalence and slurry handling methods were evaluated. The estimated levels of organisms present in RMS were considered alongside potential infective doses to assess the degree of risk posed by each pathogen and scenario.

5.2 Methods

The computer models used to simulate the transfer of pathogens through the RMS production cycle were based on the assumptions in the diagrams in Figures 5.1 and 5.2. These diagrams illustrate the factors involved in the process that could influence the inputs and outputs of the pathogen. Due to the differences between *Salmonella* spp and MAP some aspects of the models differed and therefore some aspects have been defined separately.

Key aspects of the generic model development for both MAP and *Salmonella*, along with allied assumptions, are listed below.

- A theoretical one hundred cow herd was used .
- Infection categories for individual cows were defined as 'not infected', 'low shedders', 'medium shedders' and 'high shedders'. These categories were assigned set concentrations of organisms per gram of faeces based on values defined from prior research. The number of individuals in each category was dependent on the prevalence, which could be altered in the model to investigate different scenarios.
- Slurry production per cow was defined as an average of 43.1kg of faeces and 20.6kg of urine per day (64kg slurry), based on Weiss (2004). A dry matter of 15% was recorded for slurry by Weiss (2004) which translates faecal dry matter to 22.5%. These quantities were linked with the infection categories to calculate the population of organisms in the comingled slurry. It was assumed that all of the slurry produced entered into the reception pit and consequently was used in the RMS production. (Although other fluids *eg* output of washing the milking plant and runoff from yards and roofs will enter the reception pit, the quantities and components of these

cannot be predicted so they were not included. They are likely to cause dilution of pathogens derived from slurry, so the model reflects a "worst case scenario").

- The volume of RMS needed to bed the theoretical herd was calculated using cubicle dimensions of 1.2m x 2.0m based on DairyCo recommendations for Holstein-Friesian cows along with a depth of 7.5 cm (Bradley *et al* 2014 (scoping study)) which was the average depth used. It was assumed that there was one cubicle per cow. RMS density of 0.27g/ml and a dry matter of 35% were used, based on the scoping study data.
- The model calculations required converting slurry, with a dry matter of 15%, to RMS at 35% dry matter. In this scenario it was assumed that all dry matter in the slurry was made into RMS with fluid loss only. If this resulted in over-production of RMS the excess was effectively discarded along with the same proportion of pathogens.
- A loss of 5% of the RMS (containing 5% of the organisms) was assumed in the cycle at the point where RMS is on the cubicles before it returns to the reception pit.
- The dry matter percentage of RMS increased to 40% from 35%, this simulates moisture loss before the return of the RMS to the reception pit (Bradley *et al* 2014 (scoping study)).
- The RMS cycle is repeated each day using the Figures of the previous day's RMS (minus the 5% loss and change in dry matter percentage) plus the new day's fresh slurry production, combined in the reception pit. This mixture creates the starting values for the production of the next batch of RMS.
- The assumptions above outline the primary structure of the modelling process; however, specific modifications to the model were made dependent on the pathogen being tracked. These specialised assumptions/ factors are described individually for the MAP and *Salmonella* spp models below.

5.2.1 MAP Specific Assumptions/Factors

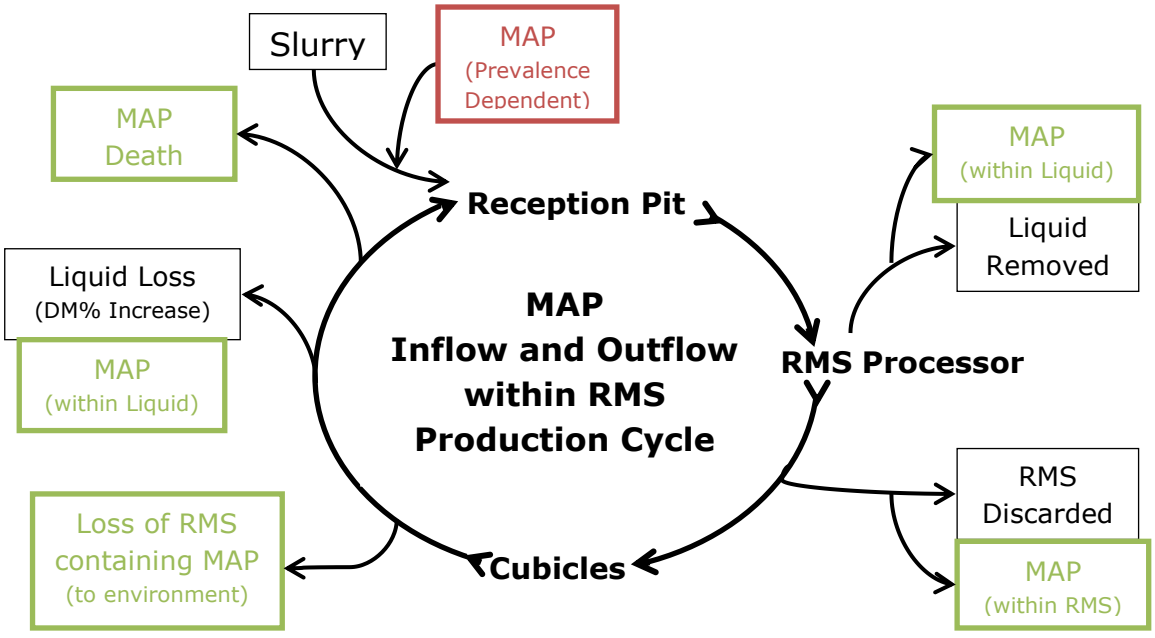
An outline of the MAP model is shown in Figure 5.1. For this model the infection categories were defined as in Table 5.1.

Table 5.1: Cow infection categories for the MAP model.

Infection Category	Organisms per Gram Faeces	Grams of Faeces Produced per Day	Dry Matter Percentage of Faeces
Not Infected	0	43,100	22.5
Low Shedder	50	43,100	22.5
Medium Shedder	25,000	45,000	20.0
High Shedder	75,000	50,000	17.5

The values stated in Table 5.1 have been defined using published research on MAP counts (Salgado *et al*, 2013). Average MAP values for were assigned for each infection category. Death of MAP organisms in RMS was added to this model. This was specified as a loss of 0.55% of organisms per day (Grewel, 2006). The ratio of organisms in the liquid and solid slurry fractions was set at 25% in liquid:75% in the solid portion (in models one, three and five) and 50% liquid:50% solid (in models two, four and six). No reported data were found for the distribution of MAP between solid and liquid fractions of cattle slurry separated by screw press separators. These illustrative levels represented no influence of separation on the initial concentration, and a situation in which MAP became concentrated (albeit to an arbitrary degree) in the solid fraction.

Figure 5.1: An illustration of the relationships in the MAP RMS model.



5.2.2 MAP Model Scenarios

- MAP models were created using the following scenarios:
- The scenarios ran for a total of 8 weeks (55 days).
- Each scenario was established using the herd MAP prevalence from day 0, from which the model simulation was left to run for the remainder of the 55 days.
- Scenarios undertaken for MAP included an 'iceberg', high prevalence herd and a single outbreak.
- Each scenario has been simulated using a different MAP in solid:fluid ratio; one scenario at 75:25 and the other at 50:50.

5.2.3 *Salmonella* Specific Assumptions/Factors

An outline of the *Salmonella* model is shown in Figure 5.2 and infection categories for this model were as defined as in Table 5.2. Values for faecal load were based on data from Kirchner *et al* (2014).

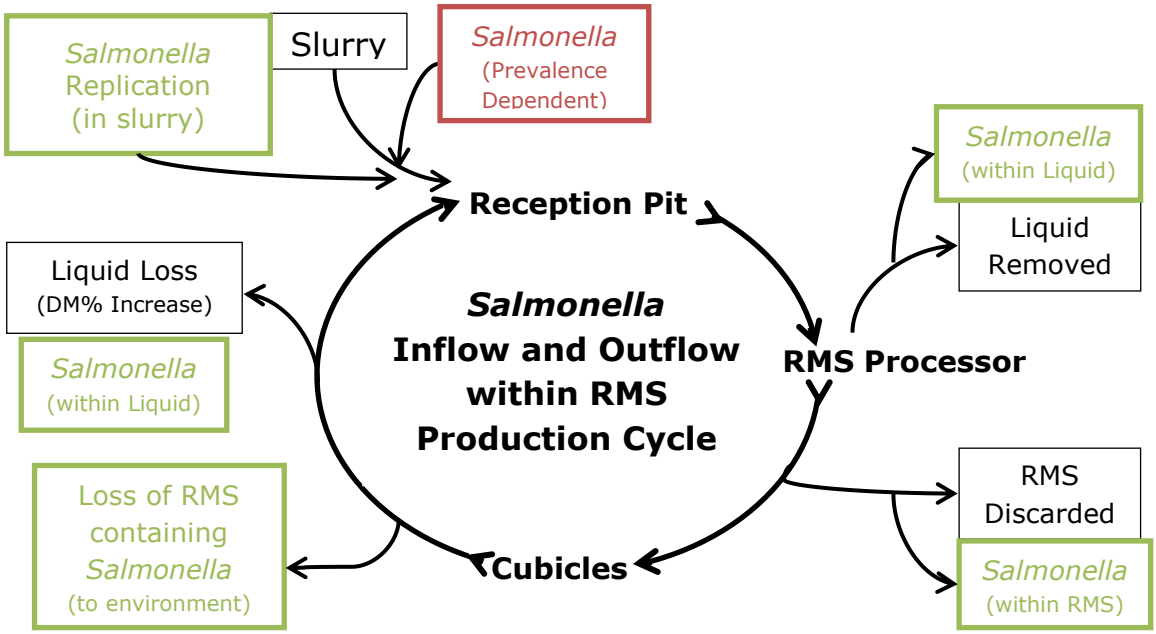
Table 5.2: Cow infection categories for the *Salmonella* spp model.

Infection Category	Organisms per Gram Faeces	Grams of Faeces Produced per Day	Dry Matter Percentage of Faeces
Not Infected	0	43,100	22.5
Low Shedder	100	43,100	22.5
Medium Shedder	100,000	43,100	22.5
High Shedder	10,000,000	4,3100	22.5

The ratio of organisms in liquid and solid slurry fractions was set at 50:50. No data on the distribution of the organism between solid and liquid fractions of separated cattle slurry could be found. Watabe *et al* (2003) reported on detection in pig slurry separated using a drum screen separator. In this situation, 11/13 samples of liquid fraction were positive, compared with 4/16 samples of the solid fraction, suggesting that more *Salmonella* was partitioned into the liquid fraction. However, in the absence of information for the situation with cattle slurry, to tend towards the likelihood of a "worst case" scenario, the decision was taken to assume no differential distribution between solid and liquid fractions. This will also have meant that the dry matter percentage of the faeces of cows in the different shedding categories will not have been influential.

No net change of *Salmonella* population has been assumed in RMS (0% change regarding replication & death). A change in replication of *Salmonella* in slurry was incorporated and investigated between 1.5% - 15%. Published data for replication of *Salmonella* in slurry were not found. This range was based on data on multiplication of *Salmonella enterica* in cow-pats (Sinton *et al*, 2007), and extended to higher levels in view of the higher moisture content of slurry, which was considered more likely to support replication, based on Sinton *et al* (2007).

Figure 5.2: An illustration of the relationships in the *Salmonella* spp RMS model



5.2.4 Salmonella Model Scenarios

- *Salmonella* models were created using scenarios as follows:
- All scenarios run for a period of 8 weeks (55 days).
- Weeks 1 (day 0-6) and 8 (day 49-55) are set as healthy herd (*ie* no *Salmonella* production in herd).
- Weeks 2-7 (day 7-48) have differing prevalences of *Salmonella* shedding cows, dependent on scenario described.
- Scenarios were created for a very severe outbreak (catastrophe), an average herd outbreak, a single outbreak, and a chronic scenario.
- Results for each scenario include a 1.5% and a 15% replication of *Salmonella* in the slurry variable.

5.3 Results

5.3.1 MAP Models

Results of the scenarios investigated in the MAP scenarios are presented graphically in Figure 5.3.

5.3.1.1 Iceberg Model

This scenario was set up based on a prevalence for the theoretical herd set at: not infected: 60%; low shedders: 30%; moderate shedders: 7%; and high shedders: 3%. This was based on a recent study which stated that if a high proportion high shedders are present in a herd, a higher level of subclinical

individuals (low and moderate shedders) are present (Crossley *et al*, 2005). This may typify a herd in the UK where a farmer may keep several high shedders *ie* high yielding cows.

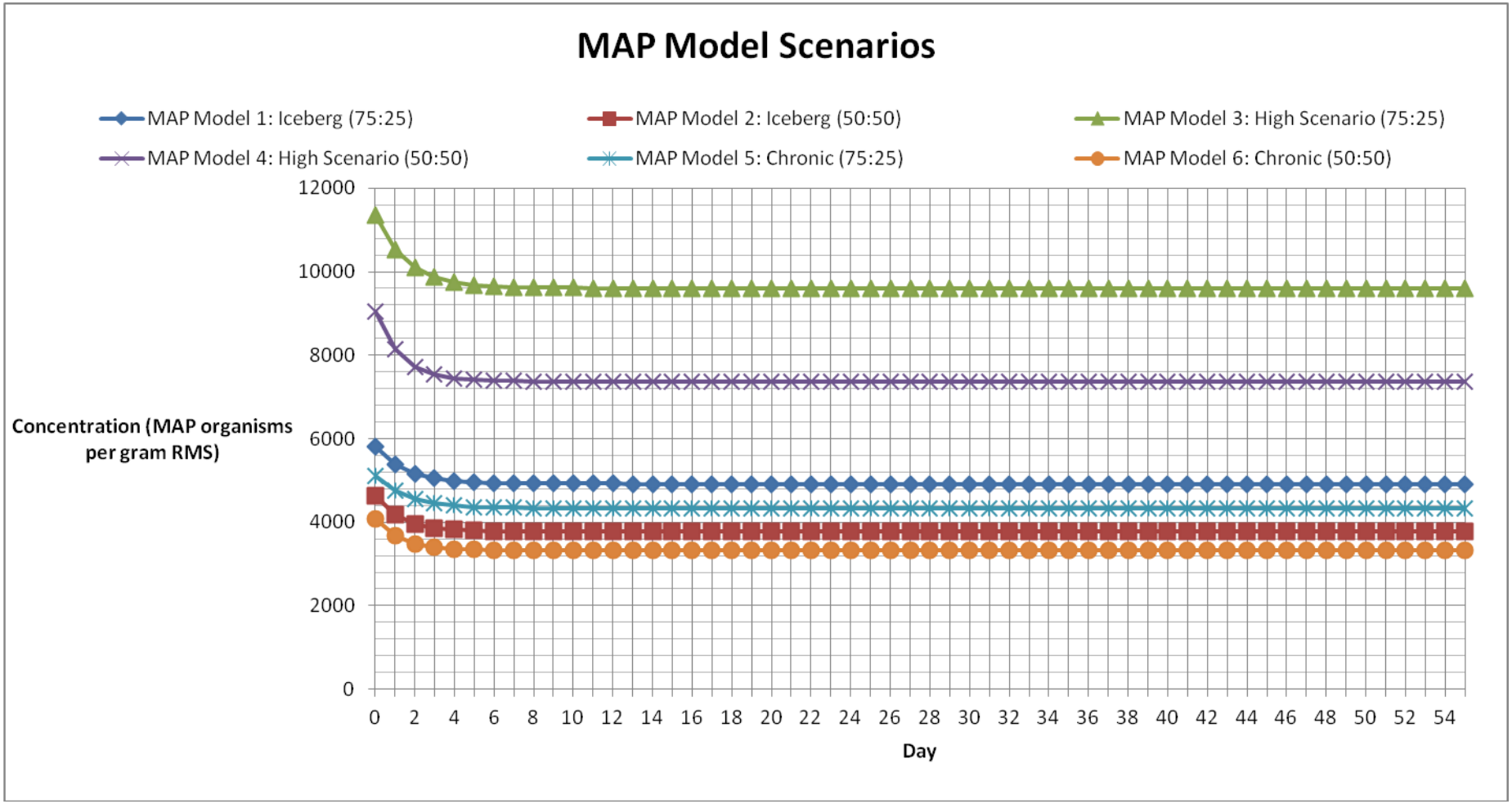
5.3.1.2 High Prevalence Model

The high MAP prevalence model was set up to assess the peak concentration if a herd is severely infected. This model used infection categories set at not infected: 90%; low shedders: 0%; moderate shedders: 0%; and high shedders: 10%. The categories have been set in this way to see the effect of only high shedders as these are likely to be the clinically obvious individuals who are likely to have been tested. It also simulates a worse-case scenario of a herd using RMS bedding.

5.3.1.3 Chronic Herd Model

This model was set up to calculate the level of MAP in RMS given a group of individuals with sub-clinical infection which may not have been detected as Johne's positive. Here infection categories have been set as: not infected: 85%; low shedders: 0%; moderate shedders: 15%; and high shedders: 0%.

Figure 5.3: Results of the MAP Scenarios from the computer simulation model.



5.3.2 *Salmonella* Models

Results of the *Salmonella* spp model scenarios are presented graphically in Figure 5.4.

5.3.2.1 Catastrophe Models

These models are based on a "worst case scenario" of a *Salmonella* outbreak in a herd. The outbreak is defined as a rise of 10% in high shedding cows per week until week 4 when a 30% level is reached. The prevalence of high shedders then reduced each week, with the final week (8) showing no shedders present in the herd (end of outbreak). Table 5.3 outlines the input Figures for the model to calculate results for each week's scenario.

Table 5.3: Proportion of shedding cows by week in the Catastrophe *Salmonella* model.

Week	Days	% High Shedders	cfu of *Salmonella* in total slurry
1	0-6	0	0
2	7-13	10	$4.31x10^{12}$
3	14-20	20	$8.62 x10^{12}$
4	21-27	30	$12.93 x10^{12}$
5	28-34	20	$8.62 x10^{12}$
6	35-41	10	$4.31 x10^{12}$
7	42-48	5	$2.155 x10^{12}$
8	49-55	0	0

5.3.2.2 Herd Outbreak Model

This model was based on a herd having an active *Salmonella* outbreak in the herd for 6 weeks (weeks 2-7) before an end of outbreak point is reached at week 8 where the herd is said to be 'cured'. The outbreak reaches a maximum of 5% high shedders which is maintained at this level for 6 weeks. This is to simulate an individual becoming a high shedder then curing, only for another individual to 'take its place' as a high shedder due to the transfer of *Salmonella* through the herd. This model aims to show how a *Salmonella typhimurium* outbreak could determine *Salmonella* populations in RMS. Table 5.4 shows the input Figures for the model to calculate results for each week's scenario.

Table 5.4: Proportion of shedding cows by week in the Herd Outbreak *Salmonella* model.

Week	Days	% High Shedders	cfu of *Salmonella* in total slurry
1	0-6	0	0
2	7-13	5	$2.155 x10^{12}$
3	14-20	5	$2.155 x10^{12}$
4	21-27	5	$2.155 x10^{12}$
5	28-34	5	$2.155 x10^{12}$
6	35-41	5	$2.155 x10^{12}$
7	42-48	5	$2.155 x10^{12}$
8	49-55	0	0

5.3.2.3 Single Outbreak Model

This model looks into the effect of one high shedder of *Salmonella* to observe the impacts that an individual cow's input (10,000,000 organisms per gram faeces) could have on *Salmonella* levels in RMS.

Criteria were defined as an outbreak of 1% prevalence in a 100 cow herd between weeks 2-7, with week 8 having 0% prevalence in the herd indicating a 'cure' week. Table 5.5 shows the input Figures for the model to calculate results for each week's scenario.

Table 5.5: Proportion of shedding cows by week in the Single Outbreak *Salmonella* model.

