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Are Sand or Composted Bedding Cubicles Suitable Alternatives to Rubber Matting for Housing Dairy Cows?

A Knowledge Summary by

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KNOWLEDGE SUMMARY

PICO question

In [Dairy Cow Management] do [Sand OR composted bedding] compared with [rubber matting] result in [fewer disease incidence] consequences?

Clinical bottom line

Clean, deep-bedded sand appears to be associated with the best outcomes in clinical mastitis, cow cleanliness, subclinical mastitis, cow lying times, hock lesions and cow preference. Recycled sand, composted manure and other deep-bedded systems also appear to have increased cow comfort and hygiene indices versus mattress systems. Deep-bedded, composted manure systems can also have better outcomes concerning Gram positive and negative bacterial growth *versus* straw and mattress systems as long as they are kept clean and renewed frequently.

Clinical Scenario

Many veterinarians are faced with dairy clients asking their advice for the best bedding medium to install in new housing upgrades for their indoor barns. Of course, the advisor has to consider cost, practicality in suitable housing design and willingness of the client to pursue the ideal path. However, there are many outcomes to consider: "Welfare" (which can have many facets), mastitis, skin lesions, lameness, optimum lying times as well as an economic and productivity benefit. Producers also have to consider logistical practicalities in slurry handling and quality assurance issues: Some milk processors will not accept Recycled Manure Solids (RMS) due to the risk of spore contamination of milk and there may be a risk of thermoduric bacterial growth in milk. There are relatively few examples in literature that address these questions and it will be useful to review the evidence before advising on specific guidelines.

Summary of the evidence

THI=Temperature Humidity Index;
BCS=Body Condition Score;
SCN=Streptococcus catalase negative;
DIM= Days in Milk;
SCC=Somatic Cell Count;
CNS=Coagulase-negative Staphylococcus;
IMI=Intra-mammary infection;
Se/Sp=Sensitivity/Specificity;
PCR=Polymerase chain reaction;
BTSCC=Bulk tank somatic cell count;
CFU=Culture Forming Units
RMS: Recycled Manure Substrate
DBMS=Deep bedded manure substrate
SBMS=Shallow bedded manure substrate
NES=New sand
RS=Recycled sand

Table 1: Outline summary of bedding type by disease event and behaviour outcomes

Bedding type	Mastitis	Lameness and Lying
Sand	• Less clinical mastitis incidence with clean sand than other media (Gao, Guarin), • No difference between culture of SCN, *Strep agalactiae* and Gmnegatives in sand vs pasture (Black). • Lowest number of organisms in new sand vs used or manure (Rowbotham) • Lower odds of CNS IMI than straw (Dufour) • Less colony forming units on sand vs box compost (van Gestelen) • Heat treating (to 80degC) sand allows heat-sensitive microorganisms to suppress growth of *E. coli* O157:H7 (Westphal) • Lower SCCs on sand systems than bedded pack and mattresses (Dufour, Wenz, Jayarao) • Less growth of *Klebsiella* and *Enterococcus* on clean sand than recycled sand, manure or shavings (Godden) • Clean sand has higher dry matter and lower organic matter than recycled sand but both support the same bacterial counts (Kristula, Carroll) • More *Streptococcus* spp. found on sand vs sawdust (Zdanowicz)	• Cows lay down for more time vs. pasture (Black) • Cows lay down for more time vs rubber matting (Bak, Solano, Jensen) • Lying time and frequency of bouts were increased in deep sand bedding vs others (Ito, Norring, Cook) • First lying bout longer on sand vs compost or mattress (van Gestelen, Cook) • Overall hoof health was better for cows on sand vs straw on mats (Norring) • For every 1-cm decrease in bedding, cows spent approximately 11 min less lying during each 24-h period (Drissler) • Time standing in stall was significantly lower in Sand cubicles vs rubber mattress (Cook, Wagner-Storch) • Preference study showed Sand > Mattress > Waterbed > Concrete (Wagner-Storch) • Another preference study showed mattresses preferred over sand (Manninen)
Deep Manure	• Less clinical mastitis incidence than new sand (Guarin) • Many more Gmnegative organisms found in manure bedding than other types of bedding (Rowbotham) • The amount of bacteria in bedded pack increases with bedding temperature and decreases with moisture (Black) • Composted manure had an association with higher BTSCC (Wenz)	• Lying bouts longer than mattresses but not as long as sand (van Gestelen) • Lower incidence of hock lesions in yard/pack barns compared with freestalls on sand (Lobeck, van Gestelen)
Shallow Manure on Mats	• Less clinical mastitis than sand (Guarin) • 9 times more streptococci than recycled sand (Rowbotham)	• See below data for mats
Wood shavings/Straw/Dirt on Mats	• More mastitis incidence than sand (Gao) • Lower odds of CNS IMI than straw (Dufour) • Cow cleanliness was decreased in cows on straw/mats vs sand (Norring) • More coliforms and *Klebsiella* found on sawdust vs sand (Zdanowicz)	• Less lying time than deep sand (Bak, Jensen) • Time standing in the stalls was greater in mattress herds than sand bedded herds (Gomez) • Higher percentage of hock lesions compared with dirt base (Lombard) • More cows stand fully in the stalls on mats vs dirt (Lombard, Cook)

1. (Gao J. 2017)	
Population:	Dairy cows and quarter milk samples
Sample size:	3190 dairy cows in 161 herds, 3288 quarter milk samples over a 2.5 year study period
Intervention details:	Sand or organic bedding materials (organic = compost, rice husk or saw dust)
Study design:	Case series
Outcome studied:	Clinical mastitis and culture results from each bedding type
Main findings: (relevant to PICO question):	• Sand bedding was cleaned 3 times per day (during milking) to remove all organic materials. In general, fresh new sand was added to the bedding once per week, according to the bedding thickness • Mean herd cumulative incidence of clinical mastitis (CICM) was 3.3% per month in the herds and median CICM was 3.0% per month (range = 1.7 to 8.1 per herd) • *E. coli* was most frequently isolated (14.4%), followed by *Klebsiella* spp. (13.0%), CNS (11.3%), *Strep. dysgalactiae* (10.5%), Staph. aureus (10.2%), and other streptococci (8.0%). *Enterobacter* spp.,*Strep. agalactiae*, and *Strep. uberis* were isolated in 5.5, 2.8, and 2.1% of samples, respectively • Of 3,288 Clinical Mastitis (CM) samples, 1,750 were collected from herds using sand bedding, whereas 1,538 samples were from herds using organic bedding materials • Mean monthly CICM was 2.9 and 3.7% for herds using sand and organic bedding material, respectively. *Streptococcus dysgalactiae* was more frequently isolated in herds using sand bedding than those using organic bedding, whereas *Klebsiella* spp. and other streptococci were more prevalent in herds using organic bedding.
Limitations:	• Restricted to farms in China • Much variation in management possible between farms and over time. • Mastitis outcome can be influenced by many other factors than bedding, so trends can be reported and associations but solid conclusions are tentative • There are many seasonal and geographical environment factors that vary across such a wide region as China that may also affect mastitis outcomes • Culturing on agar may not always show growth of causative organisms • No mastitis samples were recorded in February due to the Spring festival

2. (Guarín J.F. 2017) **USA**

Population:	Dairy cows housed indoors in Wisconsin
Sample size:	128 primarily primiparous dairy cows, 32 cows in a 4 X 4 design
Intervention details:	4 types of bedding: New sand (NES), Recycled sand (RS), Deep bedded manure solids (DBMS) and shallow bedded manure solids (SBMS) on matting
Study design:	Cohort study
Outcome studied:	Udder cleanliness score (1-4) and teat hyperkeratosis score and teat culture
Main findings: (relevant to PICO question):	• The proportion of udders that were classified as clean (score 1 or 2) was 68%, 82%, 54%, and 95% for cows housed in pens containing NES, RS, SBMS, and DBMS, respectively • No association was found between HK score and teat skin bacterial count. • Higher hygiene scores (dirtier teats) were associated with higher bacterial counts • Bacterial counts of teat skin swabs from front teats of cows in pens containing RS and SBMS were significantly less than those of rear teats of cows in pens containing DBMS or NES
Limitations:	• Low population numbers • Mostly from primiparous cows • Manual observations may be subjective

3. (Kayitsinga J. 2017) **USA**	
Population:	Dairy cows
Sample size:	Survey to 1,700 dairy farms in Michigan, Pennsylvania, and Florida in January and February 2013
Intervention details:	Questionnaire
Study design:	Case series
Outcome studied:	• Questions related to 7 major areas: sociodemographic and farm characteristics, milking proficiency, milking systems, cow environment, infected cow monitoring and treatment, farm labor, and attitudes toward mastitis and related antimicrobial use • Amount of antimicrobial drug use for clinical mastitis by intramammary or systemic administration
Main findings: (relevant to PICO question):	• Use of sand or mattresses for bedding, rather than older styles of housing, were associated with decreased systemic antimicrobial use but also there is an association of less systemic use on higher managed farm types
Limitations:	• Survey was only to Grade A certified dairy farms, so may not be representative of the whole dairy population

	• Responses were dependent on farmer opinion and self-reported frequency of antimicrobial use • There was a huge amount of variation in management practices across the surveyed population • Bedding use was only a very small part of the survey and was based on Sand or Mattress (not specific other bedding types) • Bedding associations based on multivariate regression analysis on low-level evidence foundation (survey and self opinion)

4. Black 2016	
Population:	Dry dairy cows
Sample size:	28 cows
Intervention details:	n=14 in each group . Sand (deep bedded) or pasture
Study design:	Cohort study
Outcome studied:	Accelerometer measuring steps and lying times, respiration rates and feeding displacement rates (studied for 2hours post feeding, once per week), THI from Aug 2013 to Jan 2014. BCS, locomotion and hygiene (overall cow cleanliness) all scored at 1-5.
Main findings: (relevant to PICO question):	• Freestall housed cows lay down for 2 hours longer during far-off and close-up periods ($p < 0.01$; DF: 76), but no differences occurred during calving and postpartum compared to pastured cows • No difference in bacteria cultured from either group
Limitations:	• Low cow numbers • Overproportion of primiparous cows (>60%) • Only studied in Autumn/early winter • Feedspace per cow was 60cm in headlocks for indoor and free in pasture (lower than recommended 75cm in literature and possible for bullying in pasture system, potentially reducing feed intake) • Comparing sand and pasture, not bedding types in same system, so confusing results for general indoor management and pasture • Manual observations may be subjective

5. Rowbowtham 2016	
Population:	Lactating Dairy cows in freestall pens
Sample size:	128 cows in 4 identical pens in one barn (n=32 cows per group) over 42 weeks.
Intervention details:	Each pen had either NES (new sand), RS (recycled sand), DBMS (deep bedded manure solids), SBMS (shallow bedded manure solids on mattresses)

Study design:	Cohort study
Outcome studied:	• Bacterial culture of bedding • Teat swabs from one teat per cow from each cow, once a week, pre- and post-preparation for milking • Linear regression modelling
Main findings: (relevant to PICO question):	• The median number of total Gram-negative bacteria in DBMS was 13 times as great as the median number in SBMS and 60 and 216 times as great as the median numbers in RS and NES • Not much difference between recycled and new sand in numbers of coliforms • Numbers of total gram-negative bacteria, coliforms, and *Klebsiella* spp. were significantly greater in the summer than in other seasons • 9.12 times as many streptococci in shallow-bedded manure solids as in recycled sand • Across all bacteria and bedding types, the mean number of bacteria recovered from pre-milking swabs (CFU/swab) were approximately 2 to 3 logs less than the corresponding number of bacteria in bedding • 4, 8, and 19 times as many total gram-negative bacteria recovered from cows bedded on SBMS, RS, and DBMS versus NS. • Post-prep swabs revealed less bacteria in all spp. and all bedding types • coliform bacteria (*Escherichia* spp., *Klebsiella* spp., and *Enterobacter* spp.) were only 2 to 7% of total gram-negative bacteria in bedding
Limitations:	• Primarily primiparous cows (96 vs 15 multiparous) • Other cows entered the pens when cows ended their lactations

6. (Rowbotham R.F., Associations of selected bedding types with incidence rates of subclinical and clinical mastitis in primiparous Holstein dairy cows 2016) **USA**	
Population:	Dairy cows in lactation
Sample size:	109 cows for 12 months
Intervention details:	Primarily primiparous cows randomly assigned to pens containing freestalls with 1 of 4 bedding materials: (1) deep-bedded new sand (NES, n = 27 cows), (2) deep-bedded recycled sand (RS, n = 25 cows), (3) deep-bedded manure solids (DBMS, n = 31 cows), and (4) shallow-bedded manure solids over foam-core mattresses (SBMS, n = 26 cows)
Study design:	Cohort study
Outcome studied:	• Incidence of subclinical mastitis (SM): >200k cells per quarter, sampled every 28 days from every quarter • Incidence of clinical mastitis (CM): abnormal milk appearance.

	All affected cases were cultured on agar
Main findings: (relevant to PICO question):	• The proportion of SM samples that were culture negative was numerically greater in quarters of primiparous cows bedded with NES and DBMS as compared with those bedded with RS and SBMS • Far fewer cases of CM were caused by Gm –ve bacteria for primiparous cows housed in pens containing NES than in other bedding types • Majority of SM cases were cultured negative (71%), meaning that there was no association with cultured species and bedding type for SM. • Almost half of milk samples from CM cases did not result in the identification of a pathogen • The overall incidence of CM was 0.26 cases/1,000 and was not associated with bedding types • There was a tendency for longer survival times to first case of CM for quarters of cows in pens containing NES as compared with quarters of cows bedded with RS (P = 0.056) or DBMS (P = 0.086)
Limitations:	• Primarily primiparous cows (96 vs 15 multiparous) • Other cows entered the pens when cows ended their lactations • Time in pen for RS and DBMS was significantly longer than NS and SBMS groups

7. (Bak A.S. 2016) **DEN**	
Population:	Lame Lactating and Dry Dairy cows
Sample size:	42 obviously or severely lame cows (3 were dry)
Intervention details:	19mm thick rubber mattresses in freestalls or 30cm deep bedded sand. Each cow spent 24 hours on one type and then 24 hours on the other. Then they had access to both surfaces for a further 6 days and lameness scored at the end. Concrete slatted flooring in aisles
Study design:	Cohort, cross-over study
Outcome studied:	Scales were the experimenters' own and defined in the methods section of the paper: • Lameness on 1-5 scale • Hoof lesions from trimming and manual ID • Lying times and frequency of changing position • CCTV and manual observation
Main findings: (relevant to PICO question):	• For cows kept on deep-bedded sand, the total lying time, the number of cows observed to lie in the lateral position, and the frequency of lying bouts were higher than when

	kept on the rubber mats • On sand, the lying time ranged from 28 min to 16 h, and while on rubber, it ranged from 6 min to 14 h • The number of cows performing interruptions of lying down did not differ between the 2 surfaces, but while kept on sand, the cows lay down and got up faster than the rubber mats
Limitations:	• Only 4 months of study and prior to this, cows were on loose housing. As lameness lesions take time to manifest clinically, this study cannot imply that bedding types reduce onset of lameness • Cows treated with medication including NSAID were excluded • Cows with sole haemorrhage and no other lesion were excluded • Cows that improved to lameness score 1 or 2 were excluded from the trial (10 cows in total excluded) • Manual observations may be subjective

8. (Solano L. 2016) **CAN**	
Population:	Lactating dairy cows
Sample size:	5135 cows from 10-120 DIM, 40 cows (sample) each from 141 farms across Canada
Intervention details:	Lying behavior modelled using variables: Bedding type, quantity (< or > 2cm depth) and cleanliness
Study design:	Case Series
Outcome studied:	• Automated accelerometer readings • Lying times • Lameness on 1-5 scale • Number of hock and knee injuries
Main findings: (relevant to PICO question):	• Mean herd-level daily lying time ranged from 8.2 to 13.2 h/d and individual daily lying time for cows ranged from 1.3 to 22.1 h/d. • Daily lying time was associated with the same risk factors as the other measures of lying behavior • Bout duration was shorter for cows with injured hocks • At the herd level, cows housed in stalls with sand had an increased average daily lying time of 1.44 h/d compared with cows housed in stalls with wood shavings
Limitations:	• Logistic regression and multivariate modelling on many farm and management factors • Farms had to have >=7000L/lactation average so may not be representative of all farms in country • Lameness by manual assessment • Manual observations may be subjective

9. (Cole K.J. 2016) **USA**	
Population:	Lactating dairy cows
Sample size:	18 mid-late lactation, multiparous dairy cows
Intervention details:	Fresh manure bedding vs Recycled, composted manure bedding over 6 weeks, 3 weeks on each type of bedding in a cross-over study
Study design:	Case Series
Outcome studied:	• Bacterial counts of mastitis pathogens present in bedding
Main findings: (relevant to PICO question):	• Gram-negative bacteria, coliform, and streptococcal counts in RMS windrows were reduced after 4 wk composting compared with counts in windrows before composting. • *Klebsiella* counts and DM did not differ • Composting recycled manure solids (RMS) offered minimal advantages over fresh RMS in reducing bacterial counts of common mastitis pathogens in bedding for dairy cows in freestalls
Limitations:	• Low cow numbers • Multiparous cows only • Mid-late lactation cows (most studies report more lameness in early to mid lactation)

10. (Jensen M.B. 2015) **DEN**	
Population:	Lame Lactating and dry dairy cows
Sample size:	42 obviously or severely lame cows (3 were dry)
Intervention details:	19mm thick rubber mattresses in freestalls or 30cm deep bedded sand. Each cow spent 24 hours on one type and then 24 hours on the other. Then they had access to both surfaces for a further 6 days and lameness scored at the end. Concrete slatted flooring in aisles
Study design:	Cohort, cross-over study
Outcome studied:	• Scales were Lameness on 1-5 scale (according to Thomsen P.T., Munksgaard L., Togersen F.A., (2008), Evaluation of a lameness scoring system for dairy cows, *Journal of Dairy Science*, 91, 116-126) • Hoof lesions from trimming and manual ID • Lying times and frequency of changing position • CCTV and manual observation
Main findings: (relevant to PICO question):	• The cows spent more time on the deep-bedded sand than on the rubber surface.