Week	Days	% High Shedders	cfu of *Salmonella* in total slurry
1	0-6	0	0
2	7-13	1	4.31×10^{11}
3	14-20	1	4.31×10^{11}
4	21-27	1	4.31×10^{11}
5	28-34	1	4.31×10^{11}
6	35-41	1	4.31×10^{11}
7	42-48	1	4.31×10^{11}
8	49-55	0	0

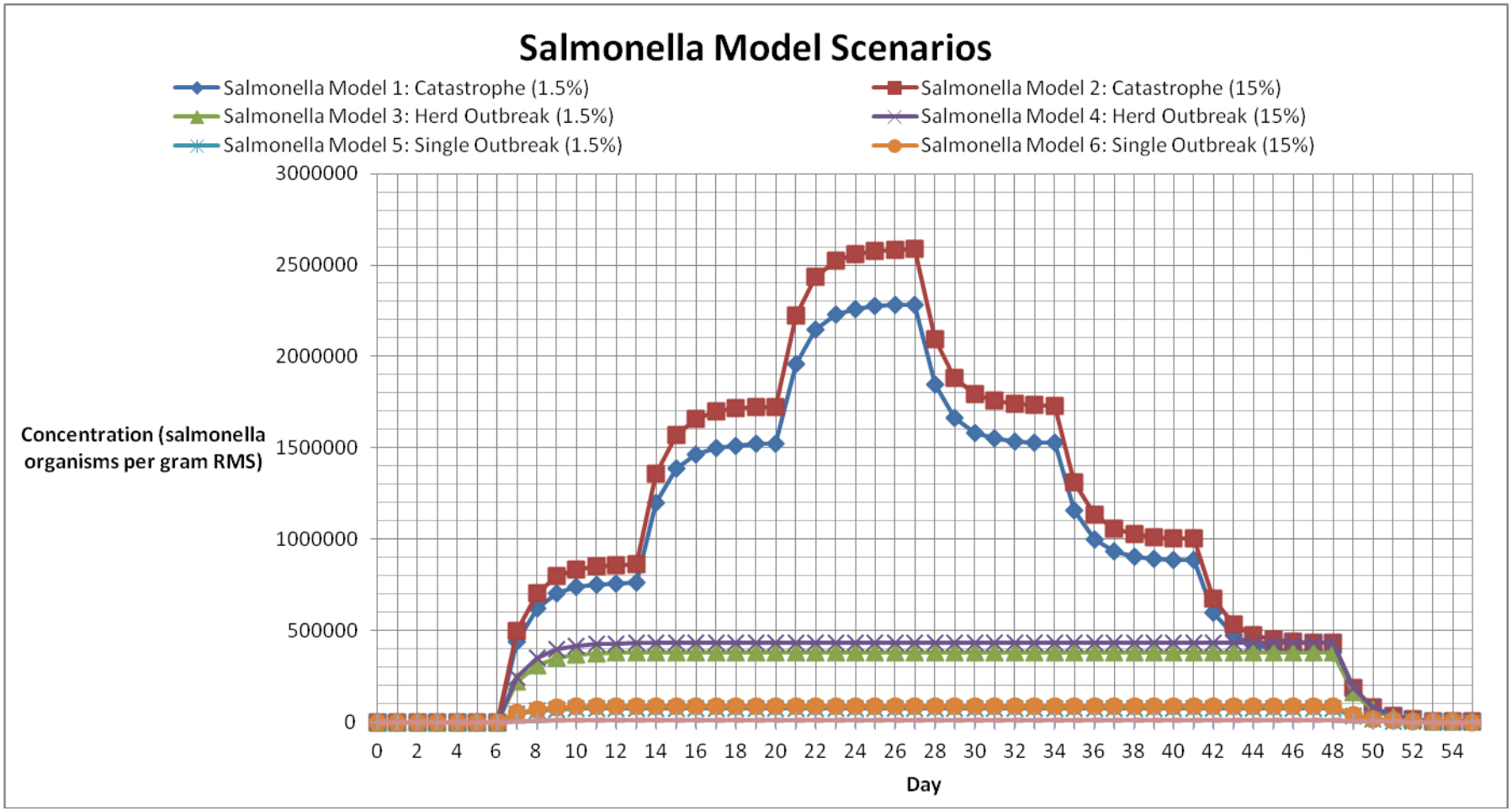
5.3.2.4 Chronic Scenarios

These model scenarios were performed to describe chronic *Salmonella* Dublin shedding within a herd. The model was set up at 10% prevalence at a medium shedding value set at 100,000 cfu of *Salmonella* per gram of faeces. Table 5.6 shows the values used to set up the scenario.

Table 5.6: Proportion of shedding cows by week in the Chronic *Salmonella* model.

Week	Days	% Medium Chronic Shedders	cfu of *Salmonella* in total slurry
1	0-6	0	0
2	7-13	10	4.31×10^{10}
3	14-20	10	4.31×10^{10}
4	21-27	10	4.31×10^{10}
5	28-34	10	4.31×10^{10}
6	35-41	10	4.31×10^{10}
7	42-48	10	4.31×10^{10}
8	49-55	0	0

Figure 5.4: Combined *Salmonella* Model Scenarios.



5.3.3 Infective Doses of MAP and *Salmonella*

Published values for the infectious oral dose of MAP are difficult to interpret because of the difficulty of quantifying MAP organisms. Literature values for cattle appear to range from 1.5 x10^6 cfu (Sweeney *et al*, 2006) to 5 x10^7 cfu (Mortier *et al*, 2013). However, results of the latter study indicated that calves younger than 6 months at the time of exposure showed higher histological lesion scores in comparison to older calves infected with the same dose. It was concluded that calves up to one year of age were susceptible to MAP infection. The infective doses for adults remain unclear.

Infective oral doses of *Salmonella* for cattle have been reported to range from 10^6 (Nazer & Osborne, 1977) to 10^{10} cfu McGuirk and Peek (2003). Again, these are levels for youngstock and Figures for adult cattle could not be found.

Although figures for human minimum infective dose typically given are 10^3 (Ryan and Ray, 2004), it is reported that on occasions infection has been caused by $<10^3$ organisms (Blaser and Newman, 1982).

5.4 Discussion and Conclusions

The in silico models were based on a variety of transparent assumptions about faecal excretion of the organisms involved, how they are partitioned in slurry constituents and many factors around the slurry handling processes. In many instances, a worst case scenario approach has been taken to attempt to capture what may represent the most problematic circumstances on farm.

The infective dose of MAP suggests that fairly large quantities of RMS would need to be ingested (of the order of 100-1000g) to reach the published values for the infective dose. Furthermore, this figure is probably representative of an infective dose for calves, and for adult cows may be substantially higher. Therefore in terms of MAP, the models constructed in this research suggest that whilst bedding of young stock using RMS should be avoided, the risk to adults may be minimal. For this reason and because of the difficulties in detecting MAP in bedding it was concluded that attempting to isolate MAP from bedding samples would not further inform this research.

The infective dose of *Salmonella* suggests that in a very severe outbreak, when levels in RMS may become high, only small quantities of RMS would need to be ingested to cause disease (of the order of <1g). Clearly this depends on many factors but these results imply that there is at least a potential danger to human and animal health from *Salmonella* in RMS if a herd suffers a severe disease outbreak. However, in a herd catastrophically infected with *Salmonella* there will be many other transmission routes, which in fact may be more significant, including feed and water, wildlife (especially birds), fomites, and human transmission.

It should be noted that neither of pathogens has been modelled in other bedding materials, so these suggested levels under various scenarios cannot be compared with the situation in alternative bedding materials.

6 Economics and Bedding Cost Calculator

6.1 Introduction

The purpose of this section is to provide an indication of the aspects to be considered when evaluating the economic implications of using RMS bedding. The intention was to provide an aid to comparing the use of different bedding materials in individual situations, rather than to carry out a full costing exercise. Clearly every case is farm specific and will need to be evaluated using the farm's own details. To this end, a Cost Calculator spreadsheet has been created which can be used to cost individual scenarios. As an aid to populating the spreadsheet, descriptions of the range of a number of parameters and costs collected from farms participating in the main survey (Chapter 2) are presented. The Cost Calculator spreadsheet is available separately.

6.2 General Overview of Farms contributing Economic Data

Average herd size and milk sales per cow per year on the day of the visit are summarised in Table 6.1. The three groups did not differ significantly in herd size. Mean milk sales per cow were significantly higher for sand farms (9446 l) than for RMS farms (8803 l) or sawdust farms (8491 l) ($P < 0.005$). There was no difference between groups in the cubicle stocking rate for the milking cows on the day of the visit.

Table 6.1: A summary of size and milk sales of survey farms.

Variable	Bedding	n	Mean	Median	SD	Min	Max	25th Percentile	75th Percentile
Average herd size									
	RMS	40	374	290	217.0	135	1000	220	480
	Sand	41	370	300	248.7	120	1550	228	435
	Sawdust	44	336	265	191.7	110	1020	205	425
Milk sales l/cow/year									
	RMS	40	8803[a]	8663	1140	6500	10833	7895	9766
	Sand	41	9446[b]	9524	1367	6567	12115	8473	10419
	Sawdust	43	8491[a]	8333	1090	5902	10435	7800	9308
Stocking rate (cows/cubicles) on day of visit									
	RMS	40	96.1	97.7	9.70	69.4	116.4	92	101
	Sand	41	99.3	98.4	11.51	75.3	137.2	94	104
	Sawdust	44	94.4	95.3	7.86	72.9	111.4	91	99

[a,b] Values with different superscripts within columns, within parameters differ ($p <= 0.05$)

For the 40 farms using RMS, the previous main bedding material for the milking cows is summarised in Table 6.2. The main bedding used previously was sawdust with 23 farms (57%) and then paper products with 7 (17.5%).

Table 6.2: A summary of bedding materials previously used by farmers before employing RMS.

Material	Number of Farms	Percentage of Farms
Sawdust	23	57.5
Paper product	7	17.5
Sand	3	7.5
Straw*	3	7.5
Gypsum	2	5.0
Oat husks	1	2.5
New unit	1	2.5

*One had previously used straw in yards

6.3 Summary of Costs and Related Information Provided by Farms Participating in the Survey.

The capital cost of separators ranged from £30,000 to £45,000. Total capital expenditure reported for setting up the RMS system was up to £80,000, dependent on the additional equipment and infrastructure. This might include pumps and stirrers, a gantry and building to house the separator, and constructing a reception pit. Costs quoted separately for structural changes ranged from £200 to £12,000. In two cases expenditure was needed to provide the three phase electricity supply required. On the farms surveyed, the time the separator was running was very variable, ranging from 2 to 10 hours / 100 cows / week. This was dependent on factors including, but apparently not restricted to, the proportion of the material used for bedding, the herd size, and groups other than milking cows for which the bedding was used.

Maintenance costs will vary dependent on the type of machine and the hours of use. The main anticipated maintenance cost for a screw press separator is the replacement of the separator screen(s), for which the survey indicated a cost of £1,500 to £2,400 per year; this could be higher if changed more than once per year. New augers within 18 months were mentioned in one instance.

Cost ranges for traditional bedding materials were £25 to £232 / tonne for sawdust and £9 to £25 / tonne for sand (depending on proximity to source, quality and whether bagged or bulk).

A labour component is not included in the calculator but will need to be considered. The reported ranges of time spent on bedding were wide. These equated to 1 - 3.5 hours / 100 cows / week for sawdust, 1 - 9 hours / 100 cows / week for sand and 1- 6 hours / 100 cows / week for RMS. Responses from the Scoping Study suggest that, depending on the previous bedding material, the labour time may be increased (*eg* by extra time spent pumping slurry, or cleaning off beds more frequently), or decreased, (*eg* if bedding frequency is reduced). Users of the cost calculator should bear in mind that a report from three new users of RMS on large farms in the Scoping Study (Bradley *et al*, 2014) was that the system required more staff input at management level than previous bedding material. It is important to monitor the performance of the machinery, the nature of the product and react accordingly, to ensure that material that is too wet is not used for bedding. This is possibly easier to ensure with a smaller staff team.

6.4 Parameters Included in the Cost Calculator

The parameters included in the cost calculator should enable it to be used in the majority of farm situations, to allow users to consider the financial implications of moving from the existing bedding system to RMS. The inputs and outputs of the calculator are shown in Figure 6.1. The calculator is split into two sections, with the first section dealing with the 'current bedding system' and the second section dealing with the proposed 'RMS bedding system'. The user enters data into the white cells.

Current Bedding System

The user enters the key information about the current bedding system to calculate the annual bedding cost. Where a dispensing machine is used this is included as a capital item with an annual charge calculated. The labour time/cost has not been included as there is not enough information available from the study and it was also felt this would over complicate the model with limited gain in the calculated outcome. The model calculates the annual bedding cost, which in this example is £4,970 per year.

There is an annual maintenance cost for the existing system of 6%. This cell can be changed and farms currently bedding on sand may wish to increase this Figure to more closely resemble the wear and tear on pumps, scrapers and floors.

RMS Bedding System

The user enters the key information about the capital cost, depreciation rate and interest rate. The default values are 10% depreciation and 5% interest. The user also enters the components contributing to operating costs which are the total kW of the various motors and pumps, the number of hours of operation per day and the number of days the system will run per year. In this example the total requirement for motors is 20 kW; they will operate for 4 hours per day for 200 days per year. The model calculates the total kW hours. The user enters the average electricity cost in p/kWh, which should be the average of the night and day tariff depending on when the pumps are running. In this example the average cost is 9 p/kW hour. The user also enters the maintenance cost, which is a % of the capital cost of the separator and pumps, with a default value of 6%, which in this example is £2,100 per year. The total annual cost for the RMS in this example is £11,040.

Figure 6.1: An illustration of the 'inputs' and 'outputs' of the Bedding Cost Calculator.

RMS bedding calculator

Current bedding system	**Sand**			**£/year**
Number of cows	100			
Days housed per year	200			
kg/cow/day	14			
Total tonnes per year	280			
Cost £/t	14			
Total cost £ per year	3920			**3920**
Current bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Dispenser	5000	10%	5.0%	750
Other capital costs	0	10%	5.0%	0
Total	**5000**			**750**
Operating costs				
Maintainance cost %	6			**300**
Total annual bedding cost				**4970**

RMS bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Separator & pumps	35000	10%	5.0%	5250
Other capital costs	15000	10%	5.0%	2250
Total	**50000**			**7500**
Operating costs				
Total motors kW	20			
Motors hours per day	4			
Days per year	200			
Total kW per year	16000			
Average elec. Cost p/kW	9			
Total elec £ per year	1440			**1440**
Maintainance cost %	6			**2100**
Total annual bedding cost				**11040**

Change in annual bedding cost				**6070**

Change in Annual Bedding Cost

The model calculates the change in the annual bedding cost, which in this example is £6,070 higher. Using the model it is easy to look at the break even number of cows, which for this example is 260 cows as shown in Figure 6.2.

Figure 6.2: An illustration of the 'inputs' and 'outputs' of the Bedding Cost Calculator to demonstrate a break even number of cows.

RMS bedding calculator

Current bedding system	**Sand**			**£/year**
Number of cows	260			
Days housed per year	200			
kg/cow/day	14			
Total tonnes per year	728			
Cost £/t	14			
Total cost £ per year	10192			**10192**
Current bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Dispenser	5000	10%	5.0%	750
Other capital costs	0	10%	5.0%	0
Total	**5000**			**750**
Operating costs				
Maintainance cost %	6			**300**
Total annual bedding cost				**11242**

RMS bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Separator & pumps	35000	10%	5.0%	5250
Other capital costs	15000	10%	5.0%	2250
Total	**50000**			**7500**
Operating costs				
Total motors kW	20			
Motors hours per day	4			
Days per year	200			
Total kW per year	16000			
Average elec. Cost p/kW	9			
Total elec £ per year	1440			**1440**
Maintainance cost %	6			**2100**
Total annual bedding cost				**11040**

Change in annual bedding cost				**-202**

6.5 Case Study 1: 580 Cow Unit previously using Sawdust

This case study is for a 580 cow unit which is housed 365 days per year and previously used 1 kg/cow/day of sawdust at a cost of £80/tonne. The annual bedding cost is calculated to be £17,986, equivalent to £32/cow. The RMS system cost £55,000 to install and uses 30 kW of motors and pumps, which operate for 2.5 hours per day for 365 days per year. The RMS annual cost is calculated to be £13,807, which gives a cost reduction of £4,179 per year. Using this data the break-even number of cows is 430.

Figure 6.3: An illustration of the 'inputs' and 'outputs' of the Bedding Cost Calculator when investigating the costs/savings associated with a change to RMS from sawdust in a 580 cow herd.

RMS bedding calculator	Farm 9			
Current bedding system	**Sawdust**			**£/year**
Number of cows	580			
Days housed per year	365			
kg/cow/day	1			
Total tonnes per year	212			
Cost £/t	80			
Total cost £ per year	16936			**16936**
Current bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Dispenser	5000	10%	5.0%	750
Other capital costs		10%	5.0%	0
Total	**5000**			**750**
Operating costs				
Maintainance cost %	6			**300**
Total annual bedding cost				**17986**
RMS bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Separator & pumps	47000	10%	5.0%	7050
Other capital costs	8000	10%	5.0%	1200
Total	**55000**			**8250**
Operating costs				
Total motors kW	30			
Motors hours per day	2.5			
Days per year	365			
Total kW per year	27375			
Average elec. Cost p/kW	10			
Total elec £ per year	2737.5			**2738**
Maintainance cost %	6			**2820**
Total annual bedding cost				**13808**
Change in annual bedding cost				**-4179**

6.6 Case Study 2: 220 Cow Unit previously using Sand

This case study is for a 220 cow unit which is fully housed and previously used 14 kg/cow/day of sand at a cost of £14/t. The annual bedding cost is calculated to be £16,789, equivalent to £76/cow/year. The RMS system cost £80,000 to install and uses 14.5 kW of motors and pumps, which operate for 1.3 hours per day for 365 days per year. The RMS annual cost is calculated to be £16,057, which gives a cost reduction of £732 per year. Using this data the break-even number of cows is 209.

Figure 6.4: An illustration of the 'inputs' and 'outputs' of the Bedding Cost Calculator when investigating the costs/savings associated with a change to RMS from sand in a 220 cow herd.

RMS bedding calculator	**Farm 36**			
Current bedding system	**Sand**			**£/year**
Number of cows	220			
Days housed per year	365			
kg/cow/day	14			
Total tonnes per year	1124			
Cost £/t	14			
Total cost £ per year	15739			**15739**
Current bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Dispenser	5000	10%	5.0%	750
Other capital costs		10%	5.0%	0
Total	**5000**			**750**
Operating costs				
Maintainance cost %	6			**300**
Total annual bedding cost				**16789**
RMS bedding system		**Depn**	**Interest**	**Annual charge**
Capital cost		**%**	**%**	**£**
Separator & pumps	55000	10%	5.0%	8250
Other capital costs	25000	10%	5.0%	3750
Total	**80000**			**12000**
Operating costs				
Total motors kW	14.5			
Motors hours per day	1.3			
Days per year	365			
Total kW per year	6880.3			
Average elec. Cost p/kW	11			
Total elec £ per year	756.83			**757**
Maintainance cost %	6			**3300**
Total annual bedding cost				**16057**
Change in annual bedding cost				**-732**

7 Antimicrobial Resistance

7.1 Introduction

One concern surrounding the use of recycled manure solids as bedding is that the potential for the closed loop offered by this process may be conducive to the persistence and perpetuation of antimicrobial resistance. As outlined in the scoping study this is an area in which there is limited understanding of the potential risks and little 'baseline' data. It also has to be acknowledged that, even if recycling manure solids as bedding is important in this process, the speed of the process of accumulation of antimicrobial resistance genes in the 'dairy environment' may mean that it takes some time for any changes to become evident.

The aim of this component of this project was to allow 'baseline' data and isolates to be collated from farms bedding on RMS and other bedding materials which could then be assessed for the presence of antimicrobial resistance and serve as a bank for future comparison.

7.2 Methods

The coliforms and *Enterococcus* spp were selected as organisms likely to be representative of environmental organisms with a known ability to harbour and transfer antimicrobial resistance. In the process of conducting the survey (see Chapter 2), bulk milk and bedding samples were collected from farms using sand, sawdust or RMS as bedding materials. These samples were used as a source of both coliform and *Enterococcus* spp isolates to enable screening for antimicrobial resistance. Data on antibiotic use in adult cattle was also collated from the questionnaires conducted during the farm visits.

Using selective media, the aim was to collect six coliform and six *Enterococcus* spp from each farm, three of each group from bedding and three of each from bulk milk. The initial aim was for all coliform isolates to be *E. coli*, but where this was not possible, other coliform species were collected. If sufficient isolates could not be obtained from milk, then if possible additional isolates were sourced from bedding on that farm with the overall aim of achieving a total of 12 isolates per farm. Isolated organisms were identified by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonics) prior to storage.

Antimicrobial sensitivities were determined using a VITEK® 2 (Biomerieux; Basingstoke UK) following the recommended protocols. This enabled direct determination of mean inhibitory concentrations (MIC) for the antibiotics selected, as opposed to the less quantitative estimations allowed by the Kirby-Bauer disc diffusion method. Coliforms were tested using the VITEK® 2 Gram-negative susceptibility card AST-GN65 and the *Enterococcus* spp with the Gram-positive susceptibility card AST-GP76. The antimicrobials tested were constrained by the availability of 'cards' provided for use in the VITEK® 2 and the organism/antibiotic combinations for which it had been validated. These are outlined in Table 7.1.

For the purposes of analysis, MICs of each antimicrobial for each organism were ranked and differences analysed using a non-parametric approach. Where univariable analysis identified differences in MIC between bedding types, multivariable analysis was also conducted using conventional models. For the purpose of multivariable analysis, and because different organisms within the coliform and *Enterococcus* spp can have different inherent resistance to antimicrobials, organisms were 'grouped'. Coliforms were

grouped as (a) *E.coli*, (b) *Klebsiella* spp and *Raoultella* spp, (c) *Serratia* spp, (d) *Citrobacter* spp and (e) Other *Enterobacteriacae*. *Enterococcus* spp were grouped as (a) *E. faecium*, (b) *E. hirae*, (c) *E. faecalis*, (d) *E. durans* and (e) Other *Enterococcus* spp. Multivariable analysis took into account a number of different farm variables and management factors, these included (amongst others) class and route of antibiotic use, geographic location of the farm, organism 'group', housing variables as well as aspects of RMS use. The purpose of this analysis was to control for such variables, rather than to understand their impact which was beyond the scope of this report.

Table 7.1: A summary of antimicrobial agents tested and their class, by pathogen.