	• On sand, cows spent the majority of the time lying, whereas on the rubber surface, they spent most of the time upright
Limitations:	• Only 4 months of study and prior to this, cows were on loose housing. As lameness lesions take time to manifest clinically, this study cannot imply that bedding types reduce onset of lameness • Cows treated with medication including NSAID were excluded • Cows with sole haemorrhage and no other lesion were excluded • Cows that improved to lameness score 1 or 2 were excluded from the trial (10 cows in total excluded) • Manual observations may be subjective

11. (Black R.A. 2014) **USA**	
Population:	Compost bedded pack barns
Sample size:	42 barns with compost bedded pack systems (CBP)
Intervention details:	Correlation models with bacterial count as standard and outcomes as variables Bacterial samples taken from 9 sites in each barn, cultured on agar
Study design:	Case Series
Outcome studied:	Temperature, moisture, carbon-to-nitrogen (C:N) ratio, space per cow, and bacterial counts from bedding material collected from compost bedded pack (CBP) barns
Main findings: (relevant to PICO question):	• Of the total bacteria sampled coliform, streptococci, staphylococci, and *Bacillus* spp. comprised 1.86%, 20.61%, 52.28%, and 25.25% of all bacteria, respectively • Coliforms and staphylococci measured a strong correlation with composite temperature (increasing with increasing temp.) and negatively with moisture • Managing the CBP system for moisture, temperature, C:N ratio, and space per cow may help to reduce some bacterial species counts, but the bacterial load in the bedding will likely remain high.
Limitations:	• Many different stocking densities and management conditions between barns, especially concerning hygiene • 32 barns were for lactating cows and 13 for hospital pens, so comparing healthy with sick cows may bring confounding variables

12. (Ito K. 2014) **CAN**	
Population:	Lactating, high producing dairy cows

Sample size:	Total sample size 3160. 40 cows on each of 40 farms in the northeastern United States (NE) and 39 farms in California (CA)
Intervention details:	Lying time and frequency of bouts were correlated to management and bedding factors
Study design:	Cross Sectional Study
Outcome studied:	Lying times and lameness scores (1-5) correlated to: management measures, including stall design, bedding, and flooring type within the pen
Main findings: (relevant to PICO question):	• Daily lying time decreased with increasing barn age and increased with the use of deep and sand bedding • Daily lying time decreased as stall stocking density increased, Daily lying time increased in the presence of rubber flooring in part of the pen, pen space per cow, and the percentage of stalls with fecal contamination, • Frequency of lying bouts decreased and average bout duration increased with the use of deep and sand bedding
Limitations:	• All farms in the study were from a quality-assurance management program, of which 40 cows were chosen from each farm. As these were already on an assurance scheme, they may not be representative of USA population of dairy farms/cows. • Surveys • Retrospective study • Manual observation of lameness • USA data, not Canadian • Data collected in selected months (Mar-May and Jul-Oct 2010) • Manual observations may be subjective

13. (Adhikari N. 2013) **USA**	
Population:	In vitro study: Inoculations of *Prototheca* gt1 (mastitis causing) and gt2 (non-mastitis causing) in broth control or bedding material
Sample size:	Four bedding types were tested: kiln-dried spruce shavings, "green" (not kiln dried) hemlock sawdust, processed manure-pack, and sand as well as broth control. 3 samples taken per type.
Intervention details:	Growth of *Prototheca*
Study design:	*In vitro* study
Outcome studied:	Quantitative bacterial growth
Main findings: (relevant to PICO question):	• Bedding type had a significant effect on *Prototheca* growth in vitro, and this effect was associated with increased growth in manure, sawdust, and sand beddings.

	• Kiln-dried spruce shavings may inhibit *Prototheca* growth
Limitations:	• In vitro only • Bedding samples taken from manufacturing sites and not dairies • Low sample numbers meant large error bars in results

14. (Dufour S. 2012) **CAN**	
Population:	Lactating dairy cows
Sample size:	• 91 farms from 4 regions of Canada. 15 cows from each farm. 3 milk samples were collected from each quarter of the selected cows at intervals of 3 wk for 2 years. Total of 59,167 quarter samples collected • Farm observations • Questionnaire on farm management
Intervention details:	SCC and culture results vs farm management practices
Study design:	Farmer Survey
Outcome studied:	SCC, culture, questionnaire and observation results on management practices of farm
Main findings: (relevant to PICO question):	• CNS IMI incidence of 0.29 new IMI/quarter-month but this was 0.36 when misclassification (due to Se/Sp of culture and other factors) was considered • Use of sand or wood-based product bedding was associated with lower odds of acquiring a CNS IMI compared with straw bedding
Limitations:	• Milk samples growing >3 types of bacteria were considered contaminated and excluded, meaning > 7,000 samples were excluded • Mastitis incidence is highly related to management practices and these varied between each farm, so multiple confounding factors were not taken into account

15. (Lobeck K.M. 2011) **USA**	
Population:	Lactating dairy cows
Sample size:	18 farms, 6 of each housing type, Jan-Nov 2008. 11,400 cows were scored each season across all farms
Intervention details:	Cross negative ventilated freestall barns – all deep sand (CV) and compost-bedded-pack barns – 5 wood sawdust, 1 wheat straw (CB), compared with conventional, naturally ventilated freestall barns (NV)
Study design:	Case Series

Outcome studied:	Scales were the experimenters' own and defined in the methods of the paper. Locomotion (1-5 scale), hock lesions (1-3 scale), body condition score (1-5 scale), hygiene, respiration rates, mortality, and mastitis prevalence, culling and mortality rates
Main findings: (relevant to PICO question):	• No difference in lameness incidence or severity by each barn type • Compost-bedded barns had lower prevalence of Hock lesions (3.8%) than CV and NV barns (31.2% and 23.9%, respectively) • No significant differences between barn systems for mastitis, culling or mortality
Limitations:	• Small region of USA • Manual observations are subjective in nature • 4 visits in 9 months per farm • Respiration rates only measured in summer • Much variation in farm management types and sizes

16. (van Gastelen S. 2011) **NED**	
Population:	Lactating dairy cows
Sample size:	2927 cows on 24 farms with freestall systems in Netherlands. 9 farms used box compost, 6 used sand, 6 used foam mattresses and 3 farms used horse manure as bedding material. Feb-May 2010
Intervention details:	box compost, sand, horse manure, and foam mattresses
Study design:	Case Series
Outcome studied:	The way cows entered the stalls, the duration and smoothness of the descent movement, and the duration of the lying bout. The cleanliness of the cows was evaluated on 3 different body parts: (1) udder, (2) flank, and (3) lower rear legs (1-4 scale), according to the Dutch Udder Health Centre Hock Lesions (1-4 scale) according to Rutherford K.M.D., Langford F.M., Jack M.C., Sherwood L., Lawrence A.B., Haskell M.J., (2008), Hock injury prevalence and associated risk factors on organic and nonorganic dairy farms in the UK, *Journal of Dairy Science*, 91, 2265-2274 Bacteriological counts of the bedding materials Bacterial count in milk
Main findings: (relevant to PICO question):	• The percentage of cows having damaged hocks was lower on farms using box compost compared with farms using sand and foam mattresses • No differences were found between the different types of bedding materials in all the cleanliness variables • The lying bout tended to be longer when using horse manure vs foam mattresses. The first lying bout tended to be longer on sand vs mattresses or box compost • Less colony-forming units on sand vs box compost

	• Manual observations are subjective in nature
Limitations:	• Only assessed in one season (Spring) • Each farm visited once for 1 day

17. (Westphal A. 2011) **USA**	
Population:	In vitro study
Sample size:	Cultures from samples of sand bedding from 1, 2500 cow dairy farm in Ohio
Intervention details:	Growth and typing of *E. coli* O157:H7 found in sand bedding from: New sand, in-use sand, washed sand, recycled sand
Study design:	In vitro study
Outcome studied:	Growth and types of *E. coli* O157:H7
Main findings: (relevant to PICO question):	• Median numbers of CFU in all types of bedding declined with time, suggesting that suppressive constituents of sand are not inhibited by processing during recycling • Heating to 80degC reduced counts of *E. coli* vs in-use and fresh sand • Ambient temperature affected non-heat treated sand contamination • The heating process allows heat-sensitive microorganisms to suppress the *E. coli*
Limitations:	• Only from one farm • Sand only and no comparisons with other types of bedding • Only cultured and studied *E. coli* O157:H7

18. (Dufour S. 2011)**CAN**	
Population:	Articles relevant to SCC related to herd health management
Sample size:	36 manuscripts included in this study: the majority used a cross-sectional study design (n = 28) or a combination of cross-sectional and before-and-after designs (n = 6). One study used strictly a before-and-after study design and only one study used a longitudinal cohort design
Intervention details:	• Five databases (PubMed, Medline, CAB, Agricola, and Web of Science) were searched on April 22, 2009 • Forest plots • Review
Study design:	Systematic Review
Outcome studied:	1. Intervention studied was a management practice applied or observed at the herd level and used as an udder health

	control strategy; 2. SCC was measured using cell counting methods rather than California Mastitis Test (CMT) or Rapid Mastitis Test 3. Mean 305-d milk production of the herds studied was ≥7,000 kg; and 4. Mean herd size of the herds studied was ≥40 milking cows
Main findings: (relevant to PICO question):	• Freestalls had lower SCCs than pasture and bedded pack • Sand > mattresses > decreased bedding moisture on their impact on decreasing SCC • Manure packed systems had lower SCC than other types (from one paper)
Limitations:	• Focused on many other management practices and impact on SCC rather than focusing on bedding type • Over time, technologies have allowed dairies to have better automated systems and recycling of bedding etc. so when reviewing articles, this has been a challenge to choose what has more weighting.

19. (Gomez A. 2010) **USA**	
Population:	Lactating Dairy Cows
Sample size:	205 cows in 16 freestall barns in Wisconsin
Intervention details:	Rubber mattress or sand cubicles
Study design:	Case Series
Outcome studied:	Continuous video surveillance collected the following data: Time lying in the stall, time standing in the stall, time standing in the alleys (including drinking), time feeding, and time milking (time out of the pen for milking and transit) during a 24-h period were measured for each cow relationships of components of the time budget with herd-level and cow-level fixed effects
Main findings: (relevant to PICO question):	• Lameness affected time feeding, with the greatest feeding times observed in non-lame cows • As lameness severity increased, time standing in the alley decreased • Time standing in the stalls was greater in the mattress herds than sand herds • Cows in MAT herds spent significantly less time lying, with shorter lying bouts than did cows in SAND herds
Limitations:	• Not randomised, • Small sample size • Not including summer (hot) data • Manual observations are subjective in nature

20. (Lombard J.E. 2010) **USA**	
Population:	Lactating dairy cows on a national management program
Sample size:	491 farms, 297 were Holstein herds with cows housed in freestalls average of 96 cows per farm and a total of 25,358 cows
Intervention details:	Bedding type, bedding quantity, stall length and width, presence of a neck rail or brisket locator, and relevant distances from the rear and bed of the stall From Mar 5 to Sept 5, 2007
Study design:	Case Series
Outcome studied:	Hygiene and hock scores (1-3 scale) (up to 100 cows/herd), number of cows housed in the pen, the number of cows standing with only the front feet in a stall, standing fully in a stall, and lying in a stall
Main findings: (relevant to PICO question):	• Rubber mats or mattresses as the stall base had a higher percentage of cows with severe hock lesions vs dirt base • Compared with farms with dirt base stalls, farms with rubber mats and mattresses had more cows standing fully in the stall • 50% of cows were lying down on farms using sand bedding compared with 40% on farms using straw, sawdust, or dry or composted manure
Limitations:	• Only herds on a national management program were selected, creating potential bias in the results • Questionnaire allows for subjective assessment • >140 manual assessors, so high risk of subjective observations and opinions

21. (Justice-Allen A. 2010) **USA**	
Population:	Barns
Sample size:	9 farms in Utah.
Intervention details:	Samples of bedding taken from back third of 6-10 stall per pen and 6 areas of hospital pens Sand from one dairy heat and chemical treated to see whether or not *Mycoplasma* spp. survived.
Study design:	Case series
Outcome studied:	Growth and identification (culture and PCR) of *Mycoplasma* spp.
Main findings: (relevant to PICO question):	• *Mycoplasma* was identified from bedding sand, dirt and recycled sand in 3 of the 9 farms and most samples from those farms • *Mycoplasma* appeared to survive in deep bedding samples for up to 8 months, peaking in cycles relating negatively to temperature and positively to moisture (deep samples were

	always moist) • Treatment with higher concentrations of disinfectants, 2% chlorhexidine and 0.5% sodium hypochlorite, eliminated 100% of *Mycoplasma* from contaminated bedding sand
Limitations:	• Only one pathogen studied • Farms mostly used sand with only a few on straw • Different pen systems investigated (stalls, pack and dry lots)

22. (Norring M., 2010) **FIN**	
Population:	Lactating dairy cows
Sample size:	18 multiparous Friesian cows on one farm
Intervention details:	3 types of bedding material: concrete, sand (particle diameter 0.1 to 0.6 mm), and soft rubber mats Each group of cows was allowed to choose between 2 of the 3 different types of materials, rotating around all material types. They were allowed 10 days for preference testing after a 5 day enforcement period so they were used to all materials.
Study design:	Cohort study
Outcome studied:	Lying time as seen by observers from video footage
Main findings: (relevant to PICO question):	• Cows were observed lying down more often on rubber mats than in concrete stalls • No overall preferences observed between rubber mats and sand stalls or between sand and concrete-based stalls
Limitations:	Low population number

23. (Cook N.B. 2008) **USA**	
Population:	Lactating dairy cows
Sample size:	59 cows from 4 farms. Non-lame, slightly lame or moderately lame cows (5 cows per category)
Intervention details:	Either rubber-crumb-filled mattress stall surfaces bedded with a small amount of sawdust (2 herds) or a Pack Mat design, which consisted of a rubber-crumb-filled mattress pad installed 5 cm below a raised rear kerb, bedded with 5 to 8 cm of sand bedding.
Study design:	Cohort study
Outcome studied:	Time-lapse video footage Lying times and number of lying bouts

	Time budgets for other activities
Main findings: (relevant to PICO question):	• cows on mattress stalls had significantly lower lying bout durations than cows on pack mat stalls • Stall standing time increased with increased lameness on all bed types • Stall standing for non-lame cows was longer in mattress stalls than pack mat (sand)
Limitations:	• Small sample size for 3 groups of cows • 4 farms mean management differences may affect results • Hard for observers to define slightly and moderately lame cows as separate groups

24. (Norring M., 2008) **FIN**	
Population:	Lactating and dry dairy cows
Sample size:	52 cows, proportions of parities adjusted per group
Intervention details:	Kept on either straw bedded concrete stalls or sand stalls
Study design:	Cohort study
Outcome studied:	Resting time by video footage, cleanliness, hock injuries, and hoof health
Main findings: (relevant to PICO question):	• Lying times and frequency of lying bouts on sand were longer and more frequent than straw • There were no differences in the dirtiness of stalls between the materials • Cows housed on straw were dirtier than those on sand • The severity of hock lesions was lower for cows on sand than for cows on straw though not the incidence (no difference) • Overall hoof health was greater for cows on sand
Limitations:	• Small sample size • Leg and hoof injuries inspected only at beginning and end of experiment (1-2 years in between)

25. (Godden S. 2008) **USA**	
Population:	Culture samples of bedding from lactating dairy cow barns
Sample size:	71 samples from 49 farms
Intervention details:	Clean sand (CS), recycled sand (RS), Digested manure solids (DMS), Shavings (SH) were sterilized and inoculated with *Klebsiella pneumoniae* and *Enterococcus faecium*, and incubated at 37degC for 24, 48 and 72 hours
Study design:	Cohort study

Outcome studied:	Bacterial growth Nutrients within bedding types
Main findings: (relevant to PICO question):	• For *K. pneumoniae*, peak growth occurred within 24 hours in all types of bedding and was stationary afterwards • For growth of *K. pneumoniae*, the order was as follows: CS < SH < RS < DS • For *Enterococcus*, growth appeared to decrease over the 72 hours in all types, decreasing greatest in shavings • Growth of *E. faecium* was greater in RS and DS than growth for CS or SH • pH in bedding types: ranging from lowest to highest as SH (4.27) < CS (8.15) < RS (8.37) < DS (8.90) • High Carbon or pH may be associated with better conditions for bacterial growth
Limitations:	• Only two species of bacteria studied

26. (Wenz J.R. 2007) **USA**	
Population:	Dairy farms across all USA
Sample size:	Of 3876 questionnaires sent out, 3466 were completed, with 1013 completing a further questionnaire and 741 able to collect milk samples and provide bulk tank SCC
Intervention details:	Questionnaire on farm management and BTSCC
Study design:	Case Series
Outcome studied:	Bulk tank SCC (BTSCC) at <200k cells/ml, 200-400k, > 400k
Main findings: (relevant to PICO question):	• Twenty-six percent and 17.8% of operations reported a BTSCC <200,000 cells/mL and >400,000 cells/mL, respectively • Farms with >500 cows and higher milk production than 9000kg/cow 305d lactation had lower BTSCC values • Mattress, sand and newspaper bedding were associated with lower BTSCC. Composted manure was associated with higher BTSCC
Limitations:	• Herds in national monitoring scheme so may have included bias to better managed herds • Questionnaire method increases risk of subjective assessment

27. (Kristula M.A. 2005) **USA**	
Population:	Bedding samples from dairy farms in USA
Sample size:	6 farm samples used for CS and 6 for RS in Winter 4 samples for RS and 7 for CS in Summer Samples taken for 6-8 days, twice
Intervention details:	Samples taken from Clean Sand (CS) or Recycled Sand (RS)

Study design:	Cohort study
Outcome studied:	The number of colony-forming units per gram of bedding of gram-negative bacteria, coliforms, Streptococcus spp., and Klebsiella spp. were estimated for each sand sample Dry matter and organic matter were also assessed
Main findings: (relevant to PICO question):	• RS and CS had the same bacterial counts when compared at any sampling time (winter or summer) • CS had higher DM than RS in winter and summer • RS had higher organic matter than CS in winter and summer
Limitations:	• Paper not clear whether the same farms were chosen to compare winter and summer samples (suspected that they were not as different numbers of farms were used) • Huge variations in types of bacteria by farm indicate large variations by facility