		Organisms Evaluated	
Antibiotic	**Antibiotic Class**	**Coliforms**	***Enterococcus* spp**
Ampicillin (AM)	Aminopenicillin	✓	
Amoxicillin/Clavulanic Acid (AMC)	Potentiated aminopenicillin	✓	
Amikacin (AN)	Aminoglycoside	✓	
Chloramphenicol (C)	Phenicol	✓	✓
Cefovecin (CFO)	Cephalosporin (3rd)	✓	✓
Ceftiofur (CFT)	Cephalosporin (3rd)	✓	
Clindamycin (CM)	Lincosamide		✓
Cefalexin (CN)	Cephalosporin (1st)	✓	
Cefpodoxime (CPD)	Cephalosporin (3rd)	✓	
Erythromycin (E)	Macrolide		✓
Enrofloxacin (ENR)	Fluoroquinolone	✓	✓
Nitrofurantoin (FT)	Nitrofuran	✓	✓
Gentamicin (GM)	Aminoglycoside	✓	
Imipenem (IPM)	Carbapenem	✓	
Marbofloxacin (MRB)	Fluoroquinolone	✓	✓
Benzylpenicillin (P)	Natural penicillin		✓
Polymyxin B (PB)	Polypeptide	✓	
Piperacillin (PIP)	Ureidopenicillin	✓	
Trimethoprim/Sulfamethoxazole (SXT)	Potentiated sulphonamide	✓	✓
Tetracycline (TE)	Tetracycline	✓	✓
Tobramycin (TM)	Aminoglycoside	✓	
Vancomycin (VA)	Glycopeptide		✓

7.3 Results

A total of 724 coliforms and 793 *Enterococcus* spp were collated and made available for sensitivity testing. Details of these isolates and their source are outlined in Tables 7.2 to 7.5. *E. coli* was the most common coliform, as intended. *E. coli* were relatively more difficult to isolate from milk, making up 44.6% of milk isolates compared to 71.9% of bedding isolates. *Enterococcus faecium* was the most common *Enterococcus* spp isolated making up 23.1% of isolates. Five species, *Enterococcus faecium*, *Enterococcus pseudoavium, Enterococcus hirae*, *Enterococcus faecalis* and *Enterococcus durans* constituted 87.2% of all *Enterococcus* spp isolates.

Table 7.2: A summary of the source of coliform isolates by on farm location and bedding type.

Bedding Type	Source	Number of Isolates
RMS	Bedding	120
RMS	Bulk milk	120
Sand	Bedding	123
Sand	Bulk milk	120
Sawdust	Bedding	120
Sawdust	Bulk milk	121

Table 7.3: A summary of the source of *Enterococcus* spp isolates by on farm location and bedding type.

Bedding Type	Source	Number of Isolates
RMS	Bedding	150
RMS	Bulk milk	121
Sand	Bedding	142
Sand	Bulk milk	126
Sawdust	Bedding	127
Sawdust	Bulk milk	127

Table 7.4: A summary of coliform species isolated for the purposes of sensitivity testing, from samples collected as part of the farm survey.

Organism	Number of Isolates
Escherichia coli	422
Klebsiella pneumoniae	51
Serratia liquefaciens	44
Klebsiella oxytoca	36
Hafnia alvei	25
Citrobacter braakii	16
Enterobacter cloacae	15
Raoultella ornithinolytica	14
Citrobacter freundii	13
Serratia marcescens	12
Enterobacter amnigenus	10
Raoultella terrigena	10
Citrobacter gillenii	7
Citrobacter koseri	7
Buttiauxella gaviniae	4
Pantoea agglomerans	4
Proteus vulgaris	4
Rahnella aquatilis	4
Serratia fonticola	4
Kluyvera intermedia	3
Raoultella planticola	3
Serratia proteamaculans	3
Proteus hauseri	2
Citrobacter farmeri	1
Enterobacter aerogenes	1
Enterobacter asburiae	1
Enterobacter cowanii	1
Enterobacter kobei	1
Enterobacter ludwigii	1
Escherichia fergusonii	1
Pantoea sp	1
Providencia heimbachae	1
Serratia odorifera	1
Serratia ureilytica	1

Table 7.5: A summary of *Enterococcus* spp species isolated for the purposes of sensitivity testing, from samples collected as part of the farm survey.

Organism	Number of Isolates
Enterococcus faecium	183
Enterococcus pseudoavium	134
Enterococcus hirae	131
Enterococcus faecalis	112
Enterococcus durans	105
Enterococcus avium	40
Enterococcus malodoratus	35
Enterococcus thailandicus	18
Enterococcus saccharolyticus	14
Enterococcus casseliflavus	6
Enterococcus devriesei	5
Enterococcus gilvus	5
Enterococcus aquimarinus	2
Enterococcus gallinarum	1
Enterococcus italicus	1
Enterococcus phoeniculicola	1

The MICs of each of the antibiotics for coliform isolates overall are illustrated in Figures 7.1 to 7.18 and summarised by 'group' in Tables 7.6 to 7.23. Univariable analysis identified significant differences in the MICs of coliform organisms between the different bedding types for ampicillin (p=0.009), amikacin (p=0.028), chloramphenicol (p=0.049), ceftiofur (p<0.001), cephalexin (p=0.010), enrofloxacin (p=0.003), nitrofurantoin (p=0.053) and piperacillin (p=0.004).

The results of multivariable analysis of MICs for different antimicrobials against coliform organisms is summarised in Table 7.24. Multivariable analysis, based on the variables recorded, confirmed that the difference in MICs between bedding groups could be explained by the additional variables included, for ampicillin, nitrofurantoin and piperacillin. However, differences were confirmed amongst the other antimicrobials tested as outlined below:

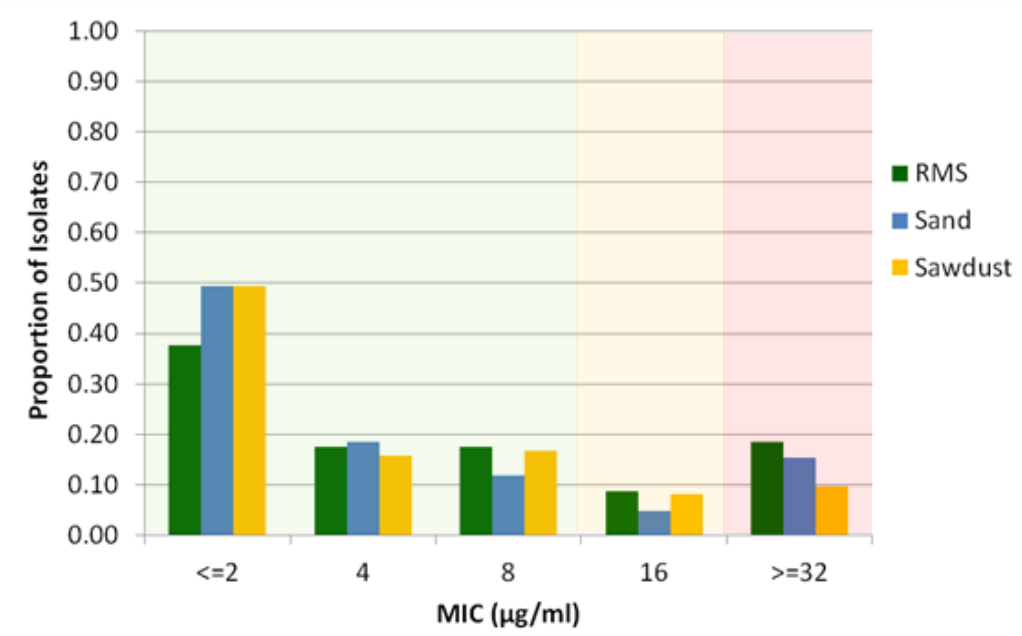
- MICs for amikacin were significantly higher for coliforms recovered from farms bedded on RMS compared to sand or sawdust.
- MICs for chloramphenicol were significantly lower for coliforms recovered from farms bedded on RMS compared to sand or sawdust.
- MICs for ceftiofur were significantly lower for coliforms recovered from farms bedded on sand compared to RMS or sawdust.
- MICs for cefalexin were significantly lower for coliforms recovered from farms bedded on sand compared to RMS or sawdust.
- MICs for enrofloxacin were significantly higher for coliforms recovered from farms bedded on sand compared to RMS or sawdust.

Table 7.6: A summary of the MICs of ampicillin for coliform isolates collected as part of the farm survey.

Ampicillin	MIC (µg/ml)						
	Not Tested	<=2	4	8	16	>=32	Total
Escherichia coli							
RMS		68	34	25		9	136
Sand		77	30	19	1	9	136
Sawdust		86	28	27	2	12	155
***Klebsiella* spp, *Kluyvera* spp and *Raoultella* spp**							
RMS		4		8	16	27	55
Sand		4			7	12	23
Sawdust		1		2	11	7	21
***Serratia* spp**							
RMS	22						22
Sand	22						22
Sawdust	19						19
***Citrobacter* spp**							
RMS	7						7
Sand	23						23
Sawdust	6						6
Other *Enterobacteriaceae*							
RMS	6	1		1	1		9
Sand	4	2	1	1		5	13
Sawdust	17	10	3	4	3		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.1: An illustration of the MICs of ampicillin for coliform isolates collected as part of the farm survey.



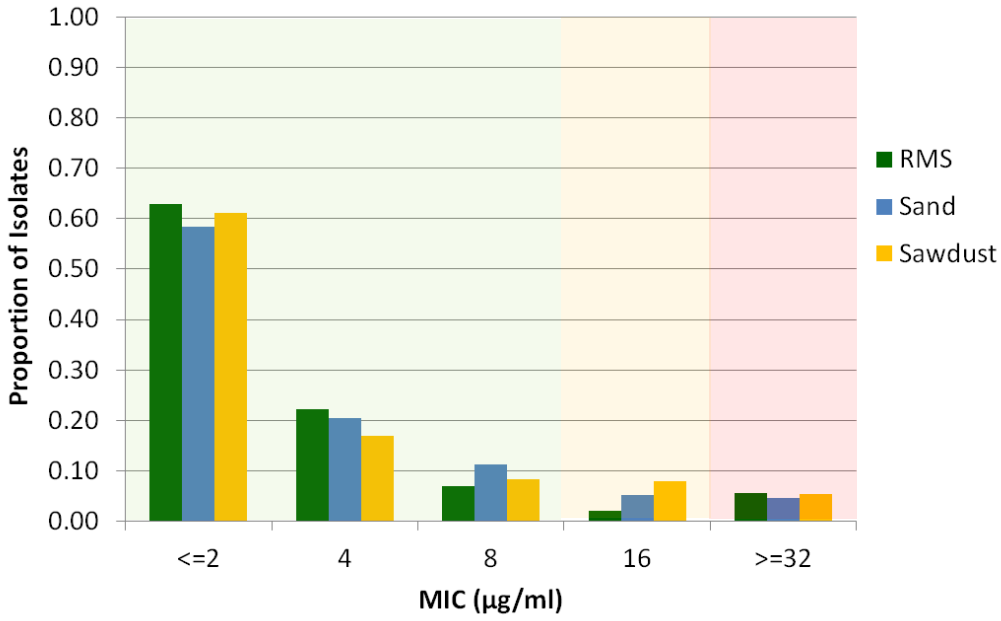
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.7: A summary of the MICs of amoxicillin/clavulanic acid for coliform isolates collected as part of the farm survey.

Amoxicillin/Clavulanic Acid	MIC (µg/ml)						
	Not Tested	<=2	4	8	16	>=32	Total
Escherichia coli							
RMS		92	35	6	1	2	136
Sand		98	29	7	1	1	136
Sawdust		116	27	8	3	1	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		45	5	3	1	1	55
Sand		20		3			23
Sawdust		19	1		1		21
***Serratia* spp**							
RMS		2	7	7	2	4	22
Sand	1		10	5	1	5	22
Sawdust		2	9	5		3	19
***Citrobacter* spp**							
RMS		2	2		1	2	7
Sand	1	2	3	7	8	2	23
Sawdust		1	1	1	2	1	6
Other *Enterobacteriaceae*							
RMS		3	2			4	9
Sand	1	5	2	2	1	2	13
Sawdust	1	7	2	6	13	8	37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.2: An illustration of the MICs of amoxicillin/clavulanic acid for coliform isolates collected as part of the farm survey.



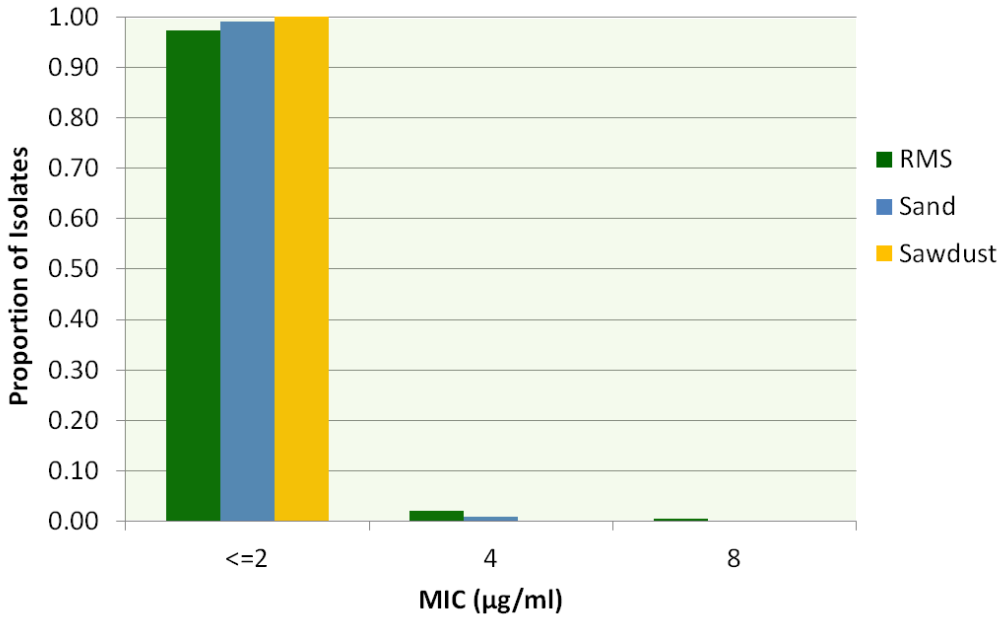
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.8: A summary of the MICs of amikacin for coliform isolates collected as part of the farm survey.

Amikacin	MIC (µg/ml)			
	Not Tested	<=2	4	8	Total
Escherichia coli					
RMS		130	5	1	136
Sand		135	1		136
Sawdust		155			155
***Klebsiella* spp and *Raoultella* spp**					
RMS		55			55
Sand		23			23
Sawdust		21			21
***Serratia* spp**					
RMS		22			22
Sand	1	21			22
Sawdust		19			19
***Citrobacter* spp**					
RMS		7			7
Sand	1	21	1		23
Sawdust		6			6
Other *Enterobacteriaceae*					
RMS		9			9
Sand		13			13
Sawdust	1	36			37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.3: An illustration of the MICs of amikacin for coliform isolates collected as part of the farm survey.



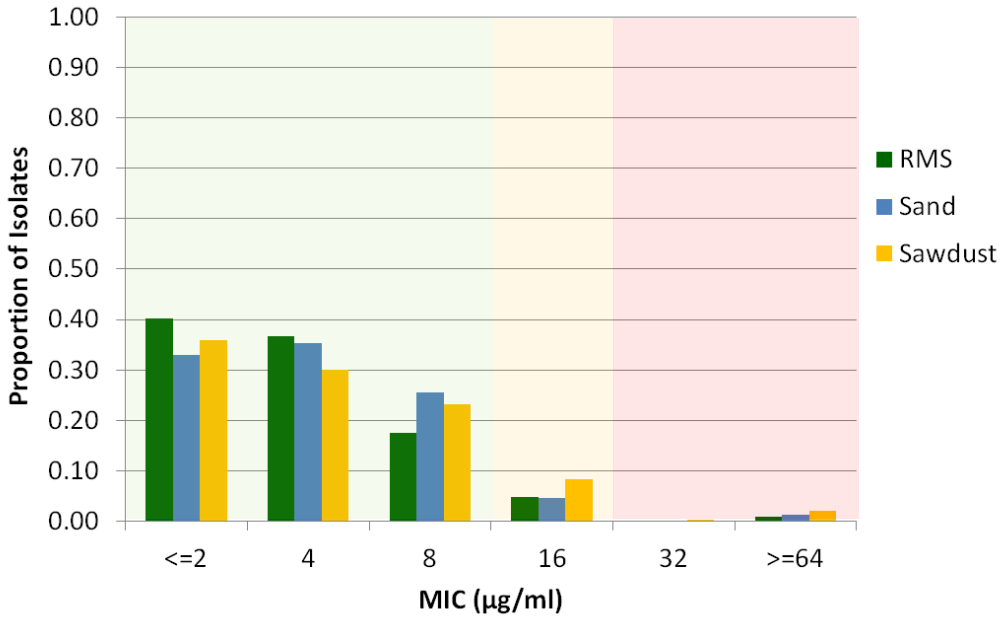
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.9: A summary of the MICs of chlorampenicol for coliform isolates collected as part of the farm survey.

Chloramphenicol	MIC (µg/ml)						
	Not Tested	<=2	4	8	16	32	>=64	Total
Escherichia coli								
RMS		36	60	30	9		1	136
Sand		32	57	36	9		2	136
Sawdust		43	53	37	17		5	155
***Klebsiella* spp and *Raoultella* spp**								
RMS		45	6	2	1		1	55
Sand		22		1				23
Sawdust		20	1					21
***Serratia* spp**								
RMS		6	8	7	1			22
Sand	1	4	7	9			1	22
Sawdust		6	4	7	2			19
***Citrobacter* spp**								
RMS		2	5					7
Sand	1	5	10	7				23
Sawdust		2	4					6
Other *Enterobacteriaceae*								
RMS		3	5	1				9
Sand		8	2	2	1			13
Sawdust	1	14	9	11	1	1		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.4: An illustration of the MICs of chlorampenicol for coliform isolates collected as part of the farm survey.



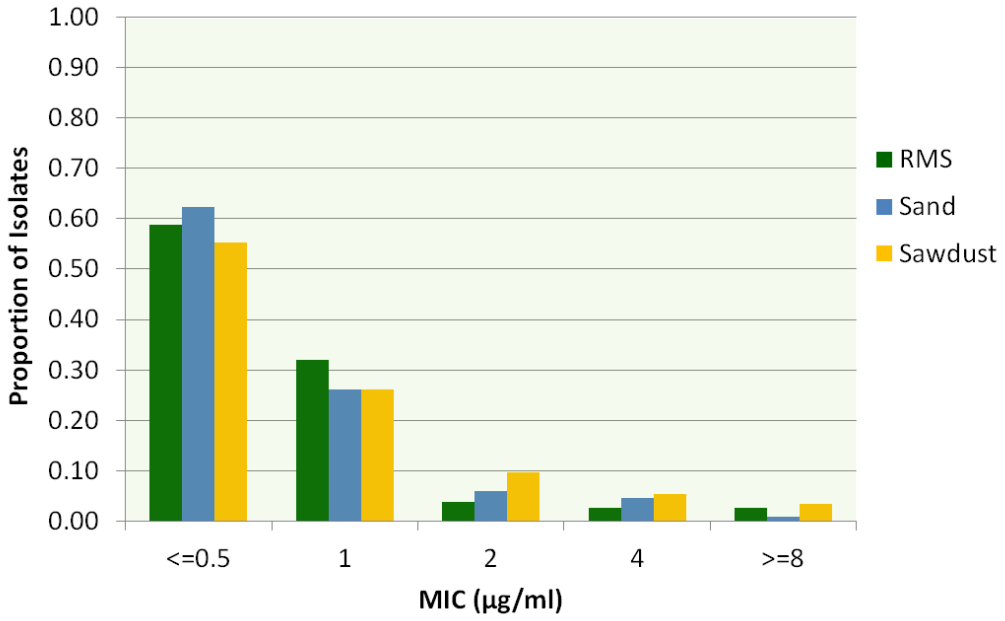
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.10: A summary of the MICs of cefovecin for coliform isolates collected as part of the farm survey.

Cefovecin	MIC (µg/ml)						
	Not Tested	<=0.5	1	2	4	>=8	Total
Escherichia coli							
RMS		90	39	4		3	136
Sand		99	34	2		1	136
Sawdust		108	35	8		4	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		34	17	1		3	55
Sand		18	5				23
Sawdust		14	4	2		1	21
***Serratia* spp**							
RMS	1	8	13				22
Sand	1	7	12	2			22
Sawdust		6	8	3	1	1	19
***Citrobacter* spp**							
RMS		2	3	2			7
Sand	1	1	4	7	10		23
Sawdust			3	2	1		6
Other *Enterobacteriaceae*							
RMS			1	2	6		9
Sand		9	1	2		1	13
Sawdust	1	3	12	8	11	2	37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.5: An illustration of the MICs of cefovecin for coliform isolates collected as part of the farm survey.



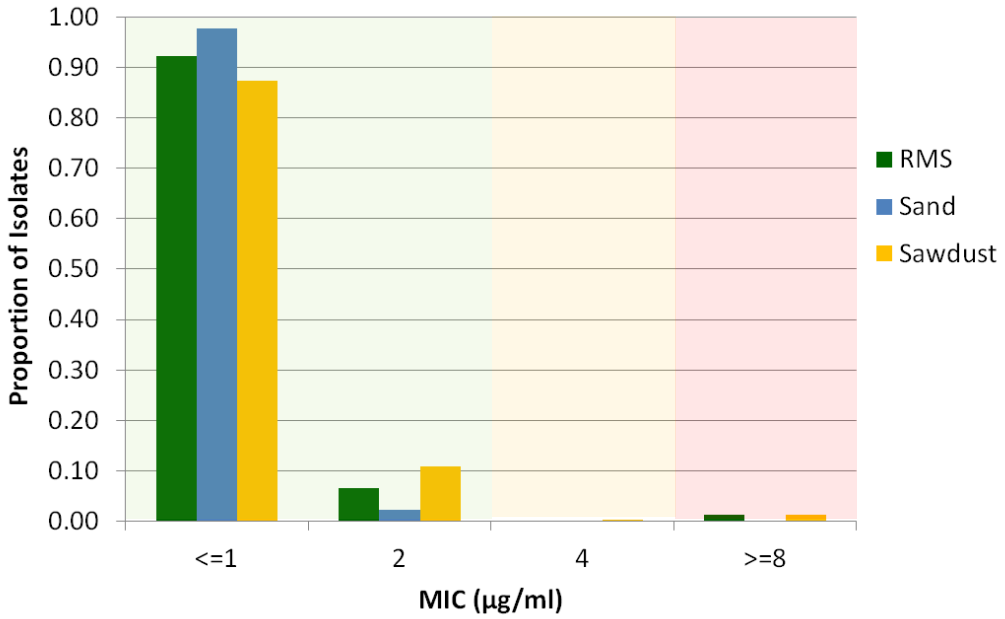
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.11: A summary of the MICs of ceftiofur for coliform isolates collected as part of the farm survey.