28. (Cook N.B., 2005) **USA**	
Population:	Lactating dairy cows
Sample size:	12 herds of 150-450 cows were observed but 6 herds using mattresses and 6 herds using sand were chosen for lameness and lying time observations. 602 cows in total were observed with 304 on mattresses and 298 on sand.
Intervention details:	Sand or rubber crumb filled mattresses
Study design:	Cohort study
Outcome studied:	Number of cows that were lying down in a stall, standing in a stall with all 4 feet, standing in a stall with the rear 2 feet in the alley (perching), and standing in the alley drinking and feeding were recorded
Main findings: (relevant to PICO question):	• Lameness prevalence in MAT herds was more than 3 times that observed in SAND herds • No significant difference was found between cow comfort indices and lying times in different systems
Limitations:	• Herds representative of local area but not of USA • Variations in stocking density were identified across the sample population • Variations in lameness across herds may have affected lying times

29. (Drissler M. 2005) **CAN**	
Population:	Lactating dairy cows
Sample size:	24 cows in 2 groups of 12, each with 12 freestalls
Intervention details:	Sand depth of cubicles in time

Study design:	Cohort study
Outcome studied:	Lying time, number and length of lying bouts and sand depth
Main findings: (relevant to PICO question):	• Over time, the stall surface took on a concave shape, and bedding depth was lowest at the center of the stall and highest at the edges. The loss of bedding was greatest after new bedding was added, and sand levels continued to decline during the entire 10-d period • For every 1-cm decrease in bedding, cows spent approximately 11 min less lying during each 24-h period • Duration of lying bouts was shorter when the stalls contained less sand
Limitations:	• Small sample size • Different observers may show inconsistencies in results

30. (Zdanowicz M. 2004) **CAN**	
Population:	Lactating dairy cows
Sample size:	16 lactating Holstein cows
Intervention details:	Housed on either sand or sawdust-bedded free stalls using a crossover design with 3 weeks per bedding type
Study design:	Cohort study
Outcome studied:	Bacterial count and culture on teat ends and bedding
Main findings: (relevant to PICO question):	• There were 2 times more coliforms and 6 times more *K. pneumoniae* bacteria on teat ends of cows housed on sawdust compared with those housed on sand • There were 10 times more *Streptococcus* spp. bacteria on teat ends of cows when housed on sand compared with sawdust • Udder cleanliness was no different on either bedding type • There was no clear relationship between teat end counts and bedding counts
Limitations:	• Small sample size

31. (Jayarao B.M. 2004) **USA**	
Population:	Lactating dairy cows
Sample size:	126 dairy herds surveyed in Pennsylvania State
Intervention details:	Questionnaire on farm management practices
Study design:	Case Series

Outcome studied:	Bulk tank SCC (BTSCC) and bacterial culture modelled against questionnaire answers
Main findings: (relevant to PICO question):	• Fifty percent of the BTSCC samples had a BTSCC <348,000 cells/mL • BTSCC samples > 200,000cells/ml were 5 times more likely to have a high CNS (coagulase negative staphylococcus) count • Sand as bedding had significantly lower BTSCC in their BTSCC compared with organic bedding such as shavings, newspaper, and straw
Limitations:	• Farms restricted to NMC milk testing program (therefore may be biased to better management systems) • Producers that used sand (quite innovative in 2004) may have had better management practices than older-style bedding, indicating that sand itself may not be better than other bedding materials

32. (Cook N.B., 2004) **USA**	
Population:	Lactating Dairy Cows
Sample size:	12 herds of between 150-450 cows each, producing > 9545kg/cow average
Intervention details:	Deep sand bedding or rubber mattress with organic bedding material covering
Study design:	Cohort study
Outcome studied:	Lying times, time per lying bout, Lameness (1-4 scale), Video footage
Main findings: (relevant to PICO question):	• There was no significant difference in time lying down in stall • Time standing in stall was significantly lower in Sand cubicles • Cows in sand herds had a significantly greater proportion of long lying bouts than did cows in mattresses • Lame cows stand for longer in stalls than healthy
Limitations:	• Selected herds with high production and potentially better management than overall population • Manual observations may be subjective

33. (Wagner-Storch A.M. 2003) **USA**	
Population:	Lactating dairy cows
Sample size:	104 cows housed in one barn at 100% stocking density. 961 observations of stall usage over a period of 9 months in 2001
Intervention details:	Rubber mattresses, waterbed, concrete + sawdust, sand, around 25 of each type in mixed positions in barn
Study design:	Cohort study

Outcome studied:	Video footage Time budgets
Main findings: (relevant to PICO question):	• Preference was Sand > Mattress > Waterbed > Concrete • Cows spent more time lying in Sand but more time occupying rubber mattresses (standing in them)
Limitations:	• Cows had no prior time to get "used" to the bedding types • At 100% stocking, some cows had no choice on cubicles and lower social hierarchy cows may have been forced to lie on worse bedding • Some cows may have visited multiple types of bedding so modelling lameness of lesions would have been affected • A robot milked herd, so position of bed in relation to robot may have confused the data • Manual observations may be subjective

34. (Hogan J.S. 1989) **USA**	
Population:	Lactating dairy cows
Sample size:	9 farms of 60-200 cows per farm for 1 year
Intervention details:	Mattresses with sawdust, straw or sand/crushed limestone cubicles
Study design:	Cohort study
Outcome studied:	Bacterial counts from bedding materials
Main findings: (relevant to PICO question):	• Bacterial counts in organic materials (sawdust, straw) were higher than inorganic (sand) materials • *Klebsiella* counts were higher in sawdust vs straw • Streptococcal counts were higher on straw than sawdust • Gram –ve counts were higher in Summer
Limitations:	• Herds were selected with lower incidence of *Staph. aureus* and *Strep. agalactiaea* • Mixed breeds: 8/9 farms had Holsteins, one had Jerseys. There may be breed preferences due to cow size vs stall size.

35. (Carroll E.J. 1978) **USA**	
Population:	Lactating dairy cows
Sample size:	Approximately 800 cows on one farm in California
Intervention details:	Fresh or used recycled manure bedding
Study design:	Case series
Outcome studied:	Bacterial counts from bedding *Klebsiella* and *Enterobacter*, *Citrobacter* and coliform spp. cultured

Main findings: (relevant to PICO question):	• Composting manure (when fresh) effectively reduces the coliform counts to near zero • When the manure becomes moist and warm, these counts can return to levels found on contaminated bedding.
Limitations:	• Samples only from one farm and unknown over which period • Only *Klebsiella* and *Enterobacter* spp. measured

36. (Fulwider W.K. 2007) **USA**	
Population:	Lactating dairy cows
Sample size:	90,162 From 113 farms in USA
Intervention details:	The bedding materials most commonly used by waterbed and mattress dairies included sawdust, rice hulls, chopped straw, or lime. Five free-stall dairies reused (recycled) sand, whereas all others bedded with new sand
Study design:	Cohort study
Outcome studied:	Hock and leg skin lesions Cow hygiene (1-5 scoring system) from Reneau J.K., Seykora A.J., Heins B.J., Endres M.I., Farnsworth, R.J., Bey R.F., (2005) Association between hygiene scores and somatic cell scores in dairy cattle, Journal of American Veterinary Medical Association, 227, 1297-1301
Main findings: (relevant to PICO question):	• Cows on mattress systems and waterbeds had higher skin lesion scores than sand bedding • Cows on mattress and waterbed systems had lower hygiene scores than sand bedded systems
Limitations:	• No dairies used recycled manure or digester solids • Average herd size was high (>800 cows) and milk production high, so management practices may have been biased towards better, more efficient systems • Subjective, observer based scoring systems

37. (De Palo P. 2006) **ITA**	
Population:	Lactating dairy cows
Sample size:	44 cows in total: 8 cows (preference test). 36 cows (4 groups of 8) for aversion test
Intervention details:	Polyethylene (EVA) or polypropylene (PVA) mats with wood shavings or manure solids
Study design:	Cohort study
Outcome studied:	1. bed occupation time of the free stalls per day; 2. average duration of each lying event per day; 3. duration of periods spent standing in the free stall, with 2 or 4 feet

	per day (respectively); 4. number of interrupted attempts at lying down per hour ; 5. mean duration of a single lying bout in minutes; and 6. time spent ruminating in the free stall, during the lying down time per day. Cow hygiene Milk bacterial count
Main findings: (relevant to PICO question):	• Cows appeared to occupy the EVA mats more than other types • Lying times decreased with increasing temperature-humidity index (THI) in EVA mats • Cow comfort index showed greatest consistency over all THI recordings with wood shavings • Cows tended to stand more, rather than lie, in mattresses, especially the PVA type
Limitations:	• Very small sample number • May be some bias from company selling EVA mattresses as results tend to differ significantly from other literature reports (lying times on sand and manure *vs*. mattresses)

38. (Calamari L. 2009) **ITA**	
Population:	Lactating dairy cows
Sample size:	56 cows
Intervention details:	straw bedded pack (ST), rubber mat (RM), mattress (MA) and sand (SA)
Study design:	Cohort study
Outcome studied:	Lying times and frequency/length of lying bouts Milk yield and milk bacterial count
Main findings: (relevant to PICO question):	• More time was spent lying on sand and straw yard than mattresses • Time of lying bout was no different between bedding types • Mattress stalls were used for standing and sand for lying • Greater milk yield in sand *vs* mattress bedding • Manual observations may be subjective
Limitations:	• Small sample population