Ceftiofur	MIC (µg/ml)				
	Not Tested	<=1	2	4	>=8	Total
Escherichia coli						
RMS		129	5		2	136
Sand		135	1			136
Sawdust		151	2		2	155
***Klebsiella* spp and *Raoultella* spp**						
RMS		53	1		1	55
Sand		23				23
Sawdust		20			1	21
***Serratia* spp**						
RMS		17	5			22
Sand	1	20	1			22
Sawdust		15	3	1		19
***Citrobacter* spp**						
RMS		7				7
Sand	1	20	2			23
Sawdust		6				6
Other *Enterobacteriaceae*						
RMS		5	4			9
Sand		12	1			13
Sawdust	1	15	21			37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.6: An illustration of the MICs of ceftiofur for coliform isolates collected as part of the farm survey.



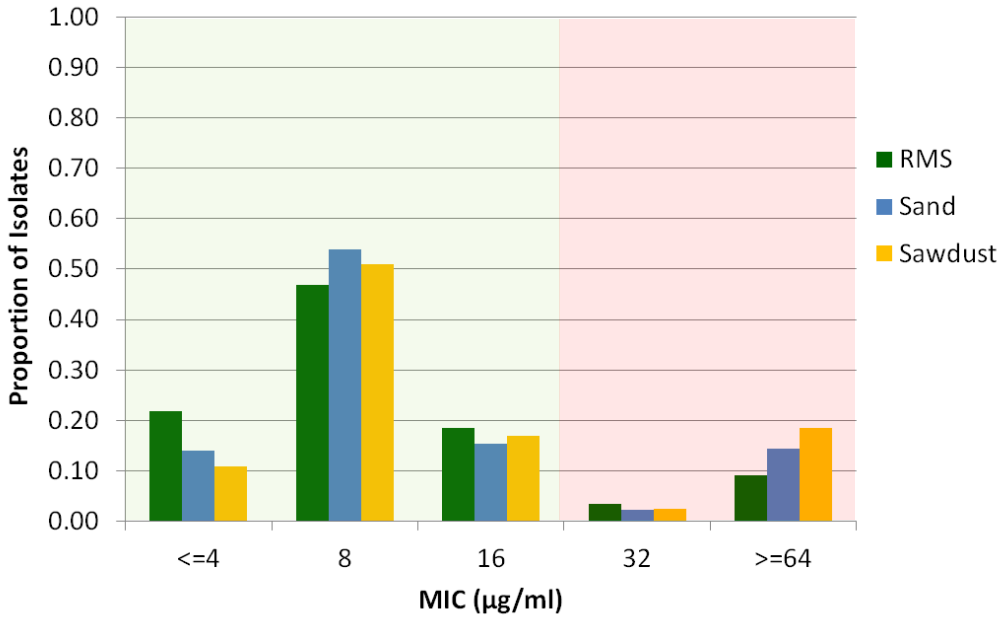
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.12: A summary of the MICs of cefalexin for coliform isolates collected as part of the farm survey.

Cefalexin	MIC (µg/ml)						
	Not Tested	<=4	8	16	32	>=64	Total
Escherichia coli							
RMS		2	102	31		1	136
Sand		7	112	17			136
Sawdust		5	116	31		3	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		47	4	2		2	55
Sand		22		1			23
Sawdust		19	1			1	21
***Serratia* spp**							
RMS	1			2	8	11	22
Sand	1			4	5	12	22
Sawdust				4	6	9	19
***Citrobacter* spp**							
RMS		1		3		3	7
Sand	1	1	1	7		13	23
Sawdust			3			3	6
Other *Enterobacteriaceae*							
RMS			1	4		4	9
Sand			3	4		6	13
Sawdust	1	2	1	5		28	37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.7: An illustration of the MICs of cefalexin for coliform isolates collected as part of the farm survey.



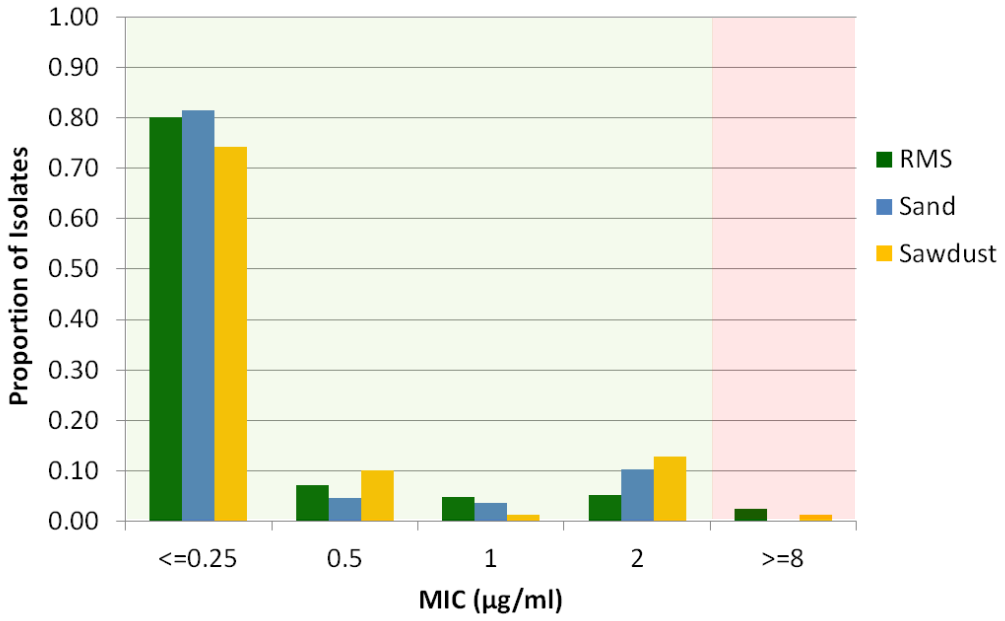
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.13: A summary of the MICs of cefpodoxime for coliform isolates collected as part of the farm survey.

Cefpodoxime	MIC (µg/ml)						
	Not Tested	<=0.25	0.5	1	2	>=8	Total
Escherichia coli							
RMS		113	14	5	1	3	136
Sand		124	7	5			136
Sawdust		133	18	1	1	2	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		50	1	1	1	2	55
Sand		23					23
Sawdust		20				1	21
***Serratia* spp**							
RMS	22						22
Sand	22						22
Sawdust	19						19
***Citrobacter* spp**							
RMS		2		2	3		7
Sand	1	2		2	18		23
Sawdust		3			3		6
Other *Enterobacteriaceae*							
RMS		1		2	6		9
Sand		9	2		2		13
Sawdust	1	6	4	2	24		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.8: An illustration of the MICs of cefpodoxime for coliform isolates collected as part of the farm survey.



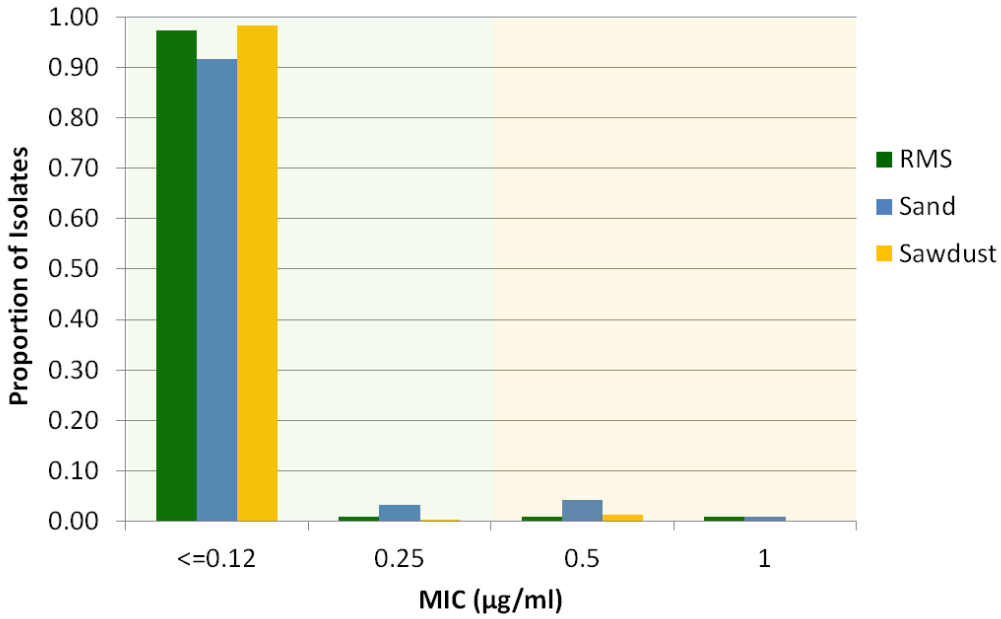
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.14: A summary of the MICs of enrofloxacin for coliform isolates collected as part of the farm survey.

Enrofloxacin	MIC (µg/ml)				
	Not Tested	<=0.12	0.25	0.5	1	Total
Escherichia coli						
RMS		133	1		2	136
Sand		134			2	136
Sawdust		155				155
***Klebsiella* spp and *Raoultella* spp**						
RMS		55				55
Sand		23				23
Sawdust		21				21
***Serratia* spp**						
RMS		19	1	2		22
Sand	1	15	5	1		22
Sawdust		18	1			19
***Citrobacter* spp**						
RMS		7				7
Sand	1	13	1	8		23
Sawdust		5		1		6
Other *Enterobacteriaceae*						
RMS		9				9
Sand		12	1			13
Sawdust	1	34		2		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.9: An illustration of the MICs of enrofloxacin for coliform isolates collected as part of the farm survey.



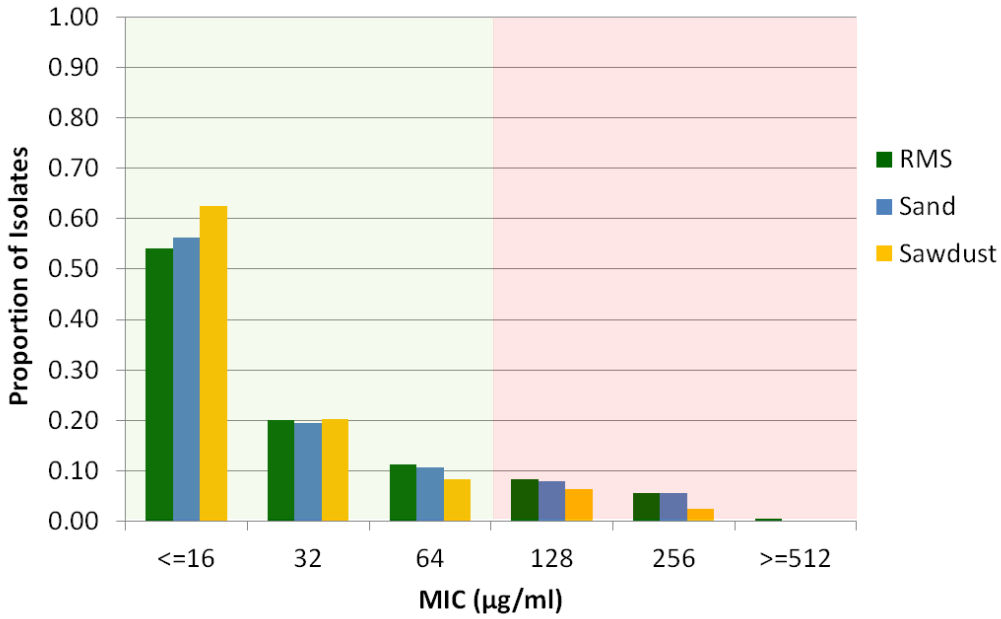
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.15: A summary of the MICs of nitrofurantoin for coliform isolates collected as part of the farm survey.

Nitrofurantoin	MIC (µg/ml)						
	Not Tested	<=16	32	64	128	256	>=512	Total
Escherichia coli								
RMS		104	27	2	2		1	136
Sand		102	28	6				136
Sawdust		115	33	7				155
***Klebsiella* spp and *Raoultella* spp**								
RMS		10	17	18	9	1		55
Sand		5	6	11	1			23
Sawdust		6	7	6	2			21
***Serratia* spp**								
RMS				2	8	12		22
Sand	1				11	10		22
Sawdust					13	6		19
***Citrobacter* spp**								
RMS		5	1	1				7
Sand	1	11	7	4				23
Sawdust		3	1	2				6
Other *Enterobacteriaceae*								
RMS		5	1	3				9
Sand		3	1	2	5	2		13
Sawdust	1	24	7	5				37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.10: An illustration of the MICs of nitrofurantoin for coliform isolates collected as part of the farm survey.



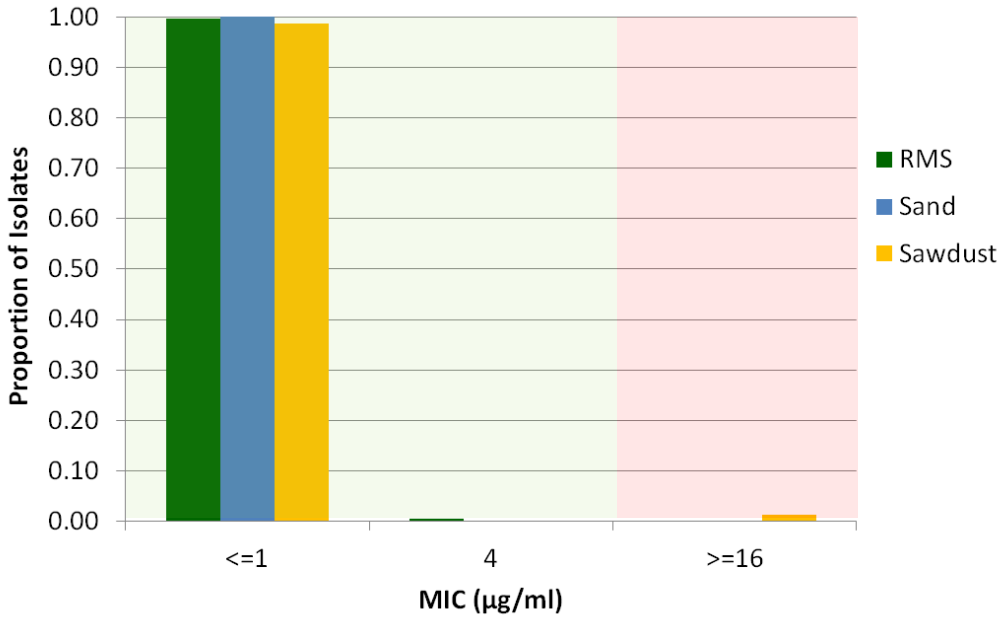
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.16: A summary of the MICs of gentamicin for coliform isolates collected as part of the farm survey.

Gentamicin	MIC (µg/ml)				
	Not Tested	<=1	4	>=16	Total
Escherichia coli					
RMS		135	1		136
Sand		136			136
Sawdust		152		3	155
***Klebsiella* spp and *Raoultella* spp**					
RMS		55			55
Sand		23			23
Sawdust		21			21
***Serratia* spp**					
RMS		22			22
Sand	1	21			22
Sawdust		19			19
***Citrobacter* spp**					
RMS		7			7
Sand	1	22			23
Sawdust		6			6
Other *Enterobacteriaceae*					
RMS		9			9
Sand		13			13
Sawdust	1	36			37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.11: An illustration of the MICs of gentamicin for coliform isolates collected as part of the farm survey.



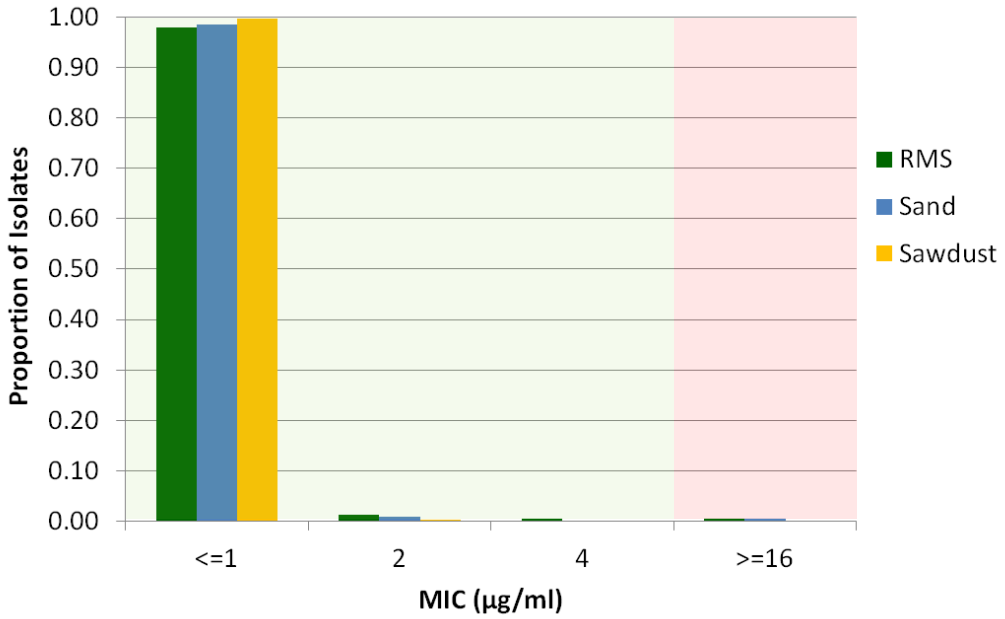
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.17: A summary of the MICs of imipenem for coliform isolates collected as part of the farm survey.

Imipenem	MIC (µg/ml)				
	Not Tested	<=1	2	4	>=16	Total
Escherichia coli						
RMS		135			1	136
Sand		135			1	136
Sawdust		155				155
***Klebsiella* spp and *Raoultella* spp**						
RMS		54		1		55
Sand		23				23
Sawdust		21				21
***Serratia* spp**						
RMS		19	3			22
Sand	1	19	2			22
Sawdust		18	1			19
***Citrobacter* spp**						
RMS		7				7
Sand	1	22				23
Sawdust		6				6
Other *Enterobacteriaceae*						
RMS		9				9
Sand	7	6				13
Sawdust	1	36				37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.12: An illustration of the MICs of imipenem for coliform isolates collected as part of the farm survey.



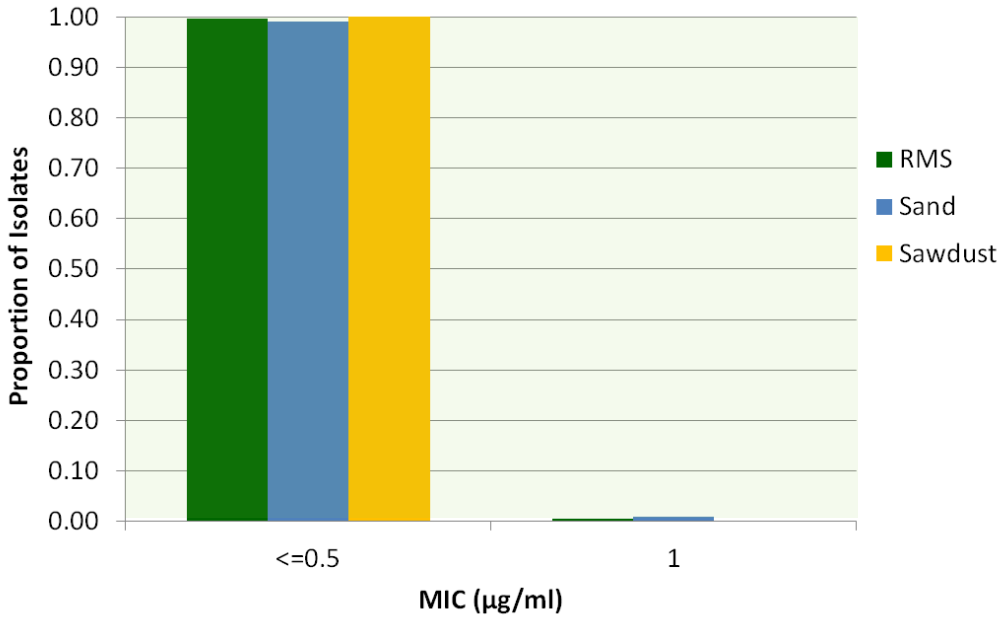
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.18: A summary of the MICs of marbofloxacin for coliform isolates collected as part of the farm survey.

Marbofloxacin	MIC (µg/ml)			
	Not Tested	<=0.5	1	Total
Escherichia coli				
RMS		135	1	136
Sand		134	2	136
Sawdust		155		155
***Klebsiella* spp and *Raoultella* spp**				
RMS		55		55
Sand		23		23
Sawdust		21		21
***Serratia* spp**				
RMS		22		22
Sand	1	21		22
Sawdust		19		19
***Citrobacter* spp**				
RMS		7		7
Sand	1	22		23
Sawdust		6		6
Other *Enterobacteriaceae*				
RMS		9		9
Sand		13		13
Sawdust	1	36		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.13: An illustration of the MICs of marbofloxacin for coliform isolates collected as part of the farm survey.



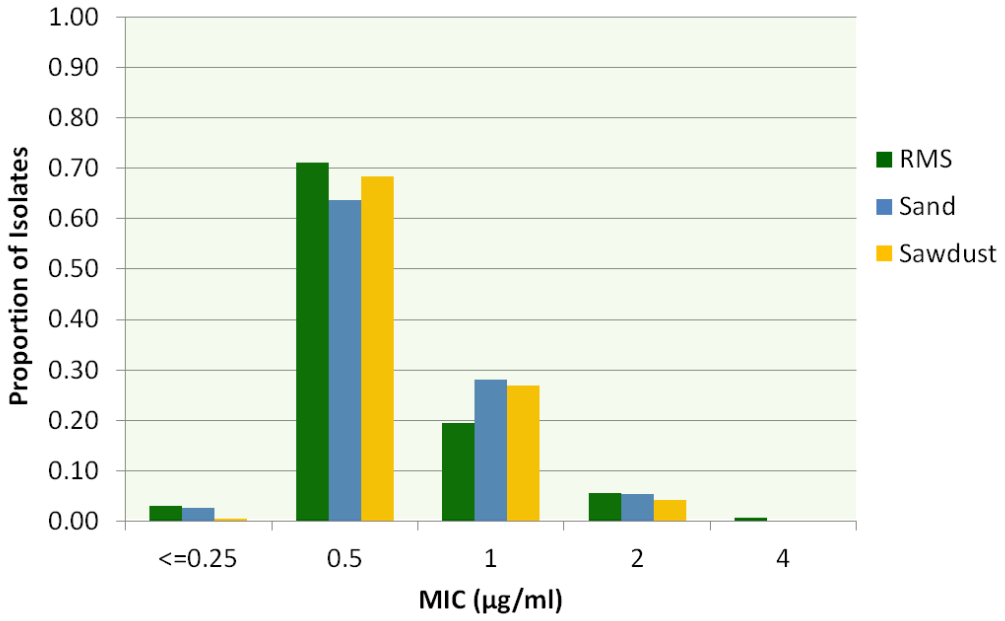
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.19: A summary of the MICs of polymixin B for coliform isolates collected as part of the farm survey.

Polymyxin B	MIC (µg/ml)						
	Not Tested	<=0.25	0.5	1	2	4	Total
Escherichia coli							
RMS	1	5	101	20	8	1	136
Sand		4	84	40	8		136
Sawdust		1	108	39	7		155
***Klebsiella* spp and *Raoultella* spp**							
RMS	31		12	11	1		55
Sand	13		9	1			23
Sawdust	12		4	5			21
***Serratia* spp**							
RMS	22						22
Sand	22						22
Sawdust	19						19
***Citrobacter* spp**							
RMS	7						7
Sand	23						23
Sawdust	6						6
Other *Enterobacteriaceae*							
RMS	9						9
Sand	13						13
Sawdust	37						37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.14: An illustration of the MICs of polymixin B for coliform isolates collected as part of the farm survey.



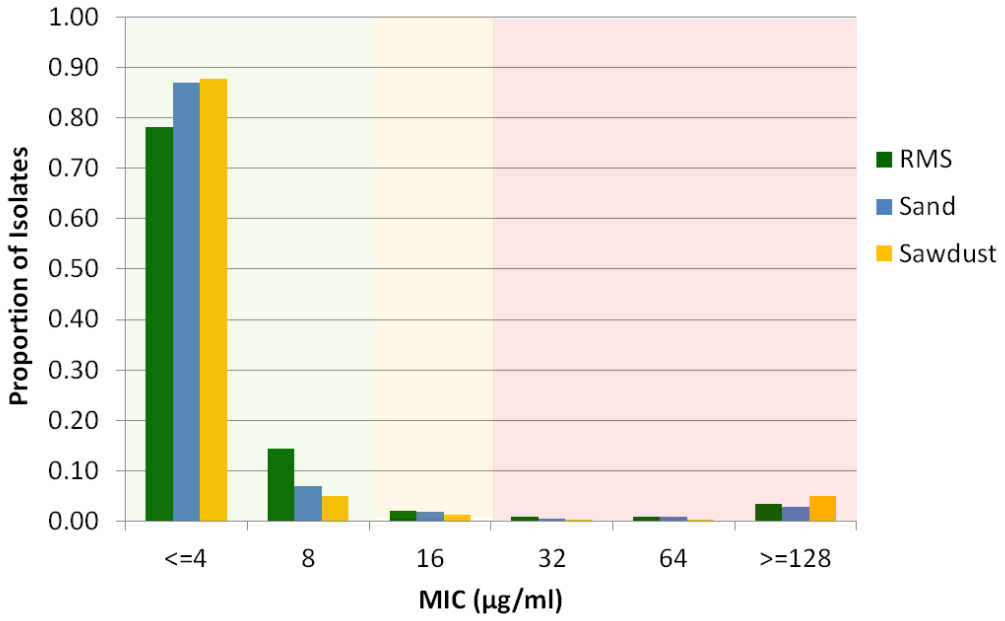
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.20: A summary of the MICs of piperacillin for coliform isolates collected as part of the farm survey.

Piperacillin	MIC (µg/ml)						
	Not Tested	<=4	8	16	32	64	>=128	Total
Escherichia coli								
RMS		125	3	1		1	6	136
Sand		126	2	1		2	5	136
Sawdust		141	3				11	155
***Klebsiella* spp and *Raoultella* spp**								
RMS		20	28	3	1	1	2	55
Sand		7	12	2	1		1	23
Sawdust		10	8	2			1	21
***Serratia* spp**								
RMS		21	1					22
Sand	1	21						22
Sawdust		17	1	1				19
***Citrobacter* spp**								
RMS		5		1	1			7
Sand	1	20	1	1				23
Sawdust		4			1	1		6
Other *Enterobacteriaceae*								
RMS		8	1					9
Sand		13						13
Sawdust	1	36						37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.15: An illustration of the MICs of piperacillin for coliform isolates collected as part of the farm survey.



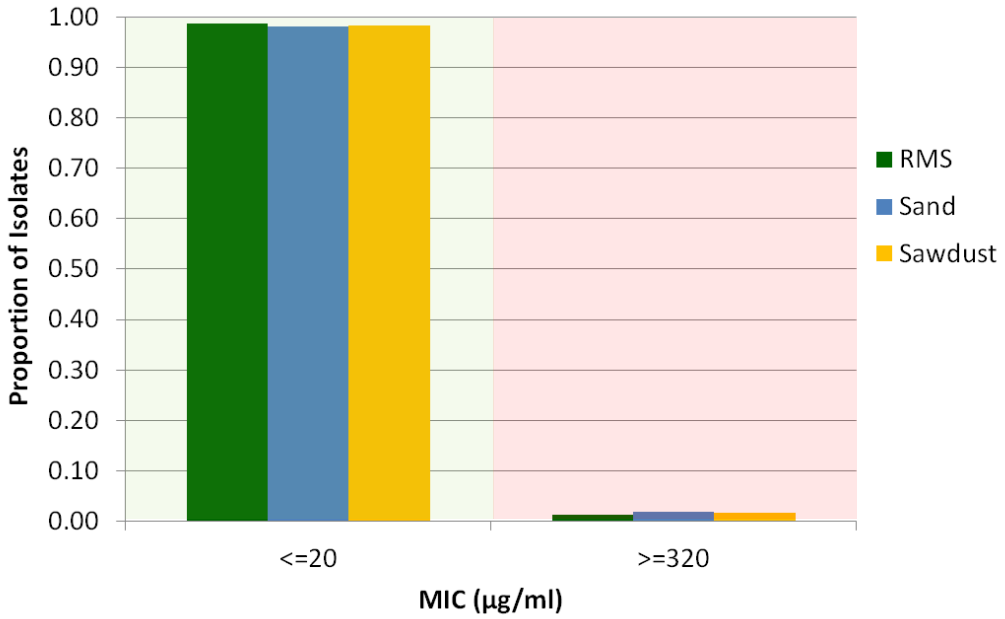
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.21: A summary of the MICs of trimethoprim/sulfamethoxazole for coliform isolates collected as part of the farm survey.

Trimethoprim/Sulfamethoxazole	MIC (µg/ml)		
	Not Tested	<=20	>=320	Total
Escherichia coli				
RMS		135	1	136
Sand		135	1	136
Sawdust		152	3	155
***Klebsiella* spp and *Raoultella* spp**				
RMS		54	1	55
Sand		22	1	23
Sawdust		20	1	21
***Serratia* spp**				
RMS	1	21		22
Sand	1	21		22
Sawdust		19		19
***Citrobacter* spp**				
RMS		7		7
Sand	1	22		23
Sawdust		6		6
Other *Enterobacteriaceae*				
RMS		8	1	9
Sand		11	2	13
Sawdust	1	36		37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.16: An illustration of the MICs of trimethoprim/sulfamethoxazole for coliform isolates collected as part of the farm survey.



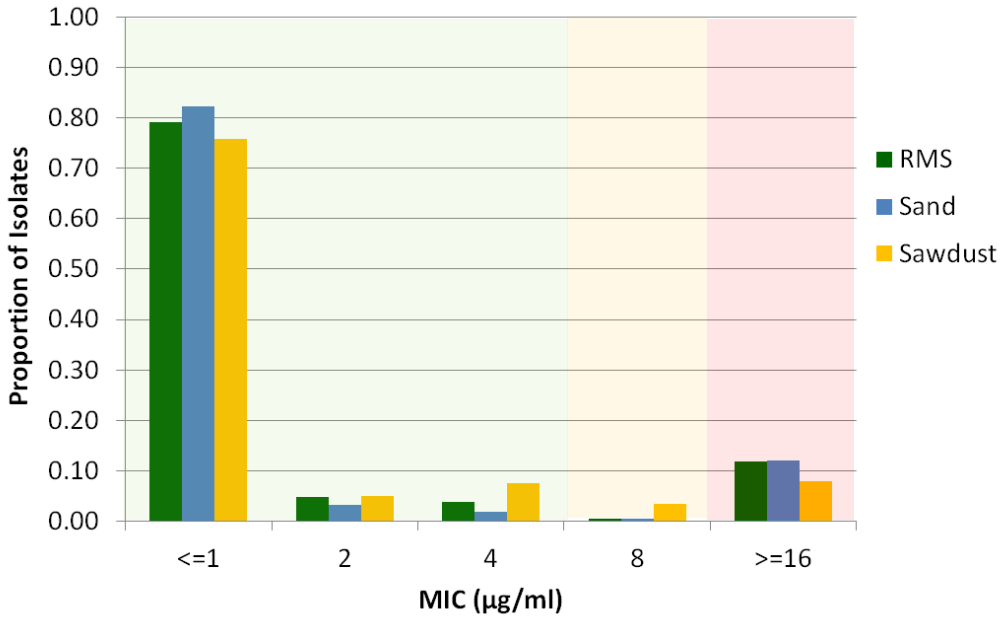
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.22: A summary of the MICs of tetracycline for coliform isolates collected as part of the farm survey.

Tetracycline	MIC (µg/ml)						
	Not Tested	<=1	2	4	8	>=16	Total
Escherichia coli							
RMS		128	1			7	136
Sand		128				8	136
Sawdust	1	138	1			15	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		39		3		13	55
Sand		21				2	23
Sawdust		18				3	21
***Serratia* spp**							
RMS		4	7	6	1	4	22
Sand	1	3	7	4		7	22
Sawdust		1	9	8	1		19
***Citrobacter* spp**							
RMS		5				2	7
Sand	1	18				4	23
Sawdust		6					6
Other *Enterobacteriaceae*							
RMS		5	3			1	9
Sand		7			1	5	13
Sawdust	1	16	2	10	7	1	37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.17: An illustration of the MICs of tetracycline for coliform isolates collected as part of the farm survey.



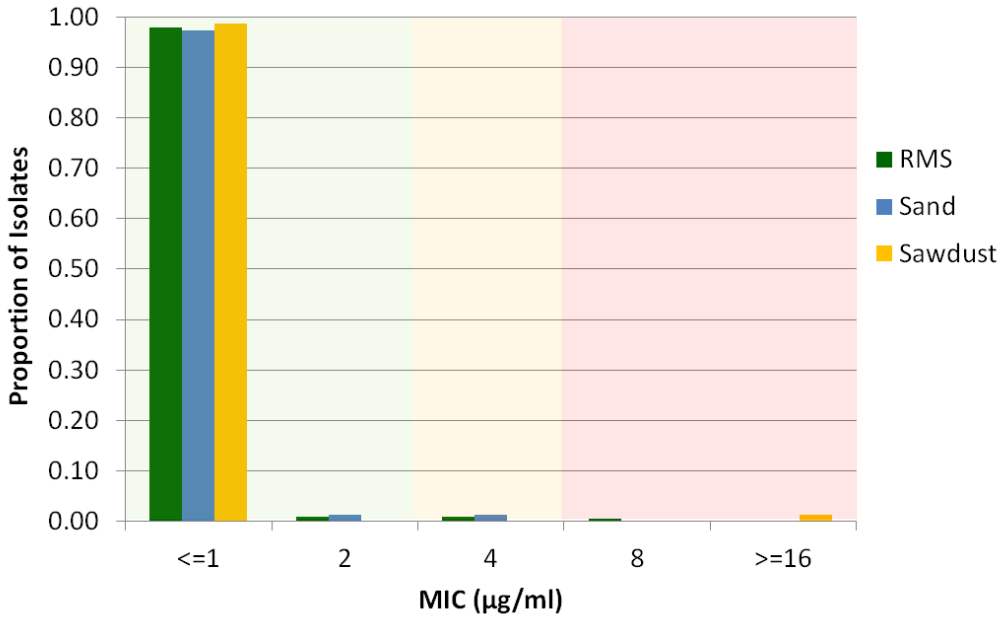
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.23: A summary of the MICs of tobramycin for coliform isolates collected as part of the farm survey.

Tobramycin	MIC (µg/ml)						
	Not Tested	<=1	2	4	8	>=16	Total
Escherichia coli							
RMS		135			1		136
Sand		136					136
Sawdust	1	151				3	155
***Klebsiella* spp and *Raoultella* spp**							
RMS		55					55
Sand		23					23
Sawdust		21					21
***Serratia* spp**							
RMS		18	2	2			22
Sand	1	16	2	3			22
Sawdust		19					19
***Citrobacter* spp**							
RMS		7					7
Sand	1	21	1				23
Sawdust		6					6
Other *Enterobacteriaceae*							
RMS		9					9
Sand		13					13
Sawdust	1	36					37

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.18: An illustration of the MICs of tobramycin for coliform isolates collected as part of the farm survey.



The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.24: A summary of the findings of multivariable analysis of MICs of different antimicrobials for coliform organisms.

Antibiotic		OR	2.5%CI	97.5%CI	
Ampicillin	Control bedding = Sand				
	RMS	1.35	0.86	2.12	NS
	Sawdust	1.03	0.65	1.63	NS
Amikacin	Control bedding = Sand				
	RMS	1.04	1.01	1.08	p<0.05
	Sawdust	1.01	0.98	1.05	NS
Chloramphenicol	Control bedding = Sawdust				
	RMS	0.75	0.61	0.93	p<0.05
	Sand	0.84	0.66	1.07	NS
Ceftiofur	Control bedding = Sand				
	RMS	1.18	1.03	1.35	p<0.05
	Sawdust	1.21	1.05	1.39	p<0.05
Cefalexin	Control bedding = Sawdust				
	RMS	0.94	0.80	1.11	NS
	Sand	0.83	0.69	1.00	p<0.05
Enrofloxacin	Control bedding = Sawdust				
	RMS	1.03	0.96	1.12	NS
	Sand	1.09	1.00	1.20	p<0.05
Nitrofurantoin	Control bedding = Sawdust				
	RMS	1.17	0.92	1.49	NS
	Sand	1.13	0.85	1.50	NS
Piperacillin	Control bedding = Sawdust				
	RMS	0.87	0.66	1.14	NS
	Sand	0.75	0.54	1.04	NS

The MICs of each of the antibiotics for *Enterococcus* spp isolates overall are illustrated in Figures 7.19 to 7.29 and summarised by 'group' in Tables 7.25 to 7.35. Univariable analysis identified significant differences in the MICs of *Enterococcus* spp organisms between the different bedding types for clindamycin ($p<0.001$) and enrofloxacin ($p=0.013$).

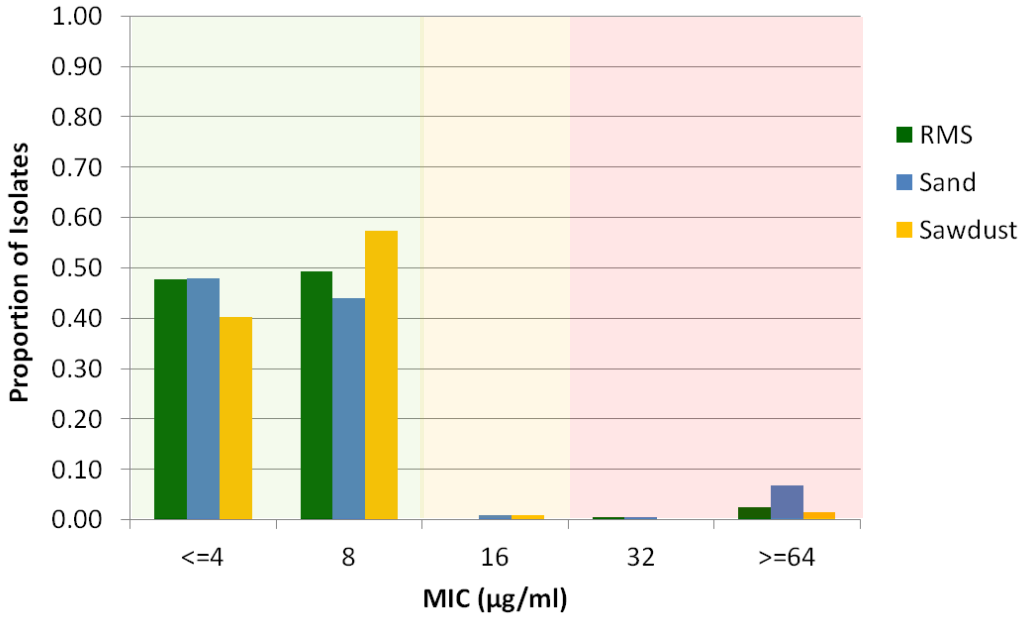
The results of multivariable analysis of MICs for different antimicrobials against *Enterococcus* spp organisms is summarised in Table 7.36. Based on the variables recorded, multivariable analysis confirmed that there were differences in the MICs for clindamycin and enrofloxacin. MICs for clindamycin were significantly higher for *Enterococcus* spp recovered from farms bedded on RMS compared to sand or sawdust. MICs for enrofloxacin were significantly higher for *Enterococcus* spp recovered from farms bedded on sawdust compared to RMS or sand.

Table 7.25: A summary of the MICs of chloramphenicol for *Enterococcus* spp isolates collected as part of the farm survey.

Chloramphenicol	MIC (µg/ml)						
	Not Tested	<=4	8	16	32	>=64	Total
Enterococcus durans							
RMS		18	11				29
Sand		35	9				44
Sawdust		14	18				32
Enterococcus faecalis							
RMS		6	25			6	37
Sand	2	4	31			2	39
Sawdust	1	7	21			2	31
Enterococcus faecium							
RMS		45	12				57
Sand		40	16				56
Sawdust	3	34	29				66
Enterococcus hirae							
RMS		34	32				66
Sand		8	11				19
Sawdust		20	20				40
Other *Enterococcus* spp							
RMS	17	13	40		1		71
Sand	45	12	24	2	1	12	96
Sawdust	28	10	33	2		1	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.19: An illustration of the MICs of chloramphenicol for *Enterococcus* spp isolates collected as part of the farm survey.



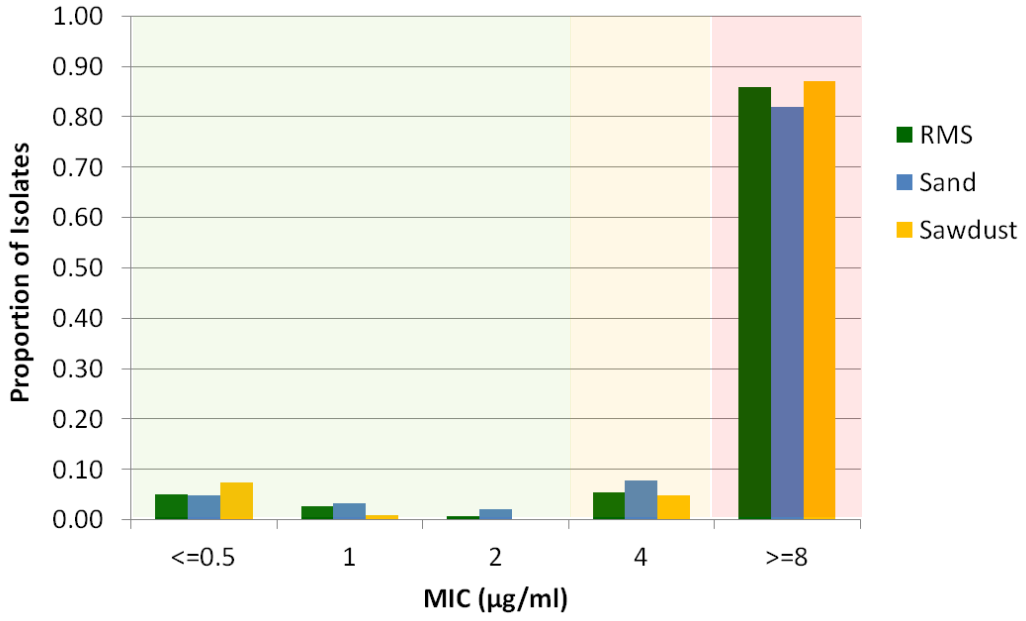
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.26: A summary of the MICs of cefovecin for *Enterococcus* spp isolates collected as part of the farm survey.

Cefovecin	MIC (µg/ml)						
	Not Tested	<=0.5	1	2	4	>=8	Total
Enterococcus durans							
RMS		1			2	26	29
Sand		1			2	41	44
Sawdust						32	32
Enterococcus faecalis							
RMS					2	35	37
Sand		1	1	1	6	30	39
Sawdust	1					30	31
Enterococcus faecium							
RMS		3	2	1	4	47	57
Sand		6		1		49	56
Sawdust	1	14	2		2	47	66
Enterococcus hirae							
RMS		6				60	66
Sand					2	17	19
Sawdust		3			1	36	40
Other *Enterococcus* spp							
RMS	5	3	5	1	6	51	71
Sand	9	4	7	3	9	64	96
Sawdust	10				8	56	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.20: An illustration of the MICs of cefovecin for *Enterococcus* spp isolates collected as part of the farm survey.



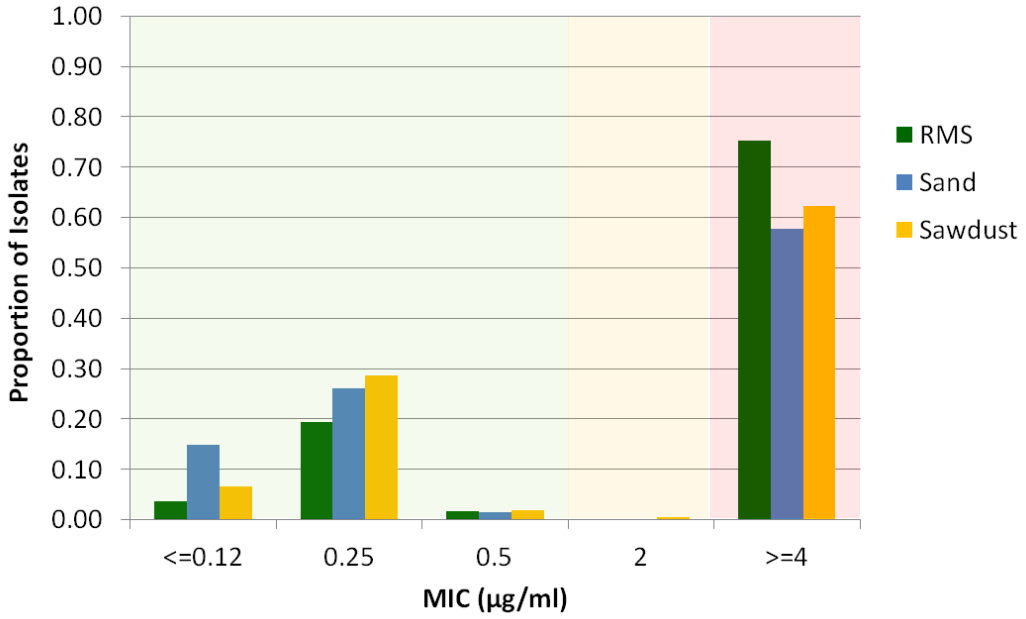
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.27: A summary of the MICs of clindamycin for *Enterococcus* spp isolates collected as part of the farm survey.

Clindamycin	MIC (µg/ml)						
	Not Tested	<=0.12	0.25	0.5	2	>=4	Total
Enterococcus durans							
RMS		5	18			6	29
Sand		24	15			5	44
Sawdust		6	20			6	32
Enterococcus faecalis							
RMS	1					36	37
Sand	7	1				31	39
Sawdust	1					30	31
Enterococcus faecium							
RMS		4	22	4		27	57
Sand		2	30	3		21	56
Sawdust	3	4	33	4	1	21	66
Enterococcus hirae							
RMS			3			63	66
Sand						19	19
Sawdust		3	1			36	40
Other *Enterococcus* spp							
RMS	17		4			50	71
Sand	51	2	6			37	96
Sawdust	30	1	6			37	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.21: An illustration of the MICs of clindamycin for *Enterococcus* spp isolates collected as part of the farm survey.



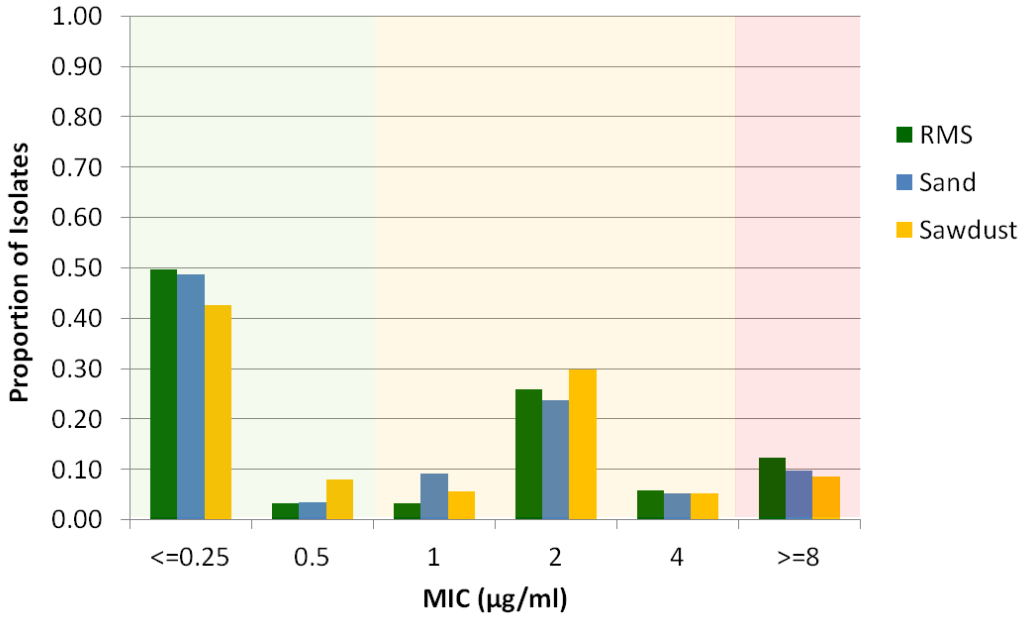
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.28: A summary of the MICs of erythromycin for *Enterococcus* spp isolates collected as part of the farm survey.

Erythromycin	MIC (µg/ml)							
	Not Tested	<=0.25	0.5	1	2	4	>=8	Total
Enterococcus durans								
RMS		28					1	29
Sand		44						44
Sawdust		28				3	1	32
Enterococcus faecalis								
RMS		4	3	6	16	1	7	37
Sand	2	8	5	11	11		2	39
Sawdust	1	2	9	8	9	1	1	31
Enterococcus faecium								
RMS		14	4	1	35	2	1	57
Sand		19	1	8	23	1	4	56
Sawdust	3	18	4	1	38		2	66
Enterococcus hirae								
RMS		66						66
Sand		19						19
Sawdust		40						40
Other *Enterococcus* spp								
RMS	16	9	1	1	12	11	21	71
Sand	45	11	1		15	10	14	96
Sawdust	28	2	4	3	16	7	14	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.22: An illustration of the MICs of erythromycin for *Enterococcus* spp isolates collected as part of the farm survey.



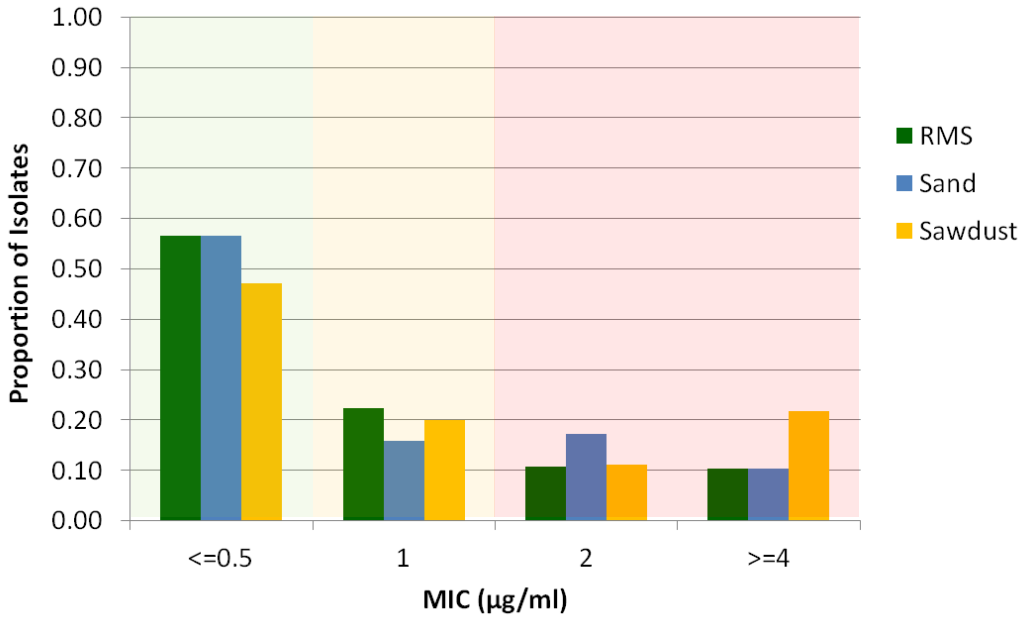
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.29: A summary of the MICs of enrofloxacin for *Enterococcus* spp isolates collected as part of the farm survey.

Enrofloxacin	MIC (µg/ml)					
	Not Tested	<=0.5	1	2	>=4	Total
Enterococcus durans						
RMS		28	1			29
Sand		43	1			44
Sawdust		32				32
Enterococcus faecalis						
RMS		27	10			37
Sand	4	27	8			39
Sawdust	1	22	8			31
Enterococcus faecium						
RMS		9	16	7	25	57
Sand		4	13	19	20	56
Sawdust	4	3	5	9	45	66
Enterococcus hirae						
RMS		64	2			66
Sand		16	2		1	19
Sawdust		37	3			40
Other *Enterococcus* spp						
RMS	18	9	25	19		71
Sand	47	25	8	16		96
Sawdust	32	3	25	14		74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.23: An illustration of the MICs of enrofloxacin for *Enterococcus* spp isolates collected as part of the farm survey.



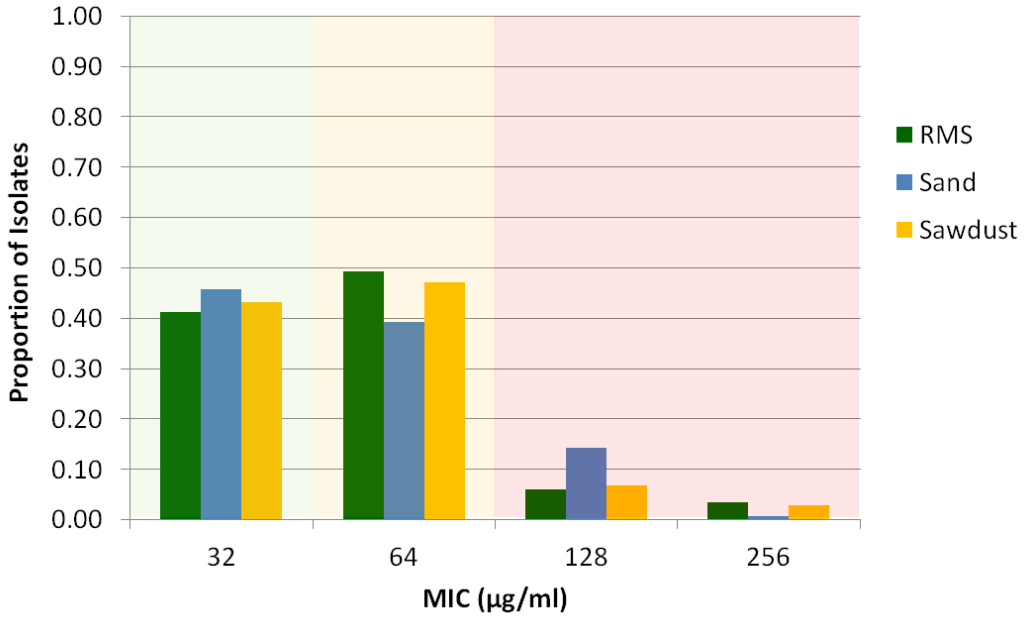
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.30: A summary of the MICs of nitrofurantoin for *Enterococcus* spp isolates collected as part of the farm survey.

Nitrofurantoin	MIC (µg/ml)					
	Not Tested	32	64	128	256	Total
Enterococcus durans						
RMS		9	9	5	6	29
Sand	1	20	14	9		44
Sawdust	2	16	7	4	3	32
Enterococcus faecalis						
RMS	29	8				37
Sand	33	5	1			39
Sawdust	18	5	8			31
Enterococcus faecium						
RMS	1	21	34	1		57
Sand	1	26	28	1		56
Sawdust	8	20	30	6	2	66
Enterococcus hirae						
RMS		23	42		1	66
Sand	1	5	13			19
Sawdust		18	22			40
Other *Enterococcus* spp						
RMS	31	21	13	6		71
Sand	63	15	5	12	1	96
Sawdust	41	16	15	2		74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.24: An illustration of the MICs of nitrofurantoin for *Enterococcus* spp isolates collected as part of the farm survey.



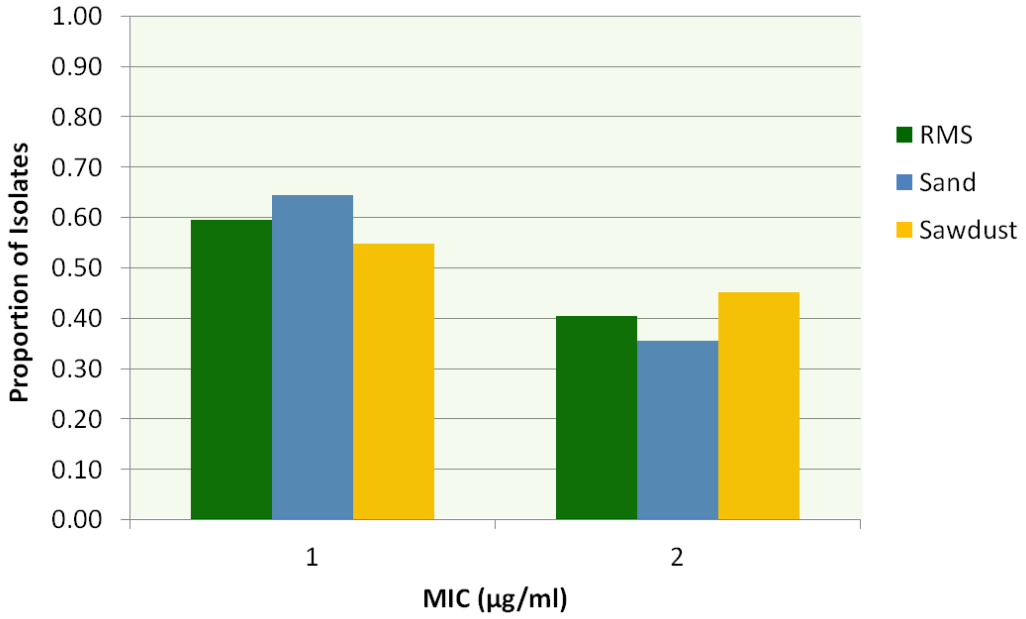
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.31: A summary of the MICs of marbofloxacin for *Enterococcus* spp isolates collected as part of the farm survey.

Marbofloxacin	MIC (µg/ml)			
	Not Tested	1	2	Total
Enterococcus durans				
RMS	25	4		29
Sand	26	16	2	44
Sawdust	27	5		32
Enterococcus faecalis				
RMS	1	24	12	37
Sand	4	27	8	39
Sawdust	3	19	9	31
Enterococcus faecium				
RMS	31	3	23	57
Sand	37	3	16	56
Sawdust	52		14	66
Enterococcus hirae				
RMS	3	60	3	66
Sand	3	15	1	19
Sawdust	2	38		40
Other *Enterococcus* spp				
RMS	27	12	32	71
Sand	38	33	25	96
Sawdust	33	7	34	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.25: An illustration of the MICs of marbofloxacin for *Enterococcus* spp isolates collected as part of the farm survey.



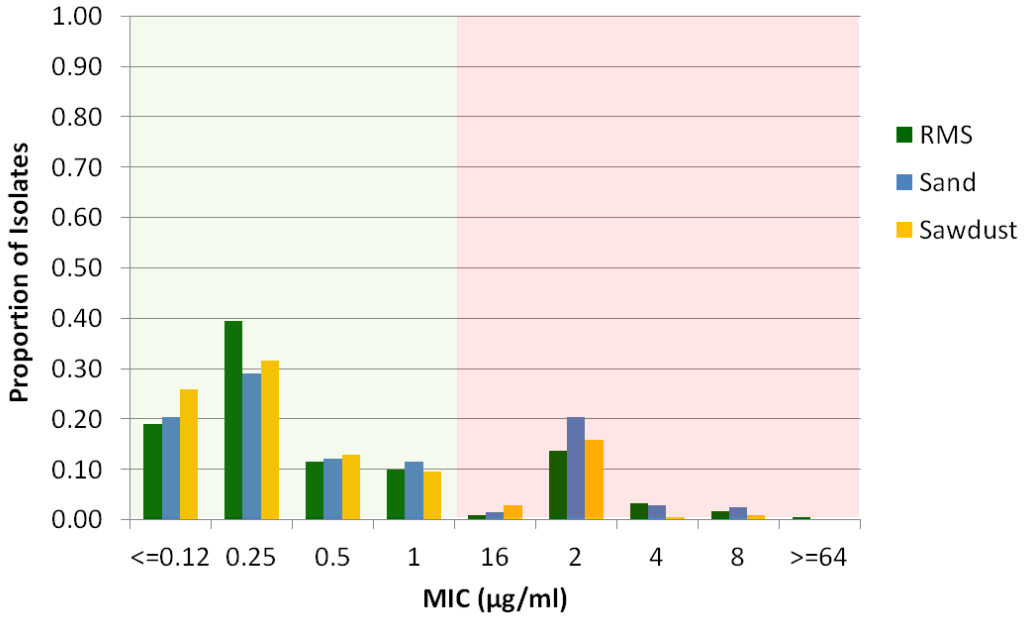
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.32: A summary of the MICs of benzylpenicillin for *Enterococcus* spp isolates collected as part of the farm survey.

Benzylpenicillin	MIC (µg/ml)									
	Not Tested	<=0.12	0.25	0.5	1	2	4	8	16	>=64	Total
Enterococcus durans											
RMS		3	13	4	3		5	1			29
Sand		5	14	5	8	1	4	5	2		44
Sawdust		9	15	3	1		1	2	1		32
Enterococcus faecalis											
RMS						31	3	3			37
Sand	2				3	34					39
Sawdust	1				1	29					31
Enterococcus faecium											
RMS		13	20	11	9	2			2		57
Sand		17	7	12	12	6	2				56
Sawdust	3	20	18	9	8	3			5		66
Enterococcus hirae											
RMS		13	33	8	11					1	66
Sand		6	12	1							19
Sawdust		15	11	8	6						40
Other *Enterococcus* spp											
RMS	19	17	29	5	1						71
Sand	45	14	27	7	1	1			1		96
Sawdust	30	10	22	7	4	1					74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.26: An illustration of the MICs of benzylpenicillin for *Enterococcus* spp isolates collected as part of the farm survey.



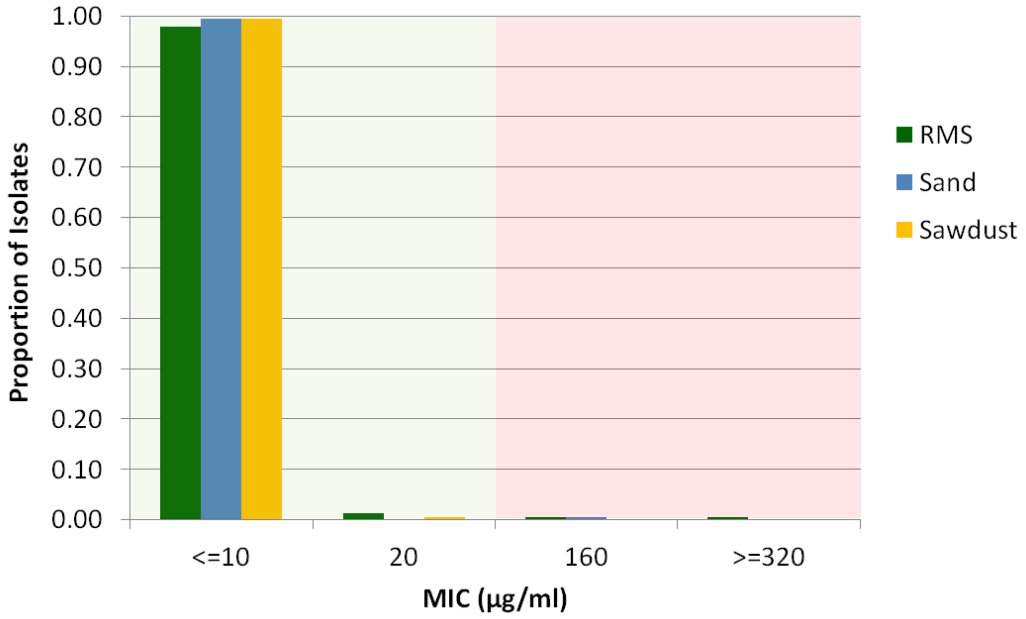
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.33: A summary of the MICs of trimethoprim/sulfamethoxazole for *Enterococcus* spp isolates collected as part of the farm survey.

Trimethoprim/Sulfamethoxazole	MIC (µg/ml)					
	Not Tested	<=10	20	160	>=320	Total
Enterococcus durans						
RMS		29				29
Sand		44				44
Sawdust		32				32
Enterococcus faecalis						
RMS		35		1	1	37
Sand	2	36		1		39
Sawdust	1	30				31
Enterococcus faecium						
RMS		57				57
Sand		56				56
Sawdust	3	63				66
Enterococcus hirae						
RMS		66				66
Sand		19				19
Sawdust		40				40
Other *Enterococcus* spp						
RMS	17	51	3			71
Sand	45	51				96
Sawdust	28	45	1			74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.27: An illustration of the MICs of trimethoprim/sulfamethoxazole for *Enterococcus* spp isolates collected as part of the farm survey.



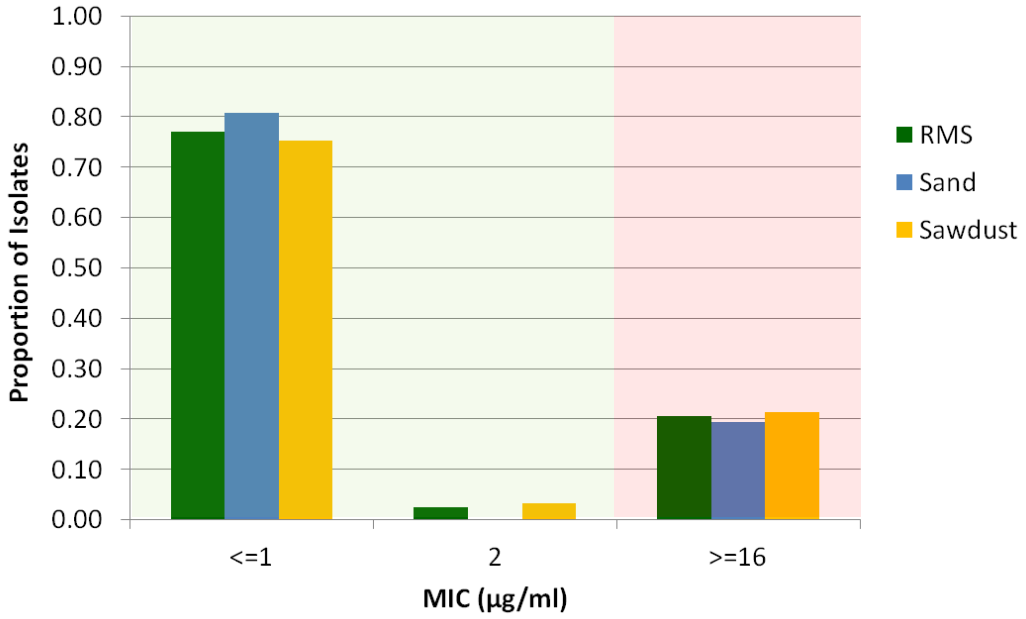
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.34: A summary of the MICs of tetracycline for *Enterococcus* spp isolates collected as part of the farm survey.

Tetracycline		MIC (µg/ml)			
	Not Tested	<=1	2	>=16	Total
Enterococcus durans					
RMS		16		13	29
Sand		27		17	44
Sawdust		22		10	32
Enterococcus faecalis					
RMS		14	1	22	37
Sand	2	20		17	39
Sawdust	1	18		12	31
Enterococcus faecium					
RMS		48	1	8	57
Sand		56			56
Sawdust	3	53	4	6	66
Enterococcus hirae					
RMS		66			66
Sand		19			19
Sawdust		40			40
Other *Enterococcus* spp					
RMS	17	43	4	7	71
Sand	45	45		6	96
Sawdust	28	26	3	17	74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.28: An illustration of the MICs of tetracycline for *Enterococcus* spp isolates collected as part of the farm survey.



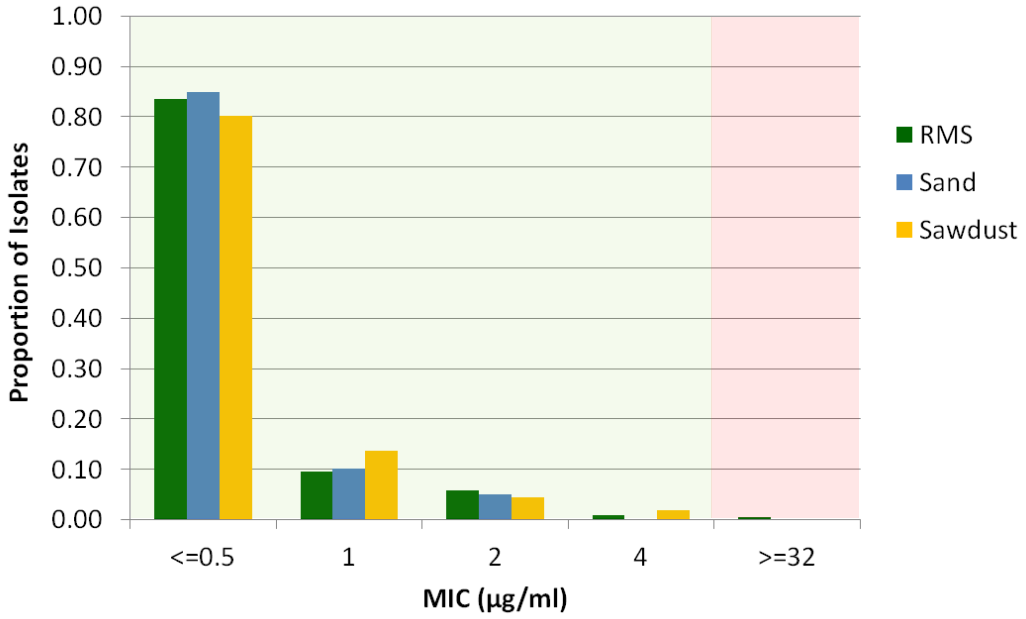
The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.35: A summary of the MICs of vancomycin for *Enterococcus* spp isolates collected as part of the farm survey.

Vancomycin	MIC (µg/ml)						
	Not Tested	<=0.5	1	2	4	>=32	Total
Enterococcus durans							
RMS		29					29
Sand		44					44
Sawdust		32					32
Enterococcus faecalis							
RMS	1	5	18	13			37
Sand	5	4	20	10			39
Sawdust	1	2	19	9			31
Enterococcus faecium							
RMS		52	5				57
Sand		56					56
Sawdust	5	52	9				66
Enterococcus hirae							
RMS		66					66
Sand		19					19
Sawdust		40					40
Other *Enterococcus* spp							
RMS	17	50		1	2	1	71
Sand	50	46					96
Sawdust	31	39			4		74

MICs for the isolates listed as 'not tested' could not be determined by the methodology used by the Vitek 2.

Figure 7.29: An illustration of the MICs of vancomycin for *Enterococcus* spp isolates collected as part of the farm survey.



The green, amber and red zones represent isolates classified as sensitive, intermediate or resistant based on published breakpoints - these are indicative only. The majority of breakpoints are those published by CLSI, when unavailable for a specific antibiotic other sources such as EUCAST or manufacturer published values were used.

Table 7.36: A summary of the findings of multivariable analysis of MICs of different antimicrobials for *Enterococcus* spp organisms.

Antibiotic		OR	2.5%CI	97.5%CI	
Clindamycin	Control bedding = Sawdust				
	RMS	1.75	1.12	2.73	p<0.05
	Sand	1.09	0.68	1.74	NS
Enrofloxacin	Control bedding = Sand				
	RMS	1.01	0.72	1.41	NS
	Sawdust	1.43	1.04	1.98	p<0.05

7.4 Discussion

After the initiation of the project, an opportunity arose which would allow access to a VITEK® 2 instrument and the determination of MICs of the coliform and *Enterococcus* spp rather than determination of sensitivities using the Kirby-Bauer disc diffusion methods. Given that in many cases break points for the classification of veterinary isolates as sensitive or resistant have not been determined it was felt that being able to determine MICs would offer a more robust approach. This is the data that has been presented in this report. The MICs reported should be considered and their interpretation undertaken in the context in which the data was collected - *ie* as a way of establishing a baseline from which any future changes could be tracked. Importantly, this part of the study was not intended to be an investigation of factors affecting MICs of organism recovered from milk and bedding from farms using different bedding systems.

The data from this study should also be interpreted in light of the fact that it is based on phenotype and not genotype (and therefore may not necessarily reflect the genetic potential of the bacterial population), and is based on a relatively small number of isolates per farm collected at a single point in time and may therefore not reflect the population as a whole. Therefore, whilst offering a useful 'snapshot' and benchmark in time, this data cannot, by itself, be considered to be a comprehensive overview of antimicrobial resistance on the sampled farms; it rather provides (as initially envisaged) a useful point from which further studies can be undertaken. As such there is a need for further research in this area, both through more in-depth analysis of the current dataset and continued monitoring of farms over time.

Despite the caveats outlined above, we can make interesting observations from the collated data. The vast majority of isolates were below the breakpoints used to define resistant strains and were defined as 'wild-type' by the Vitek 2 analysis - *ie* "the phenotype is defined as the phenotype for that species in the "wild," *ie* prior to any mutation of chromosomal genes or acquisition of new DNA that alters susceptibility to the drug class in question" (Sanders *et al*, 2000).

Whilst differences between MICs were identified between bedding groups, these differences were not clearly in favour of or against any particular bedding type - this is a particularly interesting finding that warrants further research. It could be that different beddings favour different bacterial populations which have different inherent resistances, or abilities to acquire resistance, and we have not detected

these effects despite controlling for organism type in our initial analysis of this data. Further analysis of the existing dataset may provide further insights in this area.

Whilst the analysis used in this study did control for antimicrobial use on farm, it only did so in a very 'coarse' way (*ie* had an antimicrobial class been used on farm in adult cattle (and by which route) in the previous 12 months). This somewhat crude analysis suggested complex interactions may have been occurring which may have included cross resistance and collateral sensitivity through direct or pleotropic effects. A full investigation of such possible effects and interactions is beyond the scope of this report and is an area in need of further research, both in the existing dataset and elsewhere.

Whilst some initial analysis of the impact of RMS management on MICs was undertaken, this was not envisaged in the initial study design, was not comprehensive, and has therefore not been reported – this is an area that needs to be addressed in further research.

7.5 Conclusions

This study has not generated any clear evidence that the short term use of recycled manure solids as bedding, as compared to sawdust and sand, is associated with a general increase in MICs of the major classes of antibiotics when considering coliforms and *Enterococcus* spp. An in-depth analysis of the impact of bedding type and management on antimicrobial resistance was not envisaged as part of the research outlined in the original tender prior to this study and for that reason insufficient data is currently available to make any changes to the recommendations for best practice, with respect to the potential for perpetuation of antimicrobial resistance, when using RMS as bedding.

Further research, using the dataset generated by this study and elsewhere, is needed to further our understanding of any potential interactions between bedding type and management and antimicrobial resistance in the environment.

8 General Discussion

8.1 Overview of Study Findings

This study was designed to attempt to meet the requirements of a tender to assess the "Risks, benefits and optimal management of recycled manure solids for use as bedding for dairy cattle". In as far as this short study has allowed we have managed to improve our understanding of both the potential risks and benefits of the use of RMS as bedding for adult dairy cattle as well as aspects of optimal management.

Through the large and comprehensive survey of bedding practices across three bedding materials, we now have a much better understanding of the bacterial content of RMS when used as bedding, in both deep and shallow beds and how this compares to other currently used bedding materials. We can better relate these (or not as the case may be) to management practices on farm. However, the interpretation of our findings should be tempered by the fact that we have a self-selecting group of farmers who have been successful in implementing the use of RMS as bedding and this may therefore not necessarily reflect the risks associated with use of RMS as a bedding in a more general population of farms.

We have been able to assess the impact of bacterial load in bedding on the bacterial content of bulk milk and have identified some relationships, though it is fair to say that best practice in the milk routine is more than capable of mitigating any potential risks. However, the concern must remain, that in the absence of best practice there remains a significant risk that milk quality will suffer as a result of a high bacterial load in bedding. A key finding of this study is that there appears to be as much variation within bedding systems in bacterial numbers in bedding and bulk milk as between systems.

The finding that bacterial levels in bedding, including those of thermophilic spore-forming bacteria, were not correlated with bacterial levels in milk, might be taken to suggest that there may be scope to mitigate against the transfer of the high levels of food spoilage organisms reported in composted bedding materials into milk (although a study in the Netherlands (Driehuis *et al*, 2014) reports elevated levels of heat resistant spores in BOTH bedding and milk, so it may be that in "deliberately composted material" the levels are higher than can be reduced with milking practice).

The survey has also, coupled with the controlled trial at Sewborwens Farm, afforded the opportunity (albeit somewhat limited by the current number of RMS users in the UK) to better understand the potential risks and benefits of deep *vs* shallow RMS beds with respect to both comfort and bacterial load, with RMS beds offering some clear advantages over existing more conventional bedding materials.

Detailed analysis of udder health data has allayed many fears with respect to the impact of RMS use on udder health, though the concern must still remain with respect to the impact on clinical mastitis and particularly severe *Klebsiella* spp mastitis – it is disappointing that such concerns have still not been addressed, but this is primarily due to lack of recording of clinical disease on farm. Another concern could be that this particular coliform is acknowledged as a cause of persistent intramammary infection and, as a result, its prevalence could increase with time bedded on RMS.

Within the limits of existing knowledge, using the assumptions made, illustrative modelling of possible levels of MAP and *Salmonella* spp in slurry and RMS in different scenarios has gone some way to

improving our understanding of the potential risks of RMS with respect to these diseases. With respect to MAP, the risk would not appear to be high (providing existing RMS regulations are complied with), but uncertainties remain around the potential for pathogen accumulation in the case of a *Salmonella* spp outbreak on a farm using RMS as bedding.

Analysis of antimicrobial resistance in microorganisms on farms using RMS and other bedding materials revealed that no one bedding type was associated with higher MICs overall, for the antibiotics and the coliform and *Enterococcus* spp tested, with each bedding type being associated with the highest MICs for at least one antibiotic class. Therefore, based on current evidence, there is no reason to believe that, within the relatively short timeframe within which RMS has been used in the UK, there is any increased risk of exposure to organisms harbouring antimicrobial resistance over and above any increased risk associated with the higher bacterial numbers.

Data on cow comfort indicators for different bedding materials and designs has been collated in both the survey and controlled study at Sewborwens Farm, both of which have highlighted potential benefits of the use of RMS as a bedding material - these are important considerations, though these advantages need to be viewed in the context of the ability to mitigate other potential risks.

8.2 Summary of Findings Based on Risk Assessment Framework

8.2.1 Risks to Animal Health

8.2.1.1 Udder Health

Release assessment: There is a very high load of a wide variety of environmental mastitis pathogens in RMS bedding, which was higher than on sand or sawdust beds for all pathogen groups analysed.

Exposure assessment: Pathogens on the bedding surface will inevitably come into contact with teats. It should be remembered that lack of visible contamination, or the absence of foreign material, is not necessarily proof of absence of pathogen. However, the fact that cleanliness scoring showed less contamination on udders and lower legs on RMS than on sawdust suggests that the risk of transfer of pathogens from bed to teats could be lower with RMS. Anecdotal reports of the relative difficulty of cleaning teats of cows bedded on sand, compared with RMS, along with an indication of supporting evidence from degree of *Listeria* spp transfer from sand bedding material to milk, suggests that there may be less risk of pathogen transfer through the teat orifice associated with milking, for RMS than for sand.

Consequence assessment: Neither the survey, nor the controlled experiment, gave any evidence of increased risk of elevated cow or quarter level somatic cell count as a result of using RMS bedding. In the controlled experiment, the higher number of new cases of mastitis presenting on RMS bedding approached significance; however, the relatively limited amount of clinical mastitis data available from the survey farms did not show increased risk of clinical mastitis on RMS farms. Therefore, the possibility of an increased risk of clinical mastitis (and possibly severe mastitis) cannot be ignored. It is also worthy

of note that the four farmers discontinuing use of green bedding did so in the face of large increases in cases of mastitis (and also elevated somatic cell counts).

Mitigation: Managing beds to keep them as clean as possible, and excellent teat preparation, would be expected to help with mitigation, particularly as within the RMS bedded farms, TBC in bulk milk increased with TBC on bedding. The survey did not indicate, within RMS farms, any specific management factors which would consistently reduce the surface bacterial load in RMS significantly, apart from deep beds having lower TBC, and *Streptococcus* spp counts, and RMS on mats having lower *Bacillus cereus* levels. For certain management factors (daily application of fresh bedding) there was an indication of an effect of reducing bacterial count on beds. Conditioners were not shown to be beneficial in this study. There was an indication that slow building of deep beds would reduce coliforms during the building phase.

Regardless of bedding material, fore-milking should be advised as this was associated with reduced TBC in bulk tank milk across all farms. Excellent teat preparation should be employed to minimise the risk of transfer of pathogens during milking. Across all bedding types, pre-dipping was associated with reduction in *Streptococcus* spp in bulk milk across all farms, and with reduced bulk milk somatic cell count on RMS farms, suggesting that this practice has an important role in mitigating any increased risk of mastitis that may be associated with an increased number of bacteria in bedding.

8.2.1.2 Johne's Disease (MAP)

Release assessment: Modelling based on the available data indicates that levels of MAP in RMS bedding in the most extreme herd outbreak scenarios could reach levels of up to 10,000 organisms/g but would be likely to be self-limiting (at least in the short term).

Exposure assessment: Exposure to influential amounts of RMS by the route of ingestion is unlikely for adult cows, although possible for youngstock.

Consequence assessment: The theoretical MAP levels suggest that infection of young cattle could occur. However, although the minimum infective dose for adults is unknown, it is unlikely to be exceeded through ingestion of RMS, even by self-grooming (the most likely route of transfer).

Mitigation: As already indicated by the legislation and guidelines for best practice, mitigation of any risk of MAP transfer will be best achieved by preventing the most susceptible (youngest) age groups of cattle having access to RMS.

8.2.1.3 *Salmonella* spp

Release assessment: Modelling based on the limited data available from literature indicates that levels of *Salmonella* in RMS bedding in certain herd outbreak scenarios could reach levels of concern. For example, in a "catastrophic" 8-week herd outbreak, peaking with 30% "high shedders", levels were predicted to peak at 2 x10^7 cfu/g.

Although figures for the human minimum infective dose of *Salmonella* are typically given as 10^3 (Ryan and Ray, 2004), it is reported that on occasions infection has been caused by <10^3 organisms (Blaser and Newman, 1982). This means that potentially 1 mg of RMS could be infective to a human in a catastrophic herd outbreak.

Exposure assessment: Both young cattle, and adult cows could become exposed to "influential" quantities of *Salmonella* through the intake of a very small amount of RMS – which it might be possible to ingest through self-grooming.

Consequence assessment: The theoretical levels of *Salmonella* spp generated by the models suggest that infection of young cattle could occur through ingestion of very small quantities of RMS, and, although the minimum infective dose for adults is unknown, it is possible that this could be exceeded through ingestion of RMS by self-grooming.

Mitigation: As already indicated by the legislation and guidelines for best practice, mitigation of any risk of *Salmonella* transfer will be best achieved by preventing the most susceptible (youngest) age groups of cattle either contributing slurry to, or having access to RMS. In the event of a herd outbreak of *Salmonella* mitigation of risk would be best achieved by discontinuing the use of RMS.

8.2.1.4 Dust

Dust is a potential health hazard and carrier of pathogens. At the release assessment level, subjective observations made in the survey indicated that levels of dust on surfaces of buildings where RMS was used were lower than where sawdust was used, and not different from buildings with sand.

8.2.1.5 Injury

One anecdotal report suggests that the risk of injury by slipping accidents was increased by the use of RMS in comparison with sand. Grooved or textured concrete could help to mitigate this risk.

8.2.1.6 Hock abrasion

There is evidence of reduced risk of hock swelling and abrasion on mattresses if RMS is used, in comparison with sawdust.

8.2.1.7 Reasons for culling

There was no evidence of increased risk on RMS farms of culling for infertility, mastitis, legs/feet, TB, Johne's disease or casualty losses, using a simple measure based on the reasons for the most recent culls. However, it may take time for issues influential in culling to accumulate on farms utilizing RMS as bedding.

8.2.2 Risks to Human Health

8.2.2.1 Zoonotic Pathogens

Release assessment: The survey and controlled trial have allowed a more comprehensive assessment of zoonotic organisms of interest. Total bacterial counts, coliform counts, *Staphylococcus* spp, and *Bacillus cereus* counts were higher in RMS bedding than in other bedding materials. *Streptococcus* spp counts were higher than in sand. Frequency of isolation of *Yersinia enterocolytica* and *Salmonella* spp was no different between RMS, sand or sawdust (although numbers were very small). *Listeria* spp were isolated less often from RMS than from sand.