39. (Eckelkamp E.A. 2016) **USA**	
Population:	Lactating dairy cows
Sample size:	50 cows per farm for scoring and SCC for whole herd. 15 farms, 8 compost pack and 7 sand
Intervention details:	Compost bedded pack (yards) and sand cubicle barns

Study design:	Cohort study
Outcome studied:	Herd clinical mastitis, SCC, high SCC prevalence (% of herd >200,000 cells/mL SCC), and BTSCC), locomotion, hygiene, and hock scores
Main findings: (relevant to PICO question):	• No differences between 8 compost and 7 sand bedded farms, based on clinical mastitis, SCC, herd locomotion, hygiene score, or hock health
Limitations:	• Only 50 cows per farm scored • Observer, subjective scoring

40. (Rudd L.E. 2010) **NOR**	
Population:	Lactating dairy cows
Sample size:	1923 farms, 29,326 lactations for milk yield distributed over 363 free-stalled herds in Norway. The study uses a 305 day lactation as the focus: yield volume and bedding type as variables, so cow number is not relevant to this data mining exercise: One cow may live for 3 lactations and each is considered a single investigative point against the variables.
Intervention details:	Questionnaire filled in by producer on management and stall covering: 1 = concrete, softness of 0 mm; 2 = rubber, softness of 1 to 8 mm; 3 = soft mats, softness of 9 to 16 mm; 4 = multilayer mats, softness of 17 to 24 mm; 5 = mattresses, softness over 24 mm
Study design:	Case Series
Outcome studied:	Milk yield by flooring type
Main findings: (relevant to PICO question):	• Herds on concrete free-stall bases yielded 6,727 ± 146 kg of milk from 5 to 305 days in milk. In comparison, herds showed a decrease of 0.3% on rubber, an increase of 2.4% on soft mats, an increase of 4.5% on multilayer mats, and an increase of 3.9% on mattresses • Compared with concrete, the hazard ratio of teat lesions was less on rubber, soft mats, multilayer mats, and mattresses
Limitations:	• Farmer-lead, subjective questionnaire • Milk yields modelled using Woods equation rather than actual data • Farms with lower yields may have genetic and breed differences, affecting both production and locomotion

41. (Adams A.E. 2017) **USA**

Population:	Lactating dairy cows
Sample size:	191 dairies, 22,622 cows in 17 States of USA
Intervention details:	Farm visits by trained personnel to assess lameness and hock lesions and BCS
Study design:	Case series
Outcome studied:	BCS, Locomotion and Hock Scores (1-3 scale)
Main findings: (relevant to PICO question):	• 90.4% cows were sound (locomotion score=1), 6.9% were mild/moderately lame (locomotion score=2), and 2.7% were severely lame (locomotion score=3). • 87.3%) had no hock lesions (hock score=1), 10.1% had mild lesions (hock score=2), and 2.6% had severe hock lesions (hock score=3) • 4.2% were BCS <=2.25 on a 1-5 scale • Lower locomotion scores (2 or lower) were associated with larger operations, pasture and sand stalls vs straw, compost or matting • Lower hock scores were associated with dry lot systems
Limitations:	• Multiple assessors using subjective scoring systems but trained to single scoring system

42. (Jones B.W. 2017) **USA**	
Population:	Lactating dairy cows
Sample size:	97 cows in a freestall barn
Intervention details:	46 cows in Dual Chamber Waterbeds and 51 cows on rubber mattresses
Study design:	Cohort study
Outcome studied:	Milk yield, Lying time, Rumination time, Hock scores
Main findings: (relevant to PICO question):	• Greater Lying times and lower hock scores were associated with waterbed mattresses • Rumination time was greater on rubber mattresses • Milk yield was not significantly different between the two systems
Limitations:	• Small cow numbers • Unable to determine whether or not conflicting interests are present

43. (Upadhyay D. 2017) **IND**

Population:	Lactating, Indian cross-bred dairy cows
Sample size:	24 cows in 4 groups of 6
Intervention details:	1. Concrete floor (covered feeding area) and brick paved (open) 2. Concrete (covered feeding area) and sand bed (open) 3. Rubber mat (covered feeding area) and sand bed (open) 4. Rubber mat (covered feeding area) and brick paved (open)
Study design:	Cohort study
Outcome studied:	Lameness (1-5 scale) Walking speed
Main findings: (relevant to PICO question):	• Walking speed was greater in sand bedded groups • Lameness score was greater in brick paved groups, with greater severity also
Limitations:	• Very small group numbers • Conclusions made from underpowered findings • Subjective scoring scale

44. (Burgstaller J. 2016) **AUT**	
Population:	Lactating dairy cows
Sample size:	498 datasets from 10 barns, 201 (5 barns) in compost bedded yards and 297 (5 barns) in cubicles
Intervention details:	Compost bedded yards or cubicle barns
Study design:	Case Series
Outcome studied:	Lameness score and severity Claw lesions score and severity
Main findings: (relevant to PICO question):	• No significant differences in locomotion between different types of bedding material, flooring system, breed, visit number, observer and time since last trimming • Statistically significant differences in the prevalence of claw disorders between compost bedded and freestall cubicle barns were found for white line disease (WLD; 20.4% and 46.6%, respectively), heel horn erosion (HHE; 26.9% and 59.9%, respectively), concave dorsal wall as a result of chronic laminitis (6.5% and 15.9%, respectively) and for interdigital hyperplasia (0.2% and 3.1%, respectively)
Limitations:	• Subjective scoring systems • Comparing bedding types when flooring systems would differ and barn design may have influenced the study outcomes (whether or not there were sharp turns, steep grades, flooring flaws etc.)

45. (Lim P.Y. 2015) **UK**

Population:	Lactating dairy cows
Sample size:	3691 cows from 76 farms
Intervention details:	Cubicles with concrete base and whole straw or rape straw bedding compared with cubicles with concrete bases with sand or chopped straw bedding
Study design:	Case Series
Outcome studied:	Farm data analysis Hair loss on lateral aspect of hock (using hock map), Hock lesion (score) and lameness (score) BCS, milk yield, duration of housing
Main findings: (relevant to PICO question):	• Cows on concrete based cubicles with sand or chopped straw-based bedding had lower hair loss on hock than those on concrete with whole straw/rape straw dressing • Cows on concrete based cubicles with sand or chopped straw-based bedding had lower hair loss on hock than those on mattresses with whole straw/rape straw dressing
Limitations:	• Purpose of the paper was to compare the hock map versus the scoring system for analyzing hock lesions rather than the bedding material itself

46. (Astiz S. 2014) **NED**	
Population:	Dry cows
Sample size:	423 Holstein dry cows on one farm
Intervention details:	Compost bedded loose-yard compared (242 cows) to straw bedded loose yard (181 cows)
Study design:	Randomised control trial
Outcome studied:	• Dairy data records: • milk yield, • pregnancy after first insemination (P/FAI), • somatic cell counts (SCC), • incidence of clinical mastitis, • incidence of metritis and cytological endometritis, and • mortality/culling rate
Main findings: (relevant to PICO question):	• No differences in mortality, fertility parameters or uterine health • Lower clinical mastitis rates in compost bedding • Cows yielded avg. 760kg more milk in the first 100DIM from the compost bedded system
Limitations:	• Higher milk yield could be double-counted from the lack of mastitis (therefore less dumped/future milk losses) • Milk yield error limits crosses over between the two bedding

	types, making the result less significant • Low numbers to get sufficient power for good conclusions on yield and fertility parameters (altering things in the dry period are a long way in time from getting cows pregnant and many other factors can influence the outcomes, so making conclusions would be extremely difficult)

47. (Eckelkamp E.A., 2014) **USA**	
Population:	Lactating dairy cows
Sample size:	10 cows
Intervention details:	Freestall barn with pasture access transitioning to compost bedded loose-yard
Study design:	Case Report
Outcome studied:	Lying times by activity monitor
Main findings: (relevant to PICO question):	• Hours of lying were longer on compost than freestalls • Sound cows and lame cows lay for the same times on compost but sound cows lay longer than lame cows in freestalls.
Limitations:	• Very small numbers • Many environmental changes if going from freestall + pasture to compost, loose-housing. Feeding areas, water access, design of barn, heat abatement could all affect outcomes • Cows were on the freestall system for 495 days and compost for 132 days

48. (Chapinal N. 2013) **USA**	
Population:	Lactating dairy cows
Sample size:	79 farms (unknown number of cow records)
Intervention details:	Lameness scoring and relationships between management systems and housing
Study design:	Case series
Outcome studied:	Lameness (1-5 scale) Dairy software records
Main findings: (relevant to PICO question):	• Severe lameness increased with the percentage of stalls with fecal contamination and with use of sawdust bedding, and decreased with deep bedding, sand bedding, herd size, and rearing of replacement heifers on site as well as frequency of manure removal in the pen per day • Deep bedding and sand were highly correlated as most systems used both on the same site.

Limitations:	• Subjective scoring by multiple assessors • Modelled data from records

49. (Mitev J. 2012) **TUR**	
Population:	Lactating dairy cows
Sample size:	36 cows
Intervention details:	Rubber mats, manure-straw bedding and sand
Study design:	Cohort study
Outcome studied:	Preference to bedding type Hygiene (cleanliness score) Lying times
Main findings: (relevant to PICO question):	• Lying times and number lying down were longer on matting than manure straw bedding and sand
Limitations:	• Very small cow numbers • No information on heat abatement, as this could have affected lying times (thin, rubber matting may be cooler than manure or sand). Turkey has a climatically high THI.

50. (Kara N.K. 2011) **UK**	
Population:	Lactating dairy cows
Sample size:	709 cows from 39 herds
Intervention details:	Concrete, sand and rubber mattress bedding in freestalls and tie-stalls
Study design:	Case series
Outcome studied:	Lameness score
Main findings: (relevant to PICO question):	• There were no significant differences between mean of locomotion scores on concrete, sand or rubber bedding in this study
Limitations:	• Subjective scoring system • Varying herds with very variable environments and management systems • Comparing bedding types with more than one barn type (freestall and tie-stall) means that it is difficult to make conclusions with so much variation

51. (Shane E.M. 2010) **USA**	
Population:	Lactating dairy cows in Minnesota
Sample size:	6 dairy farms (unknown number of cows)

Intervention details:	All farms have compost bedded pack but used as a substrate either sawdust wood chips, flax straw, wheat straw, oat hulls, straw dust, and soybean straw
Study design:	Case series
Outcome studied:	Organic and inorganic composition of bedding Bacterial contamination of bedding Lameness, BCS, hock lesions and hygiene
Main findings: (relevant to PICO question):	• Bedding pack material averaged 15.8% for total C, 0.93% for total N, 17.8 for C:N ratio, 37.3% for dry matter, 8.83 for pH, 4.25 mg/kg for nitrate, 955 mg/kg for ammonium, 15 g/kg for total potassium, 2.8 g/kg for total phosphorus, 8.5 dS/m for EC, 31.7 degrees C for pack temperature, 7.6 degrees C for outside temperature, and 9.42 million CFU/mL for total bacterial counts in the bedding • All types of substrate were considered suitable but regular tilling and replacing were recommended
Limitations:	• No information on cow factors

52. (Doherr M.G. 2007) **CZR**	
Population:	Lactating dairy cows
Sample size:	970 cows on 120 farms (60 organic and 60 conventional)
Intervention details:	Rubber mat/concrete beds or “other”
Study design:	Case series
Outcome studied:	Subclinical mastitis risk factors California milk test (CMT)
Main findings: (relevant to PICO question):	• Decreased odds ratios for subclinical mastitis were found on farms using systems other than rubber mat/concrete beds
Limitations:	• Authors admitted that many of the associated factors and odds ratios were likely to be related to system management variation between farms rather than the factors in the paper themselves.

53. (Espejo L.A. n.d.) **2006**	
Population:	Lactating dairy cows in Minnesota
Sample size:	5,626 cows in 53 high-production groups
Intervention details:	Freestall herds with sand stalls and freestall herds with mattresses
Study design:	Case series
Outcome studied:	Records from dairy software Lameness score (1-5 scale)

Main findings: (relevant to PICO question):	• The mean prevalence of clinical lameness was 24.6% • Prevalence of lameness in parity one averaged 12.8% and increased by 8 percentage points each parity. • Lameness was lower in freestall herds with sand stalls (17.1%) than in freestall herds with mattress stall surfaces (27.9%)
Limitations:	• Lameness scored by multiple assessors and a subjective system

54. (Tucker C.B. 2003) **USA**	
Population:	Lactating Holstein dairy cows
Sample size:	12 cows were housed individually in separate pens
Intervention details:	Each pen contained three free stalls with a different surface: deep-bedded sawdust, deep-bedded sand, and a geotextile mattress covered with 2 to 3 cm of sawdust
Study design:	Cohort study
Outcome studied:	Preference was determined, based on lying times
Main findings: (relevant to PICO question):	• Of the 12 cows used in Experiment 1, 10 preferred sawdust before and nine after the restriction phase. During the restriction phase, average lying times and number of lying events during the restriction phase were significantly lower for the sand-bedded stalls (P<=0.05), and standing times were higher on mattresses (P<=0.05), compared with sawdust • After restriction, half of cows preferred sawdust and half sand.
Limitations:	• All cows had been housed on sawdust for their previous lactation (although they were given experience of all types of bedding before the experiment) • Very small population number

55. (Manninen E. 2002) **FIN**	
Population:	Lactating dairy cows
Sample size:	44 Friesian cows, 24 in winter and 20 in summer
Intervention details:	Experiment 1: Concrete with large amount of straw, soft rubber mat with a thin layer of straw and sand (2-3 mm) without straw Experiment 2: 2cm deep rough or fine sand
Study design:	Cohort study
Outcome studied:	Preference (occupation of cubicles of different types and lying times) observed by video

Main findings: (relevant to PICO question):	• Lying times were shorter and fewer lying bouts were observed on sand than on either straw or rubber matting in Experiment 1 • In winter, cows preferred to lie on straw rather than rubber matting • When forced to lie on sand, cows had no preference to either rough or fine sand or lying in the concrete alleyway.
Limitations:	• Before the experiment, all cows were housed on rubber matting covered with peat and straw, so some may have had previous preferences • Very small population numbers • Stocking density was different in winter (100%) to summer (50%) • Sand layer was 20cm deep which seems low compared with other papers reviewed in this knowledge summary • Manual observations may be subjective

56 (Weary D.M. 2000) **CAN**	
Population:	Lactating dairy cows
Sample size:	1752 cows on 20 farms
Intervention details:	Prevalence study on hock lesions from sawdust, sand, and geotextile mattresses
Study design:	Case Series
Outcome studied:	Hock lesions by scoring and severity scoring
Main findings: (relevant to PICO question):	• 73% of cows had at least one hock lesion • 91% cows had lesions on mattresses • 24% cows had lesion on sand
Limitations:	• Subjective scoring methods • Only 4 farms out of 20 had sand, 10 on mattresses and 6 on sawdust, with 185/1752 cows on sand.

57 (Hayasaka K. 2000) **JAP**	
Population:	Lactating dairy cows
Sample size:	15 cows on 16 cubicles
Intervention details:	Preference study comparing soil or rubber chip mattress bedding with hardness of 90 *vs* 52 respectively on a rubber hardness tester
Study design:	Cohort study
Outcome studied:	Lying time and number of lying bouts on video

Main findings: (relevant to PICO question):	• Cows on matts had longer lying times with more frequent bouts of lying • Cows on matts had lower standing times and less time spent eating than soil based bedded cows • Manual observations may be subjective
Limitations:	• Very low sample numbers

58 (Gamroth M.J. 1992) **USA**	
Population:	Bacterial growth of coliforms
Sample size:	Bacterial samples grown from bedding materials
Intervention details:	Growth of coliforms on days 0, 2, 5, 8 and 11 on dried wood sawdust, composted washed manure solids (CS), chopped ryegrass straw, and shredded paper covering gypsum wall board in freestalls under farm conditions, then: Treating recycled manure solids with hydrated lime, copper, zinc and germicide to control coliform growth
Study design:	Case series
Outcome studied:	Coliform growth on untreated and treated bedding
Main findings: (relevant to PICO question):	• Coliform growth on all types of bedding materials reached faecal output levels within 2 days. • Treatment of bedding with any of the applied materials did not affect bacterial growth in the bedding material
Limitations:	Growth from coliforms only

59 (Brim M. 1989) **USA**	
Population:	Bacterial growth of bedding material samples
Sample size:	Growth of bacteria from samples
Intervention details:	Growth on samples of following types of bedding materials: Sand, 12-mesh limestone and treated with pine disinfectant, oat straw and cedar sawdust. Mulched newspaper
Study design:	Case series
Outcome studied:	Bacterial growth
Main findings: (relevant to PICO question):	• Sand, limestone (including treated) both had significantly higher growth after 6-54 hours incubation than organic bedding (straw, sawdust, newspaper). • Organic materials (sawdust, newspaper and straw) showed bimodal growth patterns, declining at 30-54 hours and increasing again beyond inorganic levels after 96-120 hours

Limitations:	• Unknown which bacteria grown and what season, temperature, humidity and levels of contamination beforehand. • From Table 3, it is inferred that there were 10 samples taken from unused pens and 30 from used, totaling 40 samples but this was for E. coli specifically. The paper is not clear on methodology how many samples were taken for all bacterial species.

Appraisal, application and reflection

At the point of writing, the most recent report from United States Dairy Association (USDA) shows the following proportions of bedding materials used on US dairies: Sand (45%), sawdust (21%), straw (14%), and manure (7%) are the 4 most common bedding materials in US freestall barns, with 87% of freestall barns using these bedding types (USDA, 2008). No more recent evidence is available at the time of publication. From the evidence reported here, 34 publications originate from the USA, compared with 7 from Canada, 3 from Finland, 2 each from Italy, Netherlands, UK and Denmark and 1 each from Austria, Norway, India, Turkey, Czech Republic, Japan and China.

The majority of research has been concentrated on the bedding systems of lactating dairy cows, with only 3 publications specifically mentioning dry cow accommodation.

Levels of evidence have ranged from one systematic review (Dufour S. 2012) through a majority of cohort studies to many case series and one randomized control trial.

As farm management practices and observers can vary from one site to another, there has been potential for subjective errors in input data in most of the papers reviewed. Efforts in multivariate regression modelling and analysis of statistical data has led many authors to attempt to reduce this in many cases. Scoring systems for lameness, cleanliness, skin lesions and teat cleanliness have all been assessed on subjective observation systems. Attempts have been made to mitigate subjective errors by using consistent guidelines to the assessors or using one single assessor have often been explained in the methodology of each paper.