Counts of bacteria in bulk milk did not vary according to bedding type. Within RMS farms, bulk milk TBC increased with used bedding TBC.

The modelling exercise suggested that high levels of *Salmonella* spp in RMS could be reached in herd outbreaks of *Salmonella*, with the level reached depending on the form of the outbreak. With the modelled replication in slurry and bedding (which are necessarily based on very broad assumptions, in the absence of evidence) the model suggests that concentrations in RMS would rise above those in fresh faeces, thus potentially increasing the release.

Exposure assessment: Farm workers will be exposed to a higher pathogen count per gram of material when handling RMS compared with sand or sawdust. However, the risks are not directly comparable on this basis due to the difference in density of the materials and the frequency of bedding application. One must also consider that all farm workers will be exposed to fresh bovine faeces, regardless of the type of bedding used, though one might not expect that to vary between bedding types. The necessity to monitor the production of RMS at the separator, and the tendency recorded in the survey for farmers to do this by "feel", suggests there may be increased contact with RMS than with other bedding materials. Exposure during distribution may not differ since similar bedding distribution equipment is used for various materials.

The exposure to pathogens through contact with bulk milk or consumption of milk before pasteurisation would be expected to be independent of bedding type.

The risk of exposure to pathogens through consumption of milk after pasteurisation would be restricted to those pathogens that survive pasteurisation. This study has given no reason to believe that the risk would vary with bedding type.

Consequence assessment: The consequences of exposure to zoonotic pathogens via a route involving contact with RMS or consumption of contaminated products are unlikely to differ from those if the pathogen is contracted by any other route.

Mitigation: Farm workers should be made aware of the high bacterial load of RMS and the potential pathogens it may contain, and take the necessary precautions in terms of use of PPE and personal hygiene.

Milk produced on farms using RMS should be pasteurised before consumption.

8.2.2.2 Dust

Release assessment: Dust is a potential health hazard and carrier of pathogens for humans as well as animals. At the release assessment level, subjective observations made in the survey indicated that levels of dust on surfaces of buildings where RMS was used were lower than where sawdust was used, and not different from buildings with sand. Farm workers frequently commented on the lack of dust working with RMS compared with sawdust. However, the potential for the high number of micro-organisms present in RMS to be transmitted in fomites or aerosols is not currently understood.

Exposure assessment: Exposure to any dust from RMS might be similar to that from other bedding materials as it is usually applied using similar dispensing machinery.

Consequence assessment: Small particles of RMS would carry a higher pathogen load than small particles of sawdust, so the consequences of ingestion or inhalation may be more severe.

8.2.3 Risks for Food Quality

Release assessment: Although the counts of heat resistant organisms (LPC and thermophilic organisms, and *Bacillus cereus*) were higher in RMS bedding than in other materials, this was not translated into an increased count in bulk milk in this research Although this runs contrary to the reports of higher levels of heat resistant organisms in milk originating from farms using composted materials, this may be due to a 'threshold' effect (*ie* in materials which have undergone deliberate composting, the levels of heat resistant organisms or their spores are so high that teat preparation procedures are insufficient to remove them).

As 'release' to bulk milk in this study was not different on RMS compared with other bedding types the risk pathway has not been extended further.

8.2.4 Risks for Animal and Human Health as a Result of Antimicrobial Resistance

Release assessment: Whilst RMS beds were found to harbour higher numbers of pathogens than other beds, there was no evidence that the MICs for the major classes of antibiotics were any higher in the coliform and *Enterococcus* spp tested. Therefore, based on current evidence there is no reason to believe that, within the relatively short timeframe within which RMS has been used in the UK, there is any increased risk of exposure to organisms harbouring antimicrobial resistance over and above any increased risk associated with the higher bacterial numbers.

Onward passage of antimicrobial resistance via bulk milk is unlikely to differ given that different beddings were not associated with differing numbers of bacteria in milk.

Exposure assessment: Exposure will be similar to when considering exposure to pathogens present in bedding. Both animals and farm workers will be exposed to a higher pathogen count per gram of material when bedded on or handling RMS compared with sand or sawdust and therefore arguably to a higher number of any resistant organisms. However, the risks are not directly comparable on this basis due to the difference in density of the materials and the frequency of bedding application. One must also consider that all farm workers will be exposed to fresh bovine faeces, regardless of the type of bedding used, though one might not expect that to vary between bedding types. Moreover, whilst there may be an increase in the number of resistant organisms, there is no reason to believe that the proportion will be any higher.

The exposure to pathogens through contact with bulk milk or consumption of milk before pasteurisation would be expected to be independent of bedding type.

Consequence assessment: There is no reason to believe that this would be different between different bedding types.

Mitigation: Measures would be the same as when considering the presence of pathogens in bedding. Farm workers should be made aware of the high bacterial load of RMS, the potential pathogens it may contain, and the fact that all bedding materials are likely to contain resistant bacteria. They should take the necessary precautions in terms of use of the use of PPE and personal hygiene.

9 Implications for Best Practice

9.1 Overview of Implications for Best Practice

It has been difficult to identify evidence for many specific management practices to reduce the high bacterial load reached in used RMS on beds. The survey and controlled trial suggested, perhaps surprisingly, that total bacterial count, *Streptococcus* spp and psychrotrophic counts were higher in shallow than deep RMS. We were unable to demonstrate any impact of bedding conditioners (*eg* lime) on bacterial numbers in used bedding. More frequent bedding was associated with lower *Streptococcus* spp counts in used RMS bedding suggesting that more frequent bedding might be beneficial.

9.2 Comments on the Current Requirements and Recommendations for Best Practice for Use of Recycled Manure Solids (RMS) as Bedding in Cubicles for Dairy Cattle

The current requirements and recommendations for use of RMS as bedding (published at http://dairy.ahdb.org.uk/media/1037862/q_a_rms_bedding.pdf - accessed 1 July 2015) are listed below, with accompanying comments on any alterations suggested as a result of the present study (in red).

9.2.1 Current Requirements

1. RMS must only be produced using raw cattle manure/slurry from housing and/or yards.

Manure from other livestock species must not be included for the production of RMS, to avoid introducing external pathogens which may affect cattle health.

Comment: No change - not specifically addressed.

2. Material that has been composted or digested must not be used

The spores of certain bacteria, particularly those that are heat-resistant may be encouraged by composting. Too high a concentration of spores can lead to losses during the manufacture of cheese and reduce the shelf life of pasteurised milk.

Putting manure through a digester will also increase temperatures, which can affect pathogen load. Until further information is available, use of RMS produced from the output of a digester is not permitted. Equally, use of digestate which contains feedstock from non-farm sources could cause an additional unacceptable risk, and is not permitted.

Comment: No change as a result of this project, since these materials *per se* were not studied.

3. RMS must only be used as bedding for cattle which are in the same epidemiological unit as those cattle from which it is generated

To minimise the risk of disease transfer, RMS must only be produced on the unit on which it is to be used and only from slurry originating from that unit. Slurry or manure must not be moved between units either before or after processing. An epidemiological unit comprises animals which come into contact

with each other directly or indirectly (e.g. shared facilities or personnel) as part of the same farm business. They may not necessarily be housed on the same site or premises.

Comment: No change - reinforced by evidence from in silico modelling of the likelihood of persistence of *Salmonella* spp in RMS bedding.

4. Movement of RMS between epidemiological units is not permitted

Similarly, to reduce the risk of transferring pathogens, slurry or manure to be used to produce bedding must not be moved between units, either before or after processing.

Comment: No change - reinforced by evidence from in silico modelling of the likelihood of persistence of *Salmonella* spp in RMS bedding.

5. RMS must not be produced from manure/slurry of herds which are subject to official restriction for notifiable diseases, other than TB

The main notifable disease of concern is foot and mouth disease, as the infective agent can occur in faeces and urine up to four days before clinical signs appear. A list of notifiable diseases is available on Defra's website (http://www.defra.gov.uk/animal-diseases/notifiable/).

Comment: No change - not specifically addressed.

6. Manure from TB Inconclusive reactors and TB reactors must be excluded from the use of RMS

As yet the specific risk of TB spread has not been studied. However, unless TB is advanced in an animal, there are unlikely to be large numbers of organisms shed in faeces. With regular testing, the chances of reaching this stage of infectivity are much reduced. However, if TB were present in slurry, it is not likely to be reduced by physical separation. Therefore, manure from TB inconclusive reactors and TB reactors must be excluded from RMS.

Comment: No change - not specifically addressed.

7. Manure from aborted cattle under brucellosis investigation must be excluded from use as RMS

On farms where RMS are being used for bedding, rigorous biosecurity is even more important in relation to suspected brucellosis cases as it is a zoonosis.

Comment: No change - not specifically addressed.

8. Other materials, such as birthing fluids and placental material, manure from calving areas, and waste milk must not be disposed of by adding these to manure/slurry going for RMS

Afterbirth and other fluid materials are a potential risk for disease transmission. Waste milk, subject to withdrawal period, must not be added to the slurry pool, as there is an increased risk of developing antibiotic resistance. Anecdotally, inclusion of waste milk in material used for bedding has been associated with increased cell count/mastitis problems.

Comment: No change based on the findings of this study.

9. There should be no shared equipment for the handling and processing of feed and RMS

If any equipment is shared (loaders etc.) it must be thoroughly cleaned between uses. Designed to prevent cross contamination of feed or forage.

Comment: No change - not specifically addressed.

10. Should any separation equipment be moved between different epidemiological units, it must be thoroughly cleaned and disinfected before moving and subsequent re-use

On the continent, movement of contaminated equipment has been linked to transfer of pathogens from one farm to another.

Comment: No change - not specifically addressed.

11. RMS must only be used as bedding for housed cattle over six months old

Regulations on calf health and welfare (Council Directive 2008/119/EC and the Welfare of Farmed Animals Regulation 2007) state that calves must have access to a lying area which is 'clean, comfortable and adequately drained and which does not adversely affect the calves'. Youngstock are particularly susceptible to disease and if infected may be highly contaminating themselves. Risks of disease transmission will be minimised by preventing calves less than six months old from having contact with faeces and slurry from adult cattle. Any calves that are inadvertently born in areas bedded on RMS must be removed as soon as possible from the area, to a location where suitable alternative bedding is provided.

Comment: No change - reinforced by indications from in silico modelling of the likelihood of persistence of *Salmonella* spp in RMS bedding. Although the in silico modelling of MAP suggested that levels of MAP in bedding would plateau at levels unlikely to be infective for adult cows (based on current understanding of MAP infection susceptibility), the regulation preventing use for cattle under six months of age should be maintained, as these would a) have a lower minimum infective dose and b) be far more likely to ingest larger amounts of material.

12. Milk from herds using RMS must be pasteurised

All bedding materials are potential sources of contamination for milk. Micro-organisms and their spores can get on to the teat from the bedding and through the milking process end up in the milk bulk tank. As a precautionary measure, use of RMS is not permitted on farms selling unpasteurised milk.

Comment: No change. Although across all bedding types there was no evidence of relationships between bacterial counts on bedding and in milk, there was evidence of correlation between TBC in bedding and in milk within RMS.

13. RMS must be produced from a slurry separator unit, designed for the purpose, which produces manure solids of at least 34% DM

Slurry is mechanically separated into a liquid fraction and a "solid" fraction, typically by using a screw or roller press action. The equipment needs to be capable of extracting sufficient water to make the solid fraction at least 34% dry matter. If the material is too wet (below 34%) it is unsuitable for use as bedding.

Comment: No change.

14. RMS must only be used on cubicle beds, and not as a deep bed in pens or yards

RMS must only be used in cubicles, either as a layer on top of mattresses, or as a cubicle bed up to 15 cm in depth. It should not be used in calving areas, due to the susceptibility of newborn calves to Johne's disease.

Comment: No change. Deep beds are likely to support more thermophilic spore formation and *B. cereus*. Furthermore it is not possible to maintain a 'dry' bedding environment in pens / yards.

9.2.2 Current Recommended Best Practices

In addition, to the requirements above which must be followed at all times, the twelve recommendations in this section should be followed as current best practice.

1. Users of RMS as dairy cow bedding should actively monitor cow health, in particular intramammary health, as well as bulk tank milk quality .

Comment: No change or upregulate? the challenges faced in the study of obtaining sufficient data on clinical mastitis cases from farms reinforces the need for record keeping to ensure that the implications of changes can be understood.

2. Farm personnel should be made aware of the importance of personal hygiene during and following the handling of RMS

Comment: No change, but potential levels of *Salmonella* spp reinforce the importance of this mitigating factor.

3. RMS should be prepared and stored under cover to avoid an increase in water content prior to application

Comment: No change - although no increased risks were identified associated with uncovered separators, this was confounded by the fact that farmers avoided preparing bedding outdoors in wet weather. If farmers with uncovered separators intend to prepare bedding only in dry weather, they are no longer in control of the frequency and timing of bedding.

4. Manure/slurry from animals under treatment should not be incorporated into RMS (this includes dry cow treatment)

Comment: No change based on the findings of this study.

5. Manure/slurry from animals/herds showing clinical signs of infection, enteric condition or outbreaks of clinical disease (e.g. *Salmonella*, VTEC E.coli. etc.) should not be incorporated

Comment: Upregulate to a requirement. In view of the results of in silico modelling, it is suggested that use of RMS bedding MUST be suspended in the event of a Salmonella outbreak.

6. There should be excellent cow preparation at milking time (e.g. pre-milking teat preparation and pre-dipping), sanitation of milking equipment and cow hygiene

Comment: Upregulate to a requirement. In view of the relationship (within farms using RMS), of TBC in milk increasing as the TBC in bedding increased, the importance of teat preparation in maintaining milk quality needs to be emphasised. It is suggested that pre-dipping should become a requirement for herds bedded on RMS.

7. There should be excellent bedding/cubicle management, including

- **Adding RMS to the beds in limited quantities to allow further drying to take place**
- **Managing beds to minimise 'heating' and therefore bacterial multiplication after application**
- **Designing and managing beds to minimise contamination with urine and fresh faecal material**
- **Frequent removal (at least daily) of freshly soiled material from bedding)**

Comment: No change

8. Ventilation should be adequate and overstocking avoided, to ensure further drying of RMS once applied to bedding and to minimise the levels of ammonia in the housed atmosphere

Comment: No change to this recommendation is suggested on the basis of the current study. Although no direct relationship between building ventilation and risk measures was identified, likely to be as a result of other confounding factors, there is no reason to alter this recommendation which is based on general understanding of physical principles.

9. Freshly separated RMS should be used as soon as practically possible (normally within 12 hours)

Comment: No change

10. Newly introduced adult animals to the herd should not have their manure mixed into the RMS system (for a period of one month), i.e. material from isolation pens should not be added to the pool for separation

Comment: No change to this recommendation is suggested on the basis of the current study. The predictions for levels of *Salmonella* as an example of an infectious disease endorse this practice.

11. Water and/or solutions used in footbath wash should not be disposed of in the slurry/manure to be used as RMS bedding

Comment: No change based on the findings of this study.

12. Manure/slurry from cattle less than 12 months old should not be used as a raw material for RMS. The material should only be used to bed cattle older than 12 months old.

Comment: No change to these recommendations is suggested on the basis of the current study. The predictions for levels of *Salmonella* endorse this practice.

9.3 Best Practice for Building RMS Beds

The farmer-led question of "What is the best way avoid heating while establishing deep beds of RMS?" proved difficult to answer categorically. So many parameters are likely to affect the temperature and dry matter content of the bedding material and although these two parameters might be expected to be

interdependent, there is clearly not a simple relationship between them allowing simple recommendations to be made. However it is probably fair to draw the following conclusion from the limited research conducted as part of this study:

- There was an indication that “slow” building of beds over a week results in lower temperatures during this building phase (compared to filling beds completely on day one).
- The effect of presence or absence of cows during the building phase does not appear to be consistent.
- There was some indication that heating during the building phase might be increased if the dry matter of the initial material was lower.
- The implications of physical conditions for bacterial numbers in bedding and cow intramammary infection remain unclear.

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APPENDIX 1 - Farm Survey Protocols

A1: Sampling Bulk Milk

- Sample the milk collected over 24 or 48 hours.
- Ensure that the bulk milk is fully cooled and agitated before taking the sample.
- Collect samples from the top of the bulk tank (if at all possible) using the disposable dipper provided (one per farm). If using the tank outlet for milk collection, let the milk drain for a short period to ensure the milk sample is representative of the milk inside the bulk tank.
- Take care to collect the sample hygienically.
- Fill one 500 ml sterile sample bottle if there is only one bulk tank.
- If there is more than one bulk tank, sample each tank into a separate sample bottle, and record volume of milk in each tank

A2: Sampling Bedding

Unused Bedding

Using a gloved hand, collect a sample of bedding material from below the surface (up to a depth of 25 cm) of the pile/bag of unused bedding. Combine samples from 3 sites / bags to provide approximately 500 ml of material. Recycled Manure Solids must be sampled during or immediately after separation.

Used Bedding

Timing of sampling

Arrange the visit so that used bedding is sampled immediately prior to the application of fresh bedding (*ie* sample the bedding at its "dirtiest").

Cubicle selection

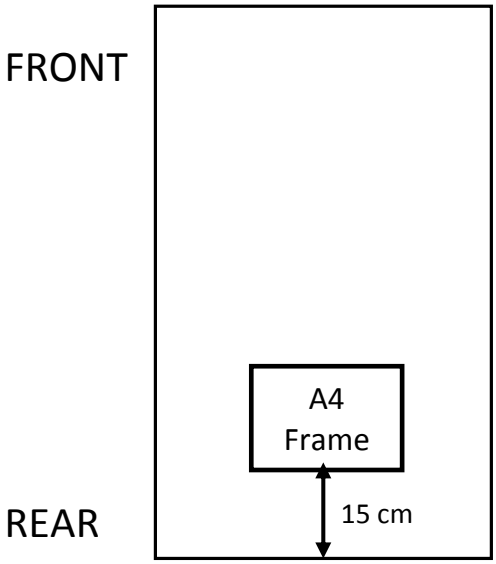
A minimum of 10 cubicles need to be sampled. These need to be distributed proportionally across the different sheds and rows within sheds, and then randomly positioned, avoiding any atypical cubicles. If there is fresh faeces in the sampling position of the selected cubicle, move to another cubicle. If there are both deep and shallow beds, sample at least 10 of each type and record the proportions of each.

Sample collection

The sampling position is illustrated in Figure A2. In total, approximately 750 ml (a full bag of loosely packed used bedding) is needed.

Place an A4 frame centrally, in landscape format, at the rear of the cubicle to be sampled, approx 15 cm from the rear edge (in the udder contact area). Collect bedding from within the frame to a maximum depth of 2.5 cm. If bedding is deep you may only need a sample from each corner of the frame. If bedding is sparse you may need to sample more cubicles.

Figure A2: Position for sampling used bedding from cubicles



A3: Cubicle Measurements and Observations

Make the following observations on the cubicles from which used bedding is collected.

1. *Percentage bedding cover - whole cubicle.*

2. *Percentage bedding cover - rear 1/3 of cubicle.*

3. *Depth of bedding in front 1/3 of cubicle* ("knee area"). Use rod to penetrate to base of cubicle and measure distance to top of bedding.

4. *Depth of bedding in rear 1/3 of cubicle* ("udder area") (in the centre of the sampling frame prior to sampling). Use rod to penetrate to base of cubicle and measure distance to top of bedding.

5. *Temperature of bedding at 5 cm depth in rear 1/3 of cubicle* ("udder area") (in the centre of the sampling frame prior to sampling). If bed is sufficiently deep, measure temperature using the thermometer provided by inserting probe to a depth of 5cm. Do not measure temperature if the cubicle has just been vacated by a cow, in this case measure the temperature of an adjacent cubicle.

A4: Observations of Shed Environment

1. Temperature. Measure external and internal temperatures using the thermometer provided.

2. Ventilation. Assess ventilation - fill in the descriptors and on the basis of these allocate an overall score.

1 Excellent	2 Good	3 Poor	4 Inadequate

Justifications for score

Roof sheets clean	
Condensation	
Cobwebs	
Odour	
Ridge type	
Side cladding	
Gable end cladding	
Slotted roof	
Wind shadows and shelter	
Multi-span	
Predominant wind direction	

3. Dust. Assess dust level by observations of structural surfaces. Score:

0 - None or minimal

1 - Some

2 - A lot

A5: Cow Scoring

Selection of cows to score

Score 30 milking cows, proportionally distributed across groups (exclude any hospital groups). Work out how many cows should be scored per group and then score the 'n'th cow encountered when moving through groups.

Cow assessment

Look at both sides of cow and record the highest score observed.

Scoring methodology

Cleanliness

Score according to Cook (2002): lower leg, upper leg and flank, udder

Hock Scores

Score according to the first column in Table A5.

Table A5: Hock scoring and mapping onto existing scores.

Score used in study	DairyCo/AHDB Score	Potterton (2011) Score	Description
Swelling			
0	0	0	None, normal anatomy clearly defined
1	0	1	Mild - thicker than normal
2	1	2	At least 2cm protruding
3	2	3	At least 5cm protruding
Hairloss and lesions			
0	0	H 0	No hairloss or lesion
0	0	H 1	Hairloss < 2cm diameter
1	1	H 2,3	Hairloss at least 2cm diameter
2	2	U 1	Skin damage, wound or scab < 2cm diameter
3	2	U 2,3	Skin damage, wound or scab at least 2cm diameter

DairyCo/AHDB Score
http://dairy.ahdb.org.uk/technical-information/animal-health-welfare/welfare-assessment