Evidence for bedding material and associations with mastitis

Outcomes reported in the reviewed papers have covered many aspects of mastitis, including clinical incidence, subclinical incidence and bulk tank somatic cell counts, udder cleanliness, teat cleanliness, bacterial culture of milk and teats and bacterial culture/PCR analysis of bedding material. The following general observations can be made from the reviewed studies that can support the clinical bottom line:

- Clinical mastitis incidence was lower in herds bedded on deep sand, freestall systems (Dufour S. 2012)(Gao J. 2017) (Rowbowtham R.F., 2016).
- Lower somatic cell counts and bulk tank somatic cell counts have been reported in clean sand, recycled sand (Wenz J.R. 2007)(Jayarao B.M. 2004), mattresses (F. A. Dufour S. 2011) (Wenz J.R. 2007), manure (Dufour S. 2011) but higher SCC in composted manure (Wenz J.R. 2007). Such variations in results reflect the enormous variation in potential for management differences farm by farm. Many of these papers obtained their information from farm questionnaires on stall management and farmers may not be accurately reporting frequency of cleaning, renewal frequency of bedding, stocking density, cow cleanliness, parlour routines and many other aspects. The only objective measurement in these studies was the BTSCC or SCC values obtained from the dairy records.
- At the time of writing, many geographical regions are reporting a requirement to reduce the use of antimicrobials on farm. Only one paper has reported a reduction in the use of antimicrobials on sand systems specifically (Kayitsinga J. 2017). However, it may be prudent to point out that this may not be caused by the use of sand but merely associated with farms that use sand (these farms may be more innovative or better managed generally than farms using other types of bedding).
- Cows have been reported to have lower cleanliness scores on sand systems (cleaner cows) (Guarín J.F.

2017) (Fulwider W.K. 2007) than straw (Norring M. 2008).

- Considering microbial contamination, many papers have studied the growth of bacteria in bedding materials, the relationship between dry matter and moisture content in these materials in different seasons and cultured bacteria on teats or in milk. From these, some general consistencies can be found:
    - Less bacterial counts have been reported from inorganic (sand/crushed limestone) bedding material *versus* organic (straw, manure) (Hogan J.S. 1989) (Godden S. 2008).
    - More bacterial counts have been found on teats on mattress systems versus sand (Zdanowicz M. 2004) and lower counts found on clean, new sand *versus* manure (Guarín J.F. 2017) (Rowbowtham R.F., 2016),(van Gastelen S. 2011). However, two reports found no differences in bacterial growth on bedding media types (Black R.F. 2014) (Rowbowtham R.F. 2016).
    - Heat treating or composting sand or manure have been found to reduce contamination in two studies (Cole K.J. 2016)(Westphal A. 2011)
    - The dry matter content and amount of organic material in the bedding as well as moisture have been found to affect bacterial growth within bedding of all types, so summer conditions appear to favour growth (Rowbowtham R.F., 2016).
    - When it comes to specific bedding types and their associations with specific bacterial species, associations have been found between increased Streptococcus spp. in manure and sand systems *vs*. more coliforms and *Klebsiella* found in straw, manure and sawdust systems (Rowbowtham R.F., 2016)(Zdanowicz M. 2004) (Carroll E.J. 1978) (Godden S. 2008)..
    - It is worth mentioning that fresh versions of any bedding material can easily support higher bacterial growth with increasing time of usage in the barn (Carroll E.J. 1978). This is supported by (Gamroth M.J. 1992) who showed that bacterial coliform levels on bedding equaled fecal output within 2 days and treatment with inorganic compounds in an attempt to delay this did not reduce further bacterial growth in bedding.
    - An attempt has been made to associate higher milk yields to softer flooring types but the data inputs were not reliable enough for convincing results (too much possibility for variation between farms and management practices to confidently predict differences by bedding type alone) in the author's opinion (Calamari L. 2009)(Rudd L.E. 2010) (Astiz S. 2014)

Evidence for associations between bedding types on cow comfort and lameness

When considering a subject such as "welfare" and cow comfort, it is difficult to actually define a measurement that can reflect a true picture of cow welfare. Because of this, the focus of this Knowledge Summary has been on comparisons of objective observations between bedding types and such variables as lying times, frequency and length of lying bouts, hock lesions, time spent standing in stalls and preference testing. The following general observations can be made from the reviewed literature:

- In preference tests, cows appear to choose bedding types in the following order: Sand > Mattresses > Waterbeds > Concrete/straw (Wagner-Storch A.M. 2003) with one paper showing no difference between sand and mattresses but mattresses are preferred to concrete/straw (low sample numbers) (Norring M. 2016). One preference test in Finland showed strong preferences to mattresses over sand but it is worth mentioning that the cows were used to mattresses before the trial and sample population was very small (Manninen E. 2002).
- Sand-bedded systems appear to have fewer lame cows (Cook N.B. 2005), (Cook N.B. 2004) although as mentioned before, this may be biased by farms with higher production/better management. However, multivariate analysis has attempted to account for this and results have been consistent among observations.
- Time spent lying in stalls is increased in sand bedded systems versus others, with increased length of time of the first lying bout also (Bak A.S. 2016)(Solano L. 2016) (Jensen M.B. 2015) (Cook N.B. 2004) (Ito K. 2014) (Gomez A. 2010) (Lombard J.E. 2010) (Calamari L. 2009).
- Cows may be seen spending more time in mattress stalls but they are standing rather than lying (Cook N.B. 2005)(Jensen M.B. 2015) (Cook N.B. 2004).

- Compost manure and sand systems can both be shown to have reduced hock lesions *versus* mattress bedding (Fulwider W.K. 2007) (Lobeck K.M. 2011)(van Gastelen S. 2011) (Lombard J.E. 2010) (Norring M. 2008) . Also, when kept clean, there may be no differences in mastitis, SCC, locomotion score and hock lesions between compost pack systems and deep sand freestall systems (Eckelkamp E.F. 2014)
- When using straw for bedding material, it appears that chopping the straw can be associated with less hock hair loss than whole straw (Lim P.Y. 2015)
- One paper concentrated on preference testing between sand, straw and rubber matted bedding and found contrary results to other papers, concluding that cows strongly preferred to lie on straw and rubber, avoiding sand where possible (Manninen E. 2002). Although stocking densities were different in these tests and numbers were low, with cows having previous experience of matting systems before the experiment. (Hayasaka K. 2000) mentioned that cows showed longer lying times on mattresses *versus* soil bedding but spent less time eating.
- One paper was specific enough to quantify the length of lying time with the depth of the sand bedding and showed that this depth decreases with increased time of use in the barn.
    - Lying time decreased by 11 minutes in a 24 hour period per 1cm depth of sand bedding (Drissler M. 2005).

Summary

In conclusion, clean sand appears to be associated with the best outcomes in clinical mastitis, cow cleanliness, subclinical mastitis, cow lying times, hock lesions and cow preference. Recycled sand, composted manure and other deep-bedded systems also appear to have increased cow comfort indices *versus* mattress systems. Manure systems can also have better outcomes with bacterial growth over straw and mattress systems as long as they are kept clean and renewed frequently. Mattress systems and concrete/straw bedding tend to have the worst outcomes for both mastitis and lameness parameters.

Methodology Section

Search Strategy	
Databases searched and dates covered:	PubMed search 1910 - July 2017 Google Scholar CAB Abstracts 1973 to 2017 Week 30
Search terms:	Search 1 ((((((cattle OR cow$ OR bovi*))) AND ((bed* OR mattress))) AND ((sand OR compost* OR rubber)))) AND mastitis Search 2 ((((((cattle OR cow$ OR bovi*))) AND ((bed* OR mattress))) AND ((sand OR compost* OR rubber)))) AND (([hock lesion] OR lame*)) Search 3 ((((((cattle OR cow$ OR bovi*))) AND ((bed* OR mattress))) AND ((sand OR compost* OR rubber)))) AND [lying time$] Search 4 ((((((cattle OR cow$ OR bovi*))) AND ((bed* OR mattress))) AND ((sand OR compost* OR rubber)))) AND (([dry matter intake] OR DMI))
Dates searches performed:	28 July 2017 (PubMed), 3rd August, 2017 (Google Scholar), 8th August,

	2017 (CAB Abstracts)

Exclusion / Inclusion Criteria	
Exclusion:	• References on Grazing (when not compared with the included PICO titles), tie-stall barns, feedlots, beef animals or calves. • Papers that did not directly compare bedding types with clear outcomes. • Papers that compared different types of the same medium (e.g. comparing 2 types of rubber mattresses) • Non peer-reviewed information sheets • Articles covering sheep or buffalo • Articles covering flooring or whole-barn systems rather than bedding specifically (*e.g.* compost or straw yards *vs* cubicle systems). This is because the influences of bedding would be mixed with flooring and barn design and confuse outcomes.
Inclusion:	• Comparisons of Lactating and dry Dairy Cow types of bedding with any search outcomes in search string • Peer-reviewed articles

Search Outcome			
Database	**Total number of results from the search strategies after deduplication**	**Excluded – not relevant to the PICO/not peer reviewed**	**Total relevant papers**
PubMed	46	11	35
Google Scholar	454	403	51
CAB Abstracts	143	118	25
Total relevant papers when duplicates removed			59

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